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Fish oil and argan oil intake differently modulate insulin resistance and glucose intolerance in a rat model of dietary-induced obesity

Samira Samane[a,b,c], Raymond Christon[b,d], Luce Dombrowski[b,e], Stéphane Turcotte[b,e], Zoubida Charrouf[f], Charles Lavigne[b,e], Emile Levy[b,g], Hélène Bachelard[b,h], Hamid Amarouch[c], André Marette[b,e,*], Pierre Selim Haddad[a,b,*]

[a]*Department of Pharmacology, Université de Montréal, Montréal, Québec, Canada H3C 3J7*
[b]*Institut des nutraceutiques et des aliments fonctionnels, Université Laval, Québec, Canada G1K 7P4*
[c]*Department of Biology, Hassan II University, Casablanca, Morocco*
[d]*INRA, Lipides membranaires et Régulation Fonctionnelle du Cœur et des Vaisseaux, UMR 1154 INRA-Physiologie, Université Paris XI, 92290 Châtenay-Malabry, France*
[e]*Department of Anatomy & Physiology and Lipid Research Unit, Laval University Hospital Research Center, Québec, Canada G1V 4G2*
[f]*Department of Chemistry, Mohammed V University, Rabat, Morocco*
[g]*Research Center, Sainte-Justine Children's Hospital, Montreal, Québec, Canada*
[h]*Department of Medicine and Lipid Research Unit, Laval University Hospital Research Center, Québec, Canada G1V 4G2*

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Abstract

We investigated the potential metabolic benefits of fish oil (FO) or vegetable argan oil (AO) intake in a dietary model of obesity-linked insulin resistance. Rats were fed a standard chow diet (controls), a high-fat/high-sucrose (HFHS) diet, or an HFHS diet in which 6% of the fat was replaced by either FO or AO feeding, respectively. The HFHS diet increased adipose tissue weight and insulin resistance as revealed by increased fasting glucose and exaggerated glycemic and insulin responses to a glucose tolerance test (intraperitoneal glucose tolerance test). Fish oil feeding prevented fat accretion, reduced fasting glycemia, and normalized glycemic or insulin responses to intraperitoneal glucose tolerance test as compared with HFHS diet. Unlike FO consumption, AO intake failed to prevent obesity, yet restored fasting glycemia back to chow-fed control values. Insulin-induced phosphorylation of Akt and Erk in adipose tissues, skeletal muscles, and liver was greatly attenuated in HFHS rats as compared with chow-fed controls. High-fat/high-sucrose diet–induced insulin resistance was also confirmed in isolated hepatocytes. Fish oil intake prevented insulin resistance by improving or fully restoring insulin signaling responses in all tissues and isolated hepatocytes. Argan oil intake also improved insulin-dependent phosphorylations of Akt and Erk; and in adipose tissue, these responses were increased even beyond values observed in chow-fed controls. Taken together, these results strongly support the beneficial action of FO on diet-induced insulin resistance and glucose intolerance, an effect likely explained by the ability of FO to prevent HFHS-induced adiposity. Our data also show for the first time that AO can improve some of the metabolic and insulin signaling abnormalities associated with HFHS feeding.

1. Introduction

The so-called metabolic syndrome or syndrome X is characterized by the combination of central obesity, insulin resistance, hypertension, and dyslipidemia [1,2]. Each of these components constitutes a risk factor for type 2 diabetes mellitus and cardiovascular disease (CVD) [2-5]. The combination of 2 or more of these risk factors significantly increases the incidence of either disease [2-4]. Obesity and type 2 diabetes mellitus are rapidly becoming a worldwide epidemic [6]. The link between the dietary regimen and the etiology of these pathologies has been extensively studied [7,8]. Notably, it has often been highlighted that the quantity and quality of ingested dietary lipids exert a significant influence in humans and in animal models of diabetes and CVD [9-11]. Indeed, dietary lipids

* Corresponding authors. Pierre Selim Haddad is to be contacted at Tel.: +1 514 343 6590; fax: +1 514 343 2291. André Marette, Tel.: +1 418 656 4141x47549; fax: +1 418 654 2176.
E-mail addresses: andre.marette@crchul.ulaval.ca (A. Marette), pierre.haddad@umontreal.ca (P.S. Haddad).

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have been shown to play a major role in CVD risk, although the exact role of the different types of fatty acids on atherosclerosis remains unclear [12]. It is currently believed that a diet enriched in long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) reduces the risk of developing CVD [13-15]. Fish oil (FO) is rich in LCn-3PUFA, particularly in eicosapentaenoic acid and docosahexaenoic acid. Numerous studies have highlighted the beneficial effects of dietary FO, eicosapentaenoic acid, and docosahexaenoic acid on the prevention and management of dyslipidemia, insulin resistance, hypertension, and CVD [16-18]. However, the cellular and molecular mechanisms by which these beneficial actions are exerted remain unclear. The effects of LCn-3PUFA on obesity-related inflammation have been recently revisited [19], and this could explain some of the protective effect of dietary FO on the metabolic syndrome.

Omega-6 fatty acids and in particular their precursor, linoleic acid (18:2n-6), have often been shown to exert proinflammatory effects [20]. On the other hand, long-chain n-6 PUFA, namely, γ-linolenic acid (18:3n-6) and arachidonic acid (20:4n-6), were reported to be protective against CVD risk factors [21,22]. Long-term feeding of olive oil, rich in oleic acid, largely accounts for the very low prevalence of CVD in people of the Mediterranean region [23]. However, the effect of monounsaturated fatty acids such as oleic acid on cardiovascular function and diabetes remains controversial [24,25]. Among vegetable oils, argan oil (AO) is traditionally used in Morocco to counter hypercholesterolemia and associated CVD. It is extracted from the almonds of the *Argania spinosa* tree, an endemic Moroccan species growing in semidesertic areas. This oil contains a high proportion of monounsaturated fatty acids (mainly oleic acid, 45%) and n-6 PUFA (35% linoleic acid) and a very low n-3 PUFA level (0.1%-0.3% linolenic acid). It is also rich in antioxidants (eg, tocopherol and plant sterols) and in several phenolic compounds (eg, ferulic, syringic, and vanillic acids) [26,27]. Several studies in humans and animals suggest that it could be used as a nutritional supplement in the prevention of CVD and cancer [11,26,28-30]. Argan oil intake has notably been shown to decrease plasma low-density lipoprotein cholesterol [10,29] and triglycerides [31] and also to lower blood pressure in spontaneously hypertensive rats [32]. However, its use in the treatment of insulin resistance and diabetes has not been investigated to date. Argan-derived saponins were shown to exert glucose-lowering actions [33], and we have recently reported that these saponins can potentiate insulin signaling in cultured hepatoma cells [30]. The present study was undertaken to investigate the preventive effects of FO and AO intake on obesity-linked insulin resistance and glucose intolerance. We used the high-fat/high-sucrose (HFHS)–fed rat, an established model of diet-induced obesity and insulin resistance [34-36]. Physiologic and insulin signaling determinations in tissues and isolated hepatocytes revealed that both FO and AO conferred some protection against obesity-linked insulin resistance but that different mechanisms of action appear to be involved.

2. Materials and methods

2.1. Materials

Phosphospecific antibodies Erk MAPK^{Thr202} and PKB/Akt^{Ser473} and corresponding nonphosphospecific antibody kits were purchased from Cell Signaling Technology (Beverly, MA). Bovine pancreas insulin and other chemicals were obtained from Sigma-Aldrich (Oakville, Ontario, Canada).

2.2. Animals

All experiments reported herein were approved by the Laval University Animal Care and Handling Committee and comply with Canadian Council on Animal Care guidelines for the care and use of animals for research purposes. Male Wistar rats (Charles River, Montreal, Quebec, Canada) weighing 200 to 250 g at the beginning of the study were housed individually in plastic cages in animal quarters maintained at 22°C with 12-hour/12-hour dark-light schedule. The animals were randomly divided into 4 groups. One group was fed standard laboratory rat chow (rodent chow 5075, Charles River). The HFHS diet was prepared in the Marette laboratory as previously described [36]. The overall composition of experimental diets is given in Table 1, whereas Table 2 presents the fatty acid composition of each diet. The HFHS diet provides more than one half of the dietary energy in the form of fat as opposed to normal rodent diet where fat represents 10% to 12% of energy. The animals were allowed to adapt to their environmental conditions and were fed the chow diet for 3 weeks before the dietary treatments were initiated. Animals were allowed free access to all diets for 4 weeks before sacrifice and were not pair-fed. Argan oil was obtained from Arganoil in Morocco, whereas Menhaden FO was purchased from Omega Protein (Refined Oils Division, Reedville, VA).

Table 1
Overall composition of dietary regimens

	HFHS	FO	AO
Casein	20.0	20.0	20.0
Lard	19.8	16.8	16.8
Corn oil	19.8	16.8	16.8
FO	–	6.0	–
AO	–	–	6.0
Sucrose	27.0	27.0	27.0
Cellulose	5.0	5.0	5.0
Vitamin + mineral	8.4	8.4	8.4

The overall composition of experimental diets is expressed as a percentage of total weight. Note that 6% of the fat composition (3% lard and 3% corn oil) was replaced by FO or AO, respectively. All other components remained unchanged.

Table 2
Fatty acid composition of dietary regimens

Fatty acids	Diets			
	Chow (g/kg)	HFHS (g/kg)	FO (g/kg)	AO (g/kg)
C 14:0 myristic acid		2.57	7.19	2.26
C 16:0 palmitic acid	7.60	69.30	67.90	66.30
C 16:1 palmitoleic acid			6.97	
C 16:2 hexadecadienoic acid			1.42	
C 16:3 hexadecatrienoic acid			1.18	
C 16:4 hexadecatetraenoic Acid			1.04	
C 18:0 stearic acid	1.50	30.89	27.81	29.93
C 18:1 oleic acid	12.60	82.07	75.34	98.14
C 18:2 linoleic acid	31.30	138.80	118.85	136.97
C 18:3 linolenic acid	2.80	2.97	3.61	2.97
C 18:4 stearidonic acid			2.08	
C 20:1 gadoleic acid		1.98	2.47	1.97
C 20:4 eicosatetraenoic acid			1.38	
C 20:5 eicosapentaenoic acid			9.62	
C 22:5 docosapentaenoic acid			2.35	
C 22:6 docosahexaenoic acid			6.50	

The composition of each diet in various saturated and unsaturated fatty acids is expressed in grams per kilogram. Values were calculated from the product specifications provided by the various manufacturers for contents of each fat component in the diet, namely, corn oil and lard, as well as FO and AO, taking into account that 3% lard and 3% corn oil were replaced by 6% FO or 6% AO in the FO and AO diets, respectively. Values represent averages calculated from the range of content provided by the manufacturers. The fatty acid composition of lard was taken from consensual values published electronically (http://fr.wikipedia.org/wiki/Saindoux). Fatty acids having content less than 1 g/kg have been omitted for the sake of clarity.

2.3. Intraperitoneal glucose tolerance test

An intraperitoneal glucose tolerance test (IPGTT) was performed on day 26 after a 12-hour fast. Rats were injected with 1.5 g D-glucose per kilogram of body weight. Blood samples were collected from saphenous vein immediately before glucose administration and 15, 30, 60, 90, and 120 minutes thereafter. The plasma was separated by centrifugation, and aliquots were stored at −80°C for further analysis. Glucose values were obtained immediately after sampling using a glucose reagent strip and a glucometer (MediSense Precision PCx, Cherry Hill, NJ). In a subset of experiments, plasma insulin levels were also determined at each time point using an insulin radioimmunoassay kit (Linco, St Charles, MO).

2.4. Tissue preparation

At the end of the 4-week dietary treatment period and an overnight fast, a subset of rats from each treatment group was randomly distributed into 2 further groups. One group was injected with saline and the other with insulin (5 U/kg) 15 minutes before sacrifice, as described previously [37], to assess insulin response. Epididymal and perirenal adipose tissues, as well as white and red gastrocnemius muscles and liver, were quickly excised, immediately frozen in liquid nitrogen, and stored at −80°C for further analysis. Tissues were ground in liquid nitrogen. Muscle and liver were then homogenized in 6 vol of lysis buffer; and adipose tissue, in 4 vol of the same buffer. The lysis buffer used contained the following: 20 mmol/L Tris-HCl (pH 7.5), 140 mmol/L NaCl, 1 mmol/L $CaCl_2$, 10% glycerol, 1% NP-40 (IGEPAL, Sigma-Aldrich, Oakville, Ontario, Canada), protease inhibitors (2 mg/mL benzamidine, 1× inhibitors protease cocktail, phenylmethanesulfonyl fluoride, 10 g/mL aprotinine), and phosphatase inhibitors (10 mmol/L sodium fluoride, 2 mmol/L sodium orthovanadate, and 10 mmol/L sodium pyrophosphate). Tissue homogenates were shaken for 1 hour at 4°C and then clarified by centrifugation at 14 000*g* for 10 minutes. Protein concentration of the lysates was determined by bicinchoninic acid method (Pierce, Rockford, IL) using bovine serum albumin (BSA) as the standard. Lysates were used for immunoblot analysis of insulin signaling components.

2.5. Isolation and culture of hepatocytes

At the end of the experimental dietary period (4 weeks), a subset of rats was anesthetized for surgery with inspired isoflurane/N_2O_2/O_2. Rat hepatocytes from each dietary group were isolated by liver collagenase perfusion as reported previously [38]. Cells were purified by centrifugation on a Percoll gradient for 15 minutes at 3500 rpm and 4°C. Cell viability was assessed by Trypan blue (0.2% solution) exclusion and was greater than 85% before each experiment. Freshly isolated hepatocytes were suspended in Williams' E medium supplemented with 25 mmol/L sodium bicarbonate, 1% BSA, and penicillin/streptomycin (pH 7.4 at 37°C). Cells were plated at a density of 7 to 9 million cells per milliliter onto rat tail collagen-coated 35-mm plastic Petri dishes and incubated for 60 minutes at 37°C in a humidified atmosphere of 5% CO_2-95% O_2. The medium was changed to remove unattached hepatocytes, and incubation was prolonged for an additional 30 minutes.

2.6. Insulin sensitivity bioassay

Isolated hepatocytes were cultured as described above. Insulin was dissolved in 0.01 mol/L HCl and added to the cultured medium to give final concentrations of 0, 0.1, 1, 10, and 100 nmol/L. Separate Petri dishes were challenged with one of these doses of insulin for 5 minutes at 37°C, and the stimulation was stopped by quickly removing the medium and washing the cells twice with ice-cold phosphate-buffered saline (PBS). Cells were disrupted in lysis buffer (same as described above for tissues). Lysis was carried out for 5 minutes on ice followed by scraping. Lysates were recovered, and insoluble material was removed by centrifugation (12 000 rpm for 12 minutes) at 4°C. The supernatant was kept at −80°C until analyzed. Supernatant protein content was determined by the method of Bradford (1976) (Bio-Rad protein kit, Saint-Laurent, Quebec, Canada) using BSA as a standard.

2.7. Immunoblot analysis

Hepatic cell lysates as well as tissues samples were subjected to electrophoresis on 10% sodium dodecyl

Table 3
Effect of dietary regimens on body and tissue weights

Weights (g)	Chow	HFHS	FO	AO
Body weight				
Initial	244 ± 4	241 ± 4	243 ± 4	241 ± 4
Final	375 ± 8	388 ± 7	379 ± 8	376 ± 6
Weight gain	131 ± 6	147 ± 6	136 ± 7	135 ± 7
n	17	16	16	16
Liver	11.9 ± 0.7	12.0 ± 0.7	11.9 ± 0.8	10.1 ± 0.3
n	9	8	9	9
Adipose tissue				
Epididymal	[a]4.8 ± 0.4	[b]6.5 ± 0.4	[a]5.2 ± 0.4	[b]6.4 ± 0.5
Perirenal	[a]4.5 ± 0.4	[b]7.5 ± 0.3	[a]5.7 ± 0.6	[b]7.3 ± 0.5
n	16	15	15	16
Skeletal muscle				
White gastrocnemius	0.90 ± 0.07	0.90 ± 0.10	1.00 ± 0.06	0.70 ± 0.06
Red gastrocnemius	0.90 ± 0.06	1.00 ± 0.10	1.00 ± 0.10	0.90 ± 0.08
Gastrocnemius total (W + R + mixed)	4.5 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	4.4 ± 0.1
n	10	10	10	10

Values are means ± SEM of 8 to 17 independent determinations (animals). Values bearing different superscript letters are significantly different from each other according to the post hoc analysis described in "Materials and methods" ($P < .05$). The absence of superscript letters signifies the lack of statistically significant differences by ANOVA. Indeed, the Fisher F test of the overall ANOVA for body, liver, and skeletal muscle weights, respectively, failed to reach statistical significance ($P > .05$), which prevented post hoc pairwise comparisons among treatment groups. W indicates white; R, red.

sulfate–polyacrylamide gels (SDS-PAGE), transferred to nitrocellulose membranes, and blotted with phosphospecific Erk MAPK^{Thr202} (1:2000) and PKB/Akt^{Ser473} (1/1000). Equal protein loading was routinely ascertained after transfer to nitrocellulose membranes by ponceau red staining. Moreover, nonphosphorylated forms of the proteins were detected after antibody stripping and incubation of membranes with panspecific antibodies, namely, anti-Akt (1:1000), anti-ERK (1:1000), or anti-MEK1/2 (1:1000). The antibody-antigen complexes were detected with anti-mouse or anti-rabbit antibodies coupled to horseradish peroxidase and detected using an enhanced chemiluminescence kit (Perkin Elmer, Boston, MA). Autoradiographs were analyzed by laser scanning densitometry using a tabletop Hewlett-Packard (Palo Alto, CA) Deskscan II and quantified with National Institutes of Health software. For Erk, the average of signals for both the p44 and p42 isoforms was used to obtain an overall measure of this pathway.

2.8. Statistical analysis

Data are presented as means ± SE. The effects of diet were analyzed by analysis of variance (ANOVA) or repeated-measure ANOVA where appropriate. Differences between individual group means were analyzed by Fisher's protected least squares difference test. A P value less than .05 was considered to be statistically significant.

3. Results

3.1. Effects of dietary oils on body and organ weights

As shown in Table 3, initial and final body weights were not significantly different among the dietary treatments. High-fat/high-sucrose feeding tended to enhance weight gain, but this effect failed to reach statistical significance. Among measured organ weights, only adipose tissues were affected by the dietary treatments. The HFHS diet significantly increased epididymal and perirenal fat ($P < .05$), confirming the development of obesity in this dietary model. The increased adiposity was prevented by FO but not by AO intake in HFHS-fed rats. Fish oil treatment had a tendency to slightly reduce food intake as compared with HFHS-fed animals (419 ± 11 vs 445 ± 9 kJ/d), but this effect failed to reach statistical significance ($P = .051$, results not illustrated). Argan oil treatment did not have any effect on this parameter (424 ± 8 kJ/d, not significant when compared with HFHS).

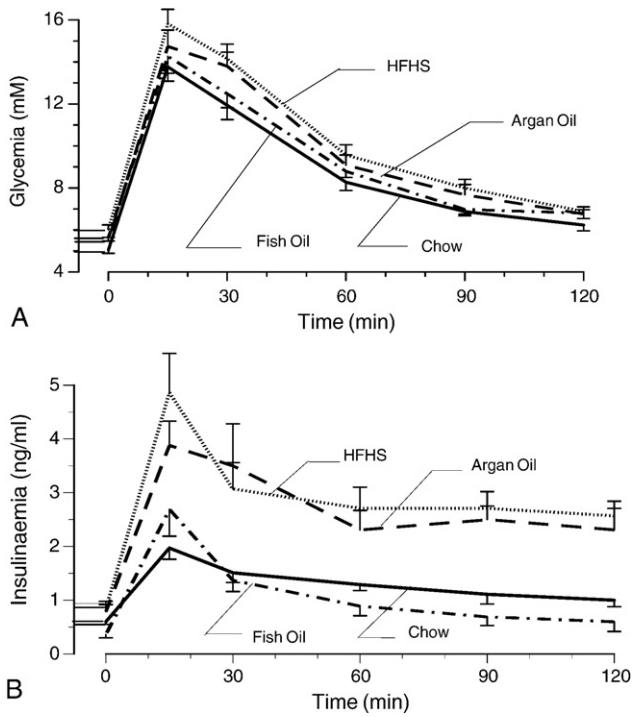

Fig. 1. Response to an IPGTT in all dietary regimen groups. Blood glucose (A) and insulin (B) concentrations were measured during an IPGTT on the 26th day of dietary treatment as described in "Materials and methods." The HFHS (dotted line) glycemia was significantly different from that of chow controls (full line) at all time points ($P < .05$), whereas AO (dashed line) values were intermediate. Fish oil (dashed-dotted line) values approached those of chow controls (full line). The HFHS (dotted line) insulinemia was significantly higher than that of chow controls (full line) at all time points ($P < .05$) except at baseline (time 0). Fish oil diet (dashed-dotted line) significantly reduced the insulin response of HFHS at all time points ($P < .05$), whereas AO (dashed line) treatment had no significant effect. The number of animals in each group and for each determination is indicated in Table 4, which also indicates the results of the AUC analysis for the IPGTT.

Table 4
Effect of dietary regimens on plasma glucose and insulin homeostasis

		Chow	HFHS	FO	AO	ANOVA *P*
Plasma glucose	Baseline (mmol/L)	[a]5.05 ± 0.16	[b]6.01 ± 0.21	[b,c]5.65 ± 0.18	[a,c]5.47 ± 0.15	.003
	n	17	16	16	16	
	IPGTT AUC	[a]1065 ± 39	[b]1231 ± 46	[a]1107 ± 39	[a,b]1177 ± 43	.003
Plasma insulin	Baseline (ng/mL)	[a,b]0.60 ± 0.49	[a]0.85 ± 0.13	[b]0.36 ± 0.57	[a]0.79 ± 0.13	.008
	n	7	7	7	8	
	IPGTT AUC	[a]155 ± 10	[b]351 ± 30	[a]130 ± 17	[b]322 ± 36	<.0001

Plasma glucose and insulin were measured at baseline and during an IPGTT as described in "Materials and methods." Values of the AUC are presented for the IPGTT. The *P* values indicated in the table refer to the significance of the Fisher F test for the overall ANOVA that allows post hoc pairwise comparisons. Values bearing different superscript letters are significantly different from each other according to the post hoc analysis described in "Materials and methods" ($P < .05$).

3.2. Effects of dietary oils on plasma glucose and insulin levels

Fig. 1 illustrates the response of plasma glucose to an IPGTT. Baseline glycemia (time 0 of IPGTT test) was significantly increased by HFHS diet as compared with chow control rats (Table 4). Argan oil treatment significantly prevented this rise in baseline glycemia, and fasting glycemia values for this group were thus not significantly

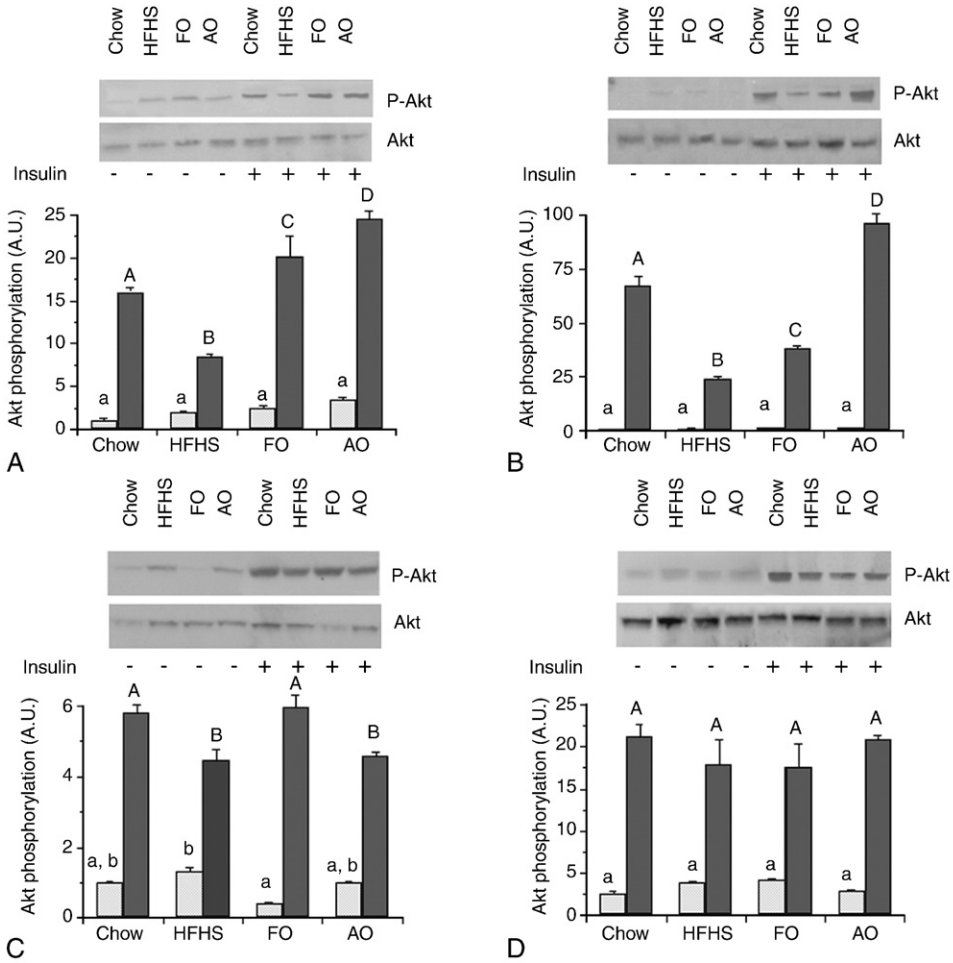

Fig. 2. Effects of dietary regimen on Akt activation in tissues. Samples of epididymal (A) and perirenal (B) fat, as well as white (C) and red (D) gastrocnemius muscle, were collected from fasting rats of all dietary regimen groups 15 minutes after intraperitoneal administration of insulin (black bars) or PBS (gray bars). Homogenates were prepared; and equal amounts of protein were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with antibodies against PKB/Akt^{Ser473}. Gels were scanned as described in "Materials and methods," and results of the densitometric analysis are presented as arbitrary units relative to the phosphorylation of PKB/Akt^{Ser473} observed in saline-treated chow controls. Values represent the mean ± SEM of 3 independent series of animals. Values bearing different superscript letters are significantly different from each other according to the post hoc analysis described in "Materials and methods" ($P < .05$). Please note that, for the sake of clarity, bar graphs have been organized to contrast responses in the absence (light bars) and presence (dark bars) of insulin for each dietary regimen and are thus not organized in the same order as the representative blots presented above each corresponding graph. AU indicates arbitrary units.

different from those of chow controls. Fish oil treatment slightly reduced baseline glycemia as compared with HFHS, but this effect failed to reach statistical significance. However, during the IPGTT test used to assess glucose tolerance, FO intake markedly reduced the glycemic responses to the glucose load as compared with untreated HFHS-fed animals (Fig. 1A). In fact, the glycemic response of the FO group was comparable to that of the chow controls when the area under the curve (AUC) parameter (taken as an index of glucose tolerance) was assessed (Table 4). On the other hand, the AO-fed group exhibited values that were intermediate between the HFHS and chow-fed control groups (not significant vs both groups). Fig. 1B shows the insulinemic responses to the IPGTT in the different dietary groups. Baseline plasma insulin levels (time 0 in Fig. 1B and values in Table 4) were significantly lower in the FO group as compared with the HFHS or AO groups. As compared with chow-fed rats, however, neither FO nor AO treatment group had a statistically significant effect on baseline insulin (Table 4). Nevertheless, FO treatment had a marked effect on the response of plasma insulin to the IPGTT (Fig. 1B). In fact, the insulin response of the FO group was comparable to that of the chow controls when the AUC parameter was compared (Table 4). Argan oil treatment failed to influence the insulin response to an IPGTT as compared with the HFHS group.

3.3. Effects of dietary oils on insulin signaling in vivo

Immunoblot analysis of 2 major pathways of insulin signaling, namely, Akt and Erk, was then carried out to assess the effects of the dietary oils on the molecular response to insulin in adipose tissues, skeletal muscles, and liver, principal metabolic targets of the hormone. In

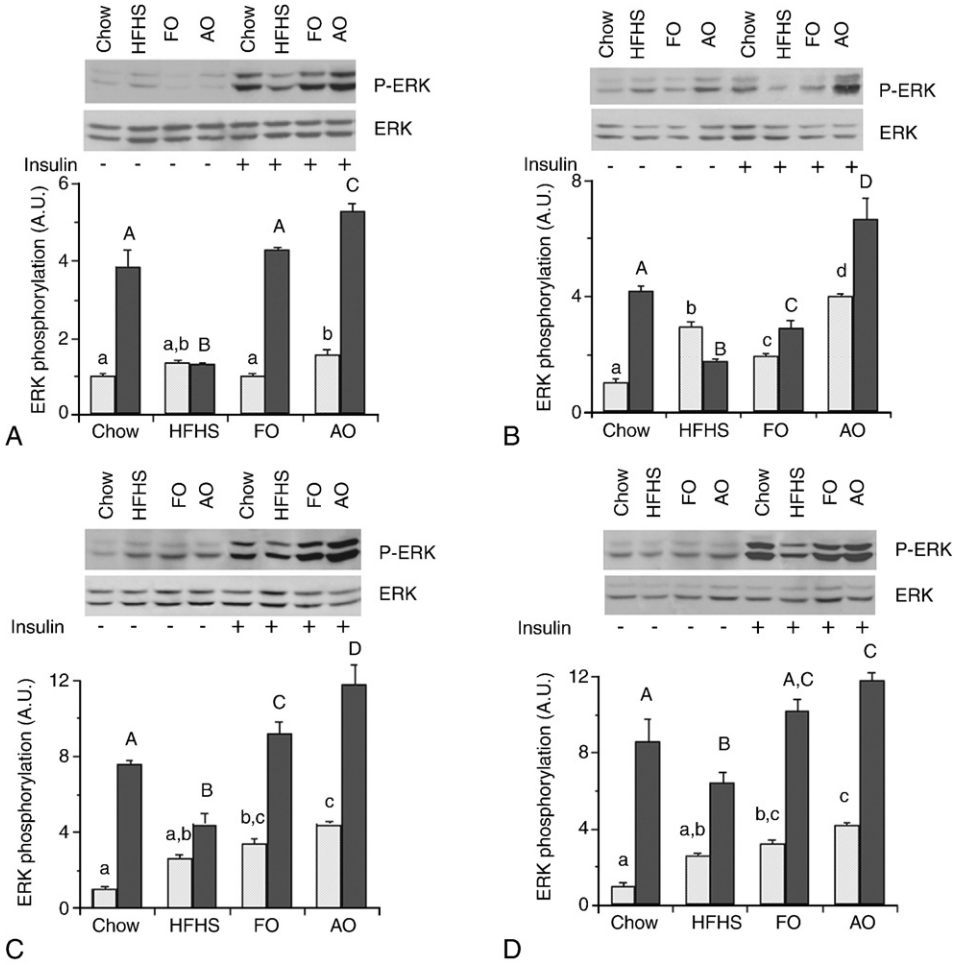

Fig. 3. Effects of dietary regimen on ERK activation in tissues. Samples of epididymal (A) and perirenal (B) fat, as well as white (C) and red (D) gastrocnemius muscle, were collected from fasting rats of all dietary regimen groups 15 minutes after intraperitoneal administration of insulin (black bars) or PBS (gray bars). Homogenates were prepared; and equal amounts of protein were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with antibodies against ERK^{Thr202}. Gels were scanned as described in "Materials and methods," and results of the densitometric analysis are presented as arbitrary units relative to the phosphorylation of ERK^{Thr202} observed in saline-treated chow controls. Values represent the mean ± SEM of 3 independent series of animals. Values bearing different superscript letters are significantly different from each other according to the post hoc analysis described in "Materials and methods" ($P < .05$). Please note that, for the sake of clarity, bar graphs have been organized to contrast responses in the absence (light bars) and presence (dark bars) of insulin for each dietary regimen and are thus not organized in the same order as the representative blots presented above each corresponding graph.

epididymal and perirenal adipose tissue, HFHS feeding greatly reduced the response of Akt to insulin as compared with the chow controls (Fig. 2). Fish oil supplementation fully restored insulin-sensitive Akt phosphorylation in epididymal adipose tissue (Fig. 2A). Improvement was less pronounced, yet still statistically significant ($P < .05$), in perirenal adipose tissue (Fig. 2B). Conversely, AO treatment returned Akt response to levels beyond that of the chow controls in both adipose tissue samples (Fig. 2A, B). None of the dietary treatments affected the basal phosphorylation state of Akt as compared with the chow controls.

In skeletal muscle, HFHS feeding reduced insulin-dependent Akt response in white gastrocnemius (Fig. 2C). In red gastrocnemius muscle, a similar tendency was observed; but the decrease in Akt activation failed to reach statistical significance (Fig. 2D). Fish oil feeding restored insulin-dependent Akt phosphorylation in white gastrocnemius muscle to the levels found in chow controls (Fig. 2C). In contrast, AO intake failed to improve the defective Akt response of the HFHS-fed group. Overall, no differences in the baseline phosphorylation state of Akt were observed among different treatment groups as compared with the chow controls.

High-fat/high-sucrose feeding completely abolished insulin-dependent activation of the Erk pathway in both adipose tissues (Fig. 3A, B). As seen for Akt phosphorylation, FO intake fully restored insulin-sensitive Erk activation in epididymal (Fig. 3A) tissue. The response of perirenal adipose tissue was similarly increased by FO diet, yet remained significantly less than that of chow controls (Fig. 3B). Argan oil intake was found to increase insulin-induced Erk phosphorylation to levels beyond that observed in adipose tissues of the chow-fed controls (Fig. 3A, B), consistent with effects on Akt phosphorylation. On the other hand, the HFHS, FO, and AO dietary treatments all increased the basal phosphorylation state of Erk in perirenal adipose tissue as compared with that of the chow controls. This was also the case for the AO treatment group in epididymal adipose tissue (Fig. 3A).

Insulin-dependent Erk activation was significantly reduced by HFHS feeding in both white and red gastrocnemius muscles (Fig. 3C, D). Animals receiving the FO diet exhibited a full recovery of Erk response to insulin in both white and red gastrocnemius muscles, whereas AO treatment enhanced insulin-stimulated Erk phosphorylation beyond the hormone response obtained in muscles of the chow-fed controls. However, it should be noted that AO intake also enhanced baseline Erk phosphorylation in both fat and muscle tissues as compared with the chow controls. When this rise in basal Erk phosphorylation is taken into account, AO feeding actually restores the action of insulin on the kinase back to the levels observed in the chow-fed controls (fold increase). None of the effects of HFHS diet and FO or AO treatment could be explained by changes in the total protein content in either Erk or Akt (Figs. 2 and 3).

3.4. Effects of dietary oils on insulin signaling in liver and isolated hepatocytes

To gain a better insight on the effect of dietary FO and AO consumption on insulin sensitivity, both Akt and Erk phosphorylation were assessed in primary cultures of hepatocytes isolated from animals in each group. Isolated hepatocytes were stimulated with 3 different concentrations

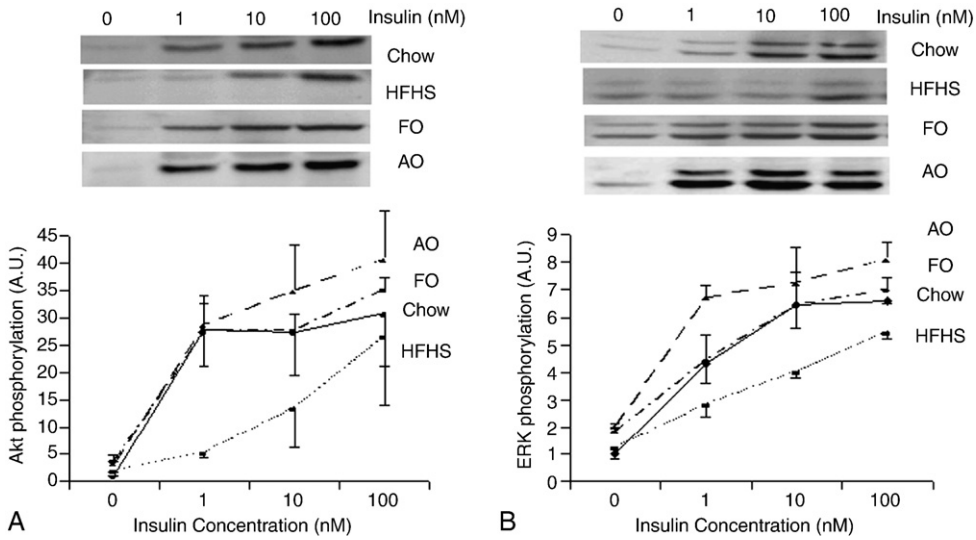

Fig. 4. Effect of in vivo dietary regimens on the insulin response of isolated hepatocytes. Hepatocytes were isolated from the livers of a subset of animals at the end of the 4-week period with each respective treatment. Primary cultures were then stimulated for 5 minutes with different concentrations of insulin. Cell lysates were prepared; and equal amounts of protein were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with antibodies against PKB/Akt^{Ser473} (A) and ERK^{Thr202} (B). Gels were scanned as described in "Materials and methods," and results of the densitometric analysis are presented as arbitrary units relative to the phosphorylation of PKB/Akt^{Ser473} or ERK^{Thr202} observed in chow controls. Values represent the mean ± SEM of 3 independent series of animals.

of insulin before being probed for phosphorylated Akt and Erk, thereby generating dose-response relationship for hormonal stimulation. Similarly to results obtained for insulin target tissues in vivo, insulin sensitivity was significantly attenuated in hepatocytes isolated from HFHS animals as compared with cells isolated from the chow-fed controls (Fig. 4). Indeed, the dose-response curves of insulin-dependent Akt and Erk phosphorylation in HFHS hepatocytes exhibited a rightward shift as compared with curves generated with cells isolated from the chow-fed controls. Both dietary FO and AO intakes were found to fully restore insulin sensitivity and the maximal hormonal response in the isolated hepatocytes. In the latter group, insulin responsiveness even tended to be enhanced beyond values observed in cells from the chow-fed controls. None of the dietary treatments had a significant impact on total Akt or Erk protein content (data not illustrated). In parallel corresponding in vivo experiments, insulin-induced Akt phosphorylation was also blunted in the liver of HFHS-fed rats; and both FO and AO intakes improved this parameter (data not illustrated).

4. Discussion

Our study shows that the intake of either dietary FO or AO can improve certain features of the metabolic syndrome in the HFHS-fed rat, a well-known model of diet-induced insulin resistance. Our results with FO expand and complete previous studies concerning its beneficial action in the metabolic syndrome, notably by showing a reduction in adiposity, as discussed further below. Previous studies have also demonstrated the beneficial effects of AO on blood lipid parameters in animals and humans [29-32,39,40]. However, to our knowledge, this is the first demonstration that AO can prevent defects in insulin signaling in both fat and liver of HFHS-fed obese rats. Importantly, our work also indicates that different mechanisms are involved in the preventive actions of FO vs AO.

As we previously reported, HFHS feeding only caused a modest increase in body weight gain but significantly enhanced epididymal and perirenal adiposity [34-36]. This model of visceral obesity is characterized by insulin resistance and perturbed glucose homeostasis, as evidenced by significantly increased fasting plasma glucose as well as exaggerated glycemic and insulin responses to an IPGTT. Cellular mechanisms underlying insulin resistance in this model were probed by assessing 2 major components of the insulin signaling pathway, which are particularly involved in the regulation of glucose metabolism and glycogen synthesis in liver, muscle, and adipose tissues. Insulin-induced phosphorylation of Akt and Erk1/2 was greatly attenuated in tissues of HFHS animals as compared with that of the chow-fed controls, consistent with our previous reports [34,35]. Similarly, insulin signal transduction was greatly reduced in primary cultures of hepatocytes isolated from HFHS animals as compared with cells obtained from the chow controls. Hence, our model appropriately reproduced several features of the metabolic syndrome and was therefore well adapted to test the effect of dietary oil intake on the mechanisms of insulin resistance.

We found that replacing 6% of the fat content of the HFHS diet by FO prevented many features of insulin resistance in HFHS-fed rats. Firstly, FO treatment prevented the gain in epididymal and perirenal fat weight induced in this dietary model. This is consistent with the data reported by Raclot and colleagues [41], but differs from other studies that either failed to observe this [42] or simply did not look at adiposity [18,43]. In FO animals, baseline glycemia also tended to be reduced as compared with that of HFHS counterparts. More strikingly, the glycemic responses of FO rats to an IPGTT were maintained at levels very similar to those observed in the chow controls. Correspondingly, the insulin response to IPGTT did not display the enhanced insulin secretion observed in HFHS animals; rather, it was almost identical to that of chow controls, indicating an improved glucose tolerance and hence insulin sensitivity. This absence of deterioration in insulin sensitivity due to the intake of FO was confirmed by enhanced insulin-dependent phosphorylation levels of Akt in epididymal fat, liver, and white gastrocnemius muscle tissues, as well as in isolated hepatocytes, as compared with those of untreated HFHS-fed rats.

Although this improvement was less evident in perirenal fat, these results corroborate the beneficial effects on insulin resistance generally attributed to LCn-3PUFA–enriched FO [18,44]. Several reports in the literature have stressed the fact that experimental conditions, in particular the type and level of dietary fat or feeding duration, strongly influence the effects of dietary fatty acids on insulin action [18,42,45-47]. In a study involving a higher FO-derived n-3 PUFA level (19%) and a lower sucrose level (5%) than used in our experimental dietary group, Taouis et al [47] showed that LCn-3PUFA FO-enriched diet prevented insulin resistance in liver as well as in muscle of high-fat–fed rats. They reported that a diet enriched in FO-derived LCn-3PUFA, contrary to that enriched in safflower oil–derived n-6 fatty acids, contributed to maintain insulin receptor, insulin receptor substrate-1 tyrosine phosphorylation, and PI 3-kinase activity in muscle but not in liver. Despite these positive effects at the tissue level, rats fed LCn-3PUFA still showed hyperglycemia and hyperinsulinemia, in contrast to the present data. However, adiposity was not reported in their study, which might explain the differences between the 2 studies.

In parallel to the improved adiposity observed in the present study, we found that FO intake prevents HFHS-induced insulin signaling defects at the level of Akt, particularly in white gastrocnemius muscle. Because Akt phosphorylation is a crucial component of insulin action on glucose transport in skeletal muscle [48], this effect likely explains the normalization of insulin sensitivity and glucose tolerance in this dietary group. Indeed, white gastrocnemius

muscle is mainly composed of type IIa and IIb fibers, which represent the largest skeletal muscle mass in the body and thus a major site of insulin-dependent glucose utilization. Akt signaling is also important for insulin-induced glucose uptake in fat [49-51] as well as for insulin stimulation of glucose metabolism in the liver [49,52]. The beneficial effects of FO on insulin sensitivity and glucose handling may thus also be explained by actions on these tissues. It is interesting to note that dietary FO was found to blunt tumor necrosis factor–α–induced insulin resistance in skeletal muscle [53]. Thus, FO intake may help prevent muscle insulin resistance by inhibiting tumor necrosis factor–α action or its release by adipose tissue, which is consistent with our observations of reduced visceral adiposity in FO-treated HFHS-fed animals. Finally, animals were not pair-fed; and FO was found to slightly reduce average caloric intake, albeit not in a statistically significant manner. Nonetheless, we cannot exclude that these slight changes in the dietary intake of FO animals may have contributed to the amelioration of obesity and the accompanying metabolic changes, despite the fact that similar body weight and weight gain were observed in all experimental groups.

Results with AO-fed animals were different from those of FO-fed congeners. Firstly, unlike FO treatment, AO supplementation did not affect adiposity as compared with the HFHS group. Similarly, AO effects on glycemic control were quite different from those of FO, with only minor improvements in the glycemic responses to an intraperitoneal glucose load. Likewise, the insulin response to an IPGTT in the AO group demonstrated an exaggerated insulin secretion that was only slightly lower than that of HFHS animals. Nevertheless, AO animals demonstrated a marked improvement in fasting plasma glucose. Argan oil feeding also significantly enhanced insulin sensitivity in both fat and liver. Effects were even seen to extend beyond the effects of FO intake on this signaling component. This hyper-responsiveness to insulin induced by AO may have contributed to maintain lipid storage through stimulation of glucose uptake in adipose tissue and hence explain the lack of effect of AO on visceral adiposity. Alternatively, the increased baseline phosphorylation state of Erk in epididymal and perirenal adipose tissue of AO-treated HFHS rats may have promoted growth and maintenance of visceral fat depots in these animals. The fact that AO improved hepatic Akt phosphorylation is consistent with the effect of this dietary regimen on fasting glycemia, and further studies will be required to clarify the mechanisms of action of AO on the liver. On the other hand, the lack of beneficial effect of AO treatment on defective insulin signaling in white gastrocnemius muscle of HFHS-fed rats could explain why it failed to significantly improve systemic insulin sensitivity, given the aforementioned importance of this tissue in systemic glucose utilization.

Several factors may explain the different responses of HFHS-fed animals to the 2 oils used in this study. Whereas the beneficial effects of FO can be mostly related to its LCn-3PUFA content [12], it is less clear whether the effects of AO can be linked to its fatty acid content. As clearly shown in Table 2, the AO diet did not have a fatty acid composition that differed greatly from HFHS diet, with the notable exception of oleic acid. Indeed, the effect of this abundant oleic acid on CVD risk factors such as insulin resistance or hypertension is rather controversial [23,24]. In addition, linoleic acid is mainly thought to be deleterious in the context of CVD [21,54,55], whereas a combination of oleic and linoleic acids (main unsaturated fatty acids of AO) has been shown to reduce endothelial function in healthy animals [56]. On the other hand, the preventive effects of AO could be attributed to the antioxidant properties of its tocopherols and polyphenols [29,57] because a reduction of oxidative stress is known to improve insulin action [58]. Indeed, the polyphenols extracted from AO have been shown in vitro to inhibit low-density lipoprotein oxidation and to stimulate the reverse transport of cholesterol [40]. In addition, there is convincing evidence for a beneficial effect of dietary AO on altered blood lipids [11]. It is, in fact, these positive considerations that prompted us to study the biological activity of AO in the context of obesity-induced metabolic syndrome. Indeed, given the involvement of dyslipidemia and oxidative stress in the pathogenesis of the metabolic syndrome and diabetes [58-60], their amelioration by AO may thus also play a role in the preventive effects observed herein.

On the other hand, it must be stressed that the whole almonds of *A spinosa* fruit have also been claimed to be useful against diabetes in Moroccan traditional medicine [30]. Indeed, the enhancement of insulin sensitivity observed herein at the hepatic level was also observed with a saponin-rich *A spinosa* press cake extract in the Hepatoma Tissue Culture rat liver cell line [30]. Hence, a more robust improvement in glycemic control may have been observed if whole *A spinosa* almonds were used in the study instead of AO. Further studies will be necessary to clarify this point. Nevertheless, taking into consideration the previously demonstrated improvement of dyslipidemia by AO, as well as the improved fasting glycemia and enhanced insulin sensitivity in fat and liver observed herein, it is possible that AO intake may have a beneficial impact on the metabolic syndrome, especially if combined with other interventions.

5. Conclusion

Our study clearly demonstrates that dietary regimens containing FO or AO can each prevent a distinct number of the deleterious effects of feeding an HFHS diet. More specifically, consuming FO prevents the development of adiposity and restores insulin sensitivity both systemically and at the level of insulin signaling components in fat, muscle, and liver. Intake of AO, in contrast, has more modest effects; notably, it does not affect adiposity or systemic insulin sensitivity but improves insulin signaling in fat and

liver beyond levels found in chow-fed animals. Fish oil and, to a lesser degree, AO thus represent promising nutritional tools to combat insulin resistance and associated comorbidities; and it is therefore critical to further clarify the mechanisms involved in their distinct beneficial actions.

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