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Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm

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Abstract

The natural ether-lipids 1-*O*-alkylglycerols (alkyl-Gro) from shark liver oil improve boar sperm motility and fertility in vitro. We examined the effects of oral shark liver oil on motility and velocity parameters of sperm together with modifications of lipid composition. Eleven boars were used as control and 11 were fed with 40 g/day for 28 days and sperm was collected on Days 0, 14 and 28 in control and treated groups. After 28 days treatment, sperm motility was improved by 2.9% as well as velocity parameters (curvilinear velocity +10.75%, progressive velocity +18.8% and average path velocity +13.5%) and sperm lipid composition was modified as follows: alkyl-Gro with saturated chains were increased (C16:0 +40.1%, C18:0 +87.2%) while alkyl-Gro with unsaturated chains remained absent, as in the control group, despite the prominence of C18:1 and C16:1 in shark liver oil. The treatment also resulted in an overall increase in the proportion of $n-3$ and $n-6$ polyunsaturated fatty acids in sperm lipids with a prominent increase of docosahexaenoic acid over time ($18.9 \pm 1.34\%$ at Day 0 to $25.7 \pm 1.11\%$ at Day 28) and compared to control ($25.7 \pm 1.11\%$ for treated versus $16.1 \pm 0.81\%$ for control at Day 28, respectively). These data demonstrate the influence of lipid intake on boar sperm composition and functions and suggest that oral intake of shark liver oil might improve reproduction.

Keywords: Boar; Spermatozoa; Motility; Alkylglycerols; Shark liver oil

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1. Introduction

The natural ether-lipids 1-*O*-alkylglycerols (alkyl-Gro) have multiple in vitro and in vivo properties: they reduce the side effects of radiotherapy, inhibit tumour growth and both stimulate and modulate immune responses [1]. Recently, we have established that alkyl-Gro modulate endothelial permeability in vitro [2]. In the field of fertility, we have previously observed beneficial effects in vitro of alkyl-Gro on boar sperm motility and fertility [3]. The mechanisms of such effects are not fully understood. 1-*O*-Alkylglycerols could serve as precursors for Platelet-activating factor (PAF) in boar sperm since the presence of PAF is correlated with sperm fertility [4] and since the effects of alkyl-Gro on sperm motility are antagonised by the specific PAF receptor antagonist SR27417 [3]. In their most abundant natural source, shark liver oil, alkyl-Gro are found in a diacylated form. In common with other fish oils, shark liver oil also is rich in $n-3$ poly-unsaturated fatty acids (PUFA). Dietary fat composition, with respect to $n-3$ PUFA, has a direct influence on the lipid composition of semen in various animal species [5,6]. In consequence, our present aim was to investigate the possibility of modifying the lipid composition and function of boar sperm with a dietary supplementation of shark liver oil containing two lipid classes with potent activities on gametes: alkyl-Gro and $n-3$ PUFA. Our data showed that boar sperm lipid composition was modified and that sperm function was improved by oral intake of shark liver oil.

2. Materials and methods

2.1. Reagents

Shark liver oil was obtained from Id-Mer (Lorient, France) after the removal of squalene from the raw oil of the Siki shark (*Centrophorus squamosus*). This oil, from a single batch, contained 25% (percent of the total weight) of alkyl-Gro as measured after saponification and separation from fatty acids. The alkyl chains, linked by an ether bond to the *sn*-1 position of the glycerol, were identified and quantified as described below and were composed of C14:0 (3%), C16:0 (7.2%), C16:1 $n-7$ (14.9%), C18:0 (1.2%) C18:1 $n-7$ (8.3%) and C18:1 $n-9$ (58.8%). Each other minor species individually represented less than 1% of the total amount. Oil fatty acid composition, analysed after saponification and transmethylation as described below, was found as follows: 14:0 (1.5%), 16:0 (16.5%), 16:1 $n-7$ (4.1%), 18:0 (2.2%), 18:1 $n-9$ (34.2%), 18:1 $n-7$ (5.6%), 18:4 $n-3$ (11.35%), 20:4 $n-3$ (10.35%), 20:5 $n-3$ (4.9%), 22:5 $n-3$ (4.35%). Each other minor fatty acids individually represented less than 0.5%. 1-*O*-Alkylglycerol 17:0 was kindly provided by Pr. Françoise Heymans (Laboratoire de Pharmacochimie Moléculaire de l'Université de Paris VI, Paris, France).

The food was formed from a basal diet consisting of wheat (22.7%), corn (15%), barley (25.6%), wheat bran (10%), soybean cattle cake (21%), vegetal oil (2%) and minerals (3.7%). Total fat represented 4.4% of dry material in which fatty acid composition was as

follows: 14:0 (0.11%), 16:0 (12.29%), 16:1 $n-7$ (0.16%), 18:0 (2.33%), 18:1 $n-9$ (20.70%), 18:1 $n-7$ (1.45%), 18:2 $n-6$ (46.24%), 18:3 $n-3$ (16.18%), 20:0 (0.19%) and 20:1 $n-9$ (0.34%).

The diluting solution for sperm samples was Beltsville Thawing Solution (BTS): glucose (3.7%), trisodium citrate dihydrate (0.6%), $NaHCO_3$ (0.125%), EDTA (0.125%), KCl (0.075%) and gentamicin (0.02%) in water (w/v). Ethylene diamine tetra-acetic acid (EDTA) was purchased from Sigma Chemical Co. (St. Louis, MO) and all solvents were obtained from Prolabo (Fontenay-sous-Bois, France). For Thin Layer Chromatography (TLC) we used silica gel 60 Å LK6 plates which were obtained from Whatman, Inc. (Clifton, NJ) and for Gas Chromatography (GC), the following equipment was utilised: GC 8000^{TOP} from Thermo Finnigan (Austin, TX). Chrompack CP-SIL-5CB capillary column (length 25 m; inside diameter 250 μm; film thickness 0.12 μm) used for alkyl-Gro analysis was purchased from Varian (Palo Alto, CA) and BPX 70 capillary column (length 30 m; inside diameter 250 μm; film thickness 0.25 μm) for fatty acids content analysis was obtained from SGE (Villeneuve Saint Georges, France).

2.2. Animal care and sperm collection

Twenty-two 2- to 3-year-old boars (LargeWhite, Pietrain and Pen Ar Lan genetic lines) were housed at a commercial boar stud (Cobiporc, St Gilles, Bretagne, France). They were housed in individual rooms with free access to water. Food (2.5 kg) was delivered to the animals every morning. Boars (11 pairs) were paired according to identical or closest birth dates and then randomly assigned to groups.

One group was used as control (they received no supplementation) and the other group received a dietary supplement of shark liver oil (40 g/day/animal), added directly to the feed, for 28 days. In each group sperm was collected on Days 0 (before treatment), 14 and 28. Ejaculates were collected in insulated beakers using the gloved-hand technique. Sperm was diluted in BTS for appropriate cell count (3×10^7 to 3.5×10^7 cells/ml) and stored at 17 °C (<2 h) until computer-assisted sperm analysis and lipid extraction. The purity of sperm population was assessed by light microscopy.

2.3. Computer-assisted analysis of motility parameters (CASA)

Sperm concentration, motility, and different movement characteristics were determined by ATS analyser (JC Diffusion International, La Ferté-Fresnel, France). This system, validated for measuring specific motility and velocity parameters of mammalian spermatozoa, was set up as described previously [3]. Diluted sperm (5 μl) was allowed to settle for 30 s in a 10-μm deep chamber (Makler counting chamber, Sefi Medical Instruments, Haifa, Israel) and 100–150 spermatozoa per sample were screened. Each measure indicated sperm number, percentage of motile spermatozoa, and the following averages: VCL (curvilinear velocity in μm/s), VSL (progressive velocity in μm/s), VAP (average path velocity in μm/s), LIN (linearity in %) and ALH (lateral amplitude of head displacement in μm). On each sample, blind measures of motility and velocity parameters were performed in triplicate with ATS system at 37 °C.

2.4. Analysis of ether-lipids in sperm

A 10^9 spermatozoa sample from each boar was washed twice in BTS, then lipids were extracted according to Bligh and Dyer [7] and 5 μg of 17:0 alkyl-Gro added as an internal standard. The extracts were treated by acetolysis as described previously by Kumar et al. [8]. Briefly, lipid extracts were incubated for 5 h at 150 °C in 0.5 ml of an acetic anhydride/acetic acid mixture (3/2; v/v). The resulting solution was extended in chloroform then washed three times with water and twice with 0.2 M Na_2CO_3. The alkyldiacetyl-Gro were separated on silica gel plates by TLC (hexane/ether/acetic acid: 40/10/0.2; v/v/v), eluted from silica with diethyl ether, dried and resuspended in a small volume of hexane. The samples were analysed by GC (200–250 °C; 2 °C/min; He 1 ml/min, column described above). Acetylated 1-*O*-alkylglycerol species were identified according to their retention time by comparison with standards analysed in the same conditions and were quantified using the alkyl-Gro 17:0 internal standard.

2.5. Analysis of sperm fatty acid composition

Lipids from 3×10^8 spermatozoa were extracted according to Bligh and Dyer [7]. The extract was saponified in 1 ml of methanolic 1 M NaOH for 45 min at 70 °C then the fatty acids were methylated by addition of 1 ml methanolic BF_3 (15 min at 70 °C). The samples were allowed to cool to room temperature and 3 ml water added. Methylated fatty acids were extracted with diethyl ether, the ether removed under nitrogen and methyl esters dissolved in n-pentane for further analysis by GC (120–210 °C; 4 °C/min, He 1 ml/min, column described above). Fatty acids were identified according to their retention time compared to those of standards analysed in the same conditions.

2.6. Statistical analysis

Data are presented as mean ± S.E.M. for each group of indicated number of animals whose semen was collected, each measurement being performed in triplicate. The significance of the differences observed between groups was assessed by repeated measures ANOVA followed by individual *t*-tests for each time point. Data at Day 0 were used as covariates and linear or quadratic regressions were used to assess the effects of treatment along time.

Analysis of treatment effect on lipidic compositions were performed by comparison of linear regression and Student paired *t*-tests for comparison between or inside groups, respectively.

3. Results

The shark liver oil was perfectly accepted by the animals and did not influence the amounts of basal diet consumed.

Table 1
Effects of shark liver oil supplementation on boar sperm percent motility, ALH and LIN

		Day 0	Day 14	Day 28
CTR	Motility	86.5 ± 1.84%	89.9 ± 0.87%	85 ± 1.2%
	ALH	1.96 ± 0.07 μm	2.01 ± 0.09 μm	2.05 ± 0.09%
	LIN	52.9 ± 1.09%	51.83 ± 1.05%	50.61 ± 1.23%
SLO	Motility	85.5 ± 1.12%	87.7 ± 1.01%	87.9 ± 1.01%*,§
	ALH	2.18 ± 0.08 μm	2.2 ± 0.06 μm	2.18 ± 0.08 μm
	LIN	51.7 ± 0.85%	51.43 ± 0.84%	52.5 ± 0.86%

Computer-assisted sperm analysis were performed in triplicate. Data are presented as means ± S.E.M. ($n = 11$).
CTR: control group; SLO: shark liver oil supplemented group.
$^{*}P < 0.05$ (CTR vs. SLO); $^{\S}P < 0.05$ (Day 0 vs. Day 28).

3.1. Effects of dietary supplementation with shark liver oil on motility and velocity of boar sperm

3.1.1. Motility

The motility of sperm (percent of mobile cells) was slightly increased (+2.4%, $P < 0.05$) after 28 days treatment (Table 1). Although there were no differences between control and treated group at Days 0 and 14, sperm from boars of the treated group at Day 28 exhibited a motility significantly greater (+2.9%, $P < 0.05$, $n = 11$) than those of the control group.

3.1.2. Velocity parameters

Analysis of velocity parameters showed that VCL, VSL and VAP were significantly increased by the treatment (P of ANOVA <0.01, 0.05, and 0.05, respectively) (Fig. 1). In the treated group the main increase was observed between Days 0 and 14, then the effects seemed to stabilise between Days 14 and 28, whereas values in the control group tended to decline over time. Thus, VCL, VSL and VAP were respectively 8.4% ($P < 0.01$), 7.9% ($P < 0.05$) and 8.9% ($P < 0.01$) greater after 14-day supplementation in the treated as compared with control animals. These differences increased to 10.75, 18.8 and 13.5%, respectively ($P < 0.001$) after 28 days of treatment. The other parameters, ALH and LIN, were neither influenced by the oral intake of shark liver oil nor by time (Table 1).

3.2. Effects of supplementation on the ether-lipid composition of sperm

Table 2 shows the composition of control and treated boar sperm at Day 0 and its change after 28 days of the experiment. One should note the prominence of C16 chains as compared to C18 chains and the absence of unsaturated C16 and C18 alkyl chains in these ether-lipids.

Dietary supplementation with shark liver oil for 28 days resulted in an increase of C16:0 and C18:0 alkyl-Gro in sperm between Days 0 and 28. The increase reached 40.1% ($P < 0.05$) and 87.2% ($P < 0.01$) for C16:0 and C18:0, respectively. In the control group, we also observed non significant variations of alkyl-Gro content: +20.6% and −1.1% for C16:0 and C18:0, respectively. Furthermore, comparison between control and treated boars

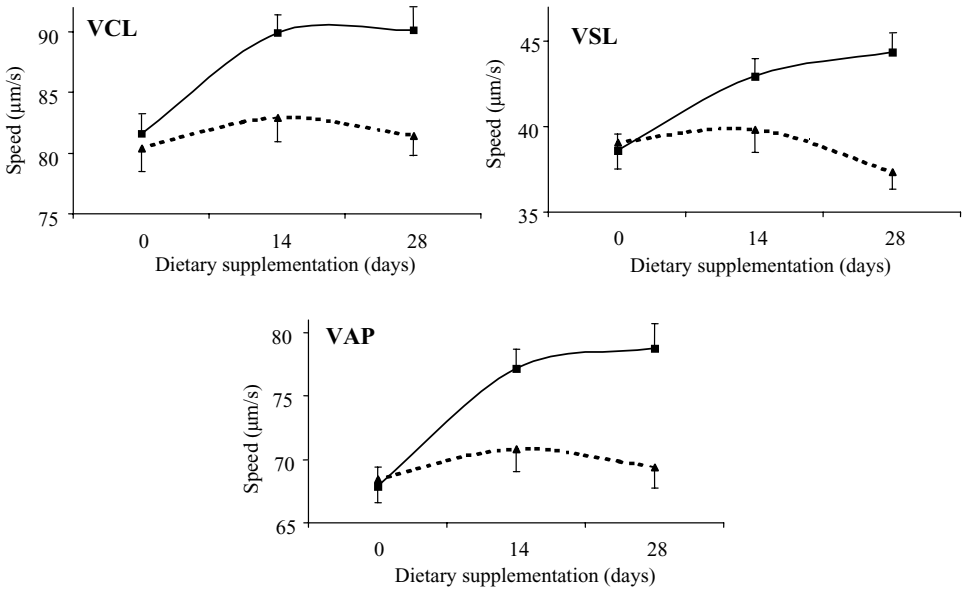

Fig. 1. Effects of dietary shark liver oil on velocity parameters of boar sperm. Boars were fed without (-▲-) or with shark liver oil supplementation (40 g/day/animal) (-■-). Velocity parameters were determined in triplicate on Days 0, 14 and 28 with an ATS analyser (means ± S.E.M.). Significance of differences between sperm of treated boars and controls for curvilinear velocity (VCL), progressive velocity (VSL) and average path velocity (VAP) was $P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively (repeated measures ANOVA, $n = 11$).

by regression analysis showed a significant difference for both C16:0 and C18:0 alkyl-Gro due to treatment ($P < 0.05$).

Interestingly, unsaturated alkyl-Gro were still not detected after treatment although they represented the major part of alkyl-Gro content of the shark liver oil, suggesting that the different species of alkyl-Gro had different fates.

Table 2
Alkylglycerol composition of sperm at Days 0 and 28 in control and treated groups

		CTR (μg/10^9cells)	SLO (μg/10^9cells)
Alkyl-Gro			
16:0	Day 0	5.76 ± 0.97	5.33 ± 0.62§
	Day 28	6.95 ± 0.68	7.47 ± 0.59*,§
16:1	Day 0	ND	ND
	Day 28	ND	ND
18:0	Day 0	0.89 ± 0.22	0.55 ± 0.20§
	Day 28	0.88 ± 0.17	1.03 ± 0.21**,§
18:1	Day 0	ND	ND
	Day 28	ND	ND

Lipid extract from sperm was treated by acetolysis and resulting alkyldiacetyl-Gro were isolated by TLC and analysed by GC ($n = 11$). CTR: control group; SLO: shark liver oil supplemented group; ND: not detected. Difference between Days 0 and 28 in treated group: $^{*}P < 0.05$; $^{**}P < 0.01$.
Significance of difference between linear regression observed for each group: $^{\S}P < 0.05$.

Table 3
Fatty acid composition (% of total fatty acids) of sperm from control and shark liver oil-supplemented boars

FA	CTR			SLO		
	Day 0[a]	Day 28[a]	Δ[a]	Day 0[a]	Day 28[a]	Δ[a]
14:0	5.4 ± 0.73	5.4 ± 0.45	0	6.2 ± 0.43	6.3 ± 0.24	+0.1
16:0	37.3 ± 1.64	37.2 ± 1.21	−0.1	36.3 ± 1.96	29.8 ± 0.90	$-6.5^{*,\S}$
16:1 $n-7$	1.0 ± 0.19	0.4 ± 0.07	-0.6^{*}	0.7 ± 0.06	0.4 ± 0.04	-0.3^{**}
18:0	19.6 ± 1.79	22.3 ± 0.65	+2.7	17.3 ± 0.66	13.2 ± 0.41	$-4.1^{**,\S\S}$
18:1 $n-9$	2.6 ± 0.39	2.8 ± 0.57	+0.2	2.7 ± 0.23	3.1 ± 0.17	+0.4
18:1 $n-7$	2.1 ± 0.55	2.5 ± 0.27	+0.4	1.1 ± 0.1	0.9 ± 0.03	−0.2
18:2 $n-6$	1.4 ± 0.22	1.7 ± 0.14	+0.3	1.8 ± 0.18	1.9 ± 0.09	+0.1
20:0	0.5 ± 0.08	0.7 ± 0.03	+0.2	0.5 ± 0.03	0.5 ± 0.02	$0^{\S\S\S}$
20:3 $n-6$	1.9 ± 0.58	0.7 ± 0.03	-1.2^{*}	1.6 ± 0.2	1.0 ± 0.05	-0.6^{*}
20:4 $n-6$	1.2 ± 0.24	1.2 ± 0.06	0	1.5 ± 0.12	2.0 ± 0.08	$+0.5^{**}$
22:4 $n-6$	0.9 ± 0.19	0.8 ± 0.04	−0.1	0.9 ± 0.07	1.2 ± 0.04	+0.3
22:5 $n-6$	8.3 ± 1.19	8.2 ± 0.34	−0.1	10.5 ± 1.35	13.6 ± 0.97	$+3.1^{*}$
22:5 $n-3$	–	–	–	–	0.4 ± 0.04	$+0.4^{**,\S\S\S}$
22:6 $n-3$	17.7 ± 1.30	16.1 ± 0.81	−1.6	18.9 ± 1.34	25.7 ± 1.11	$+6.8^{**,\S\S\S}$

Lipid extracts from sperm collected at Days 0 and 28 were treated by saponification and methylation. Resulting methyl-ester fatty acids were analysed by GC. Data are expressed as means of relative percents ± S.E.M. ($n = 11$).

FA: fatty acid; CTR: control group; SLO: shark liver oil-supplemented group.

Δ is the difference between values at Days 0 and 28 inside each group (paired t-test) with $^{*}P < 0.05$ and $^{**}P < 0.01$.

Significance of difference between linear regression observed for each group: $^{\S}P < 0.05$; $^{\S\S}P < 0.01$; and $^{\S\S\S}P < 0.001$.

[a] Percent of total fatty acids.

3.3. Effects on the fatty acid composition of sperm lipids

Treatment significantly modified the fatty acid composition profile of the sperm from the shark liver oil supplemented group whereas the composition in the control group remained nearly unchanged (Table 3). The main changes in the percent distribution were observed in the $n-3$ and $n-6$ PUFA which were increased by 38 and 20%, respectively. The strongest variations were observed for docosahexaenoic acid (DHA) (22:6 $n-3$) and for the $n-6$ corresponding PUFA, docosapentaenoic acid (DPA) (22:5 $n-6$), which were increased by 36 and 29.5%, respectively. The neat greater proportions of $n-3$ PUFA in the treated group as compared to control group was confirmed by analysis of differences between regressions ($P < 0.001$). By contrast saturated and mono-unsaturated fatty acids dropped by 17.4 and 2.2%, respectively.

4. Discussion

The aim of the present study was to assess whether oral administration of shark liver oil might modify sperm lipid composition and improve sperm function. Indeed sperm lipids were modified and sperm functions were improved by such treatment.

Previous data have shown that treating boar sperm in vitro with a mixture of naturally occurring alkyl-Gro resulted in an improvement of motility and velocity parameters of sperm, together with increased fertility when treated sperm was used for artificial insemination [3]. It is well established that dietary fat can influence sperm lipid composition: it has been shown that a diet enriched with $n-3$ fatty acids results in an increase of their proportion in sperm lipids of several species including the boar [6,9]. Furthermore, this increase may correlate with beneficial effects on sperm functions [6] and fertility [9].

Sperm lipids have different, and crucial, functions in sperm maturation and physiology. Lysophospholipids play an important role during the acrosome reaction and subsequent membrane fusion [10]. More generally, phospholipids are involved in regional variations in membrane fluidity observed during cell maturation [11]. These modifications in membrane fluidity exert a significant influence on membrane functions [12] and changes in organisation of lipid regional asymmetry in sperm plasma membrane are observed during capacitation. Bilayer translocation of phospholipids is also a major event during capacitation [13]. Furthermore, ether phospholipids have a major role in sperm physiology: they are precursors of PAF through the remodelling pathway [14]. The prominent influence of PAF on motility, capacitation and/or acrosome reaction and even fertility is abundantly documented in various species [4,15,16]. Polyunsaturated fatty acids are also important in sperm maturation [17] and physiology. Phospholipase A_2 is activated during capacitation and free PUFA, together with their metabolites, are involved in calcium flux and membrane fusion associated with the acrosome reaction [18–20].

Since both purified alkyl-Gro and $n-3$ PUFA can improve sperm function, it was of particular interest to study the effects of orally administered shark liver oil which contains both of them.

In this study, we report that a 28 days dietary supplementation with 40 g/day of shark liver oil containing alkyl-Gro and $n-3$ PUFA significantly increased the percent motility and the velocity parameters VCL, VSL and VAP of boar sperm. On the other hand, the CASA parameters ALH and LIN were not influenced by the diet. Since LIN and ALH increases are associated with spermatozoon hyperactivation often observed under capacitating conditions [21], our data suggest that dietary supplementation with shark liver oil did not induce the irreversible physiological state of capacitation.

Our data show that this oral lipid intake increased alkyl-Gro content in sperm and modified its fatty acid composition. We found that, before treatment, alkyl chains in boar sperm lipids contained only the saturated alkyl chains C16:0 and C18:0. Dietary supplementation with shark liver oil resulted in an increase of both saturated alkyl-Gro.

Surprisingly, although the treatment contained prominently C18:1 and C16:1 alkyl chains we did not detect these mono-unsaturated chains in sperm after supplementation. We have previously shown that THP-1 cells incubated with alkyl-Gro are able to incorporate both saturated and mono-unsaturated alkyl chains [22]. Other studies reported as well that dietary supplementation with alkyldiacylglycerols in rats increased the etherlipids in various tissues independently of the nature of the alkyl chains [23].

The composition of alkyl chains in sperm is poorly documented. We have shown here for the first time that, in contrast with other tissues, sperm contained essentially 16:0 and 18:0 moieties even after supplementation with unsaturated alkyl moieties. Supplementation with a high 18:1 alkyl-Gro diet and very low 18:0 resulted in a 87.2% increase in 18:0 alkyl

chain in sperm, suggesting that sperm 18:0 could result from the metabolism of other alkyl-Gro. Demonstration, mechanisms and significance of such transformations need more investigations. The predominance of saturated chains has also been observed in the plasmalogens of sperm in several species [24]. The increase in alkyl-Gro might explain partially the improvement in sperm motility and velocity. We have previously demonstrated that in vitro alkyl-Gro are incorporated into boar sperm and increase the pool of lyso-PAF [3]. Our present observations might be linked with a role of alkyl-Gro as PAF precursors since PAF with C16:0 alkyl chain is the strongest agonist on PAF receptor and that PAF concentration was also correlated with fertility [4].

Fatty acid distribution in sperm was also modified and this could as well be involved in the beneficial effects of the treatment. We found fatty acid composition of sperm in accordance with previous data [25]. $n-3$ and $n-6$ PUFA and particularly DHA (22:6 $n-3$) and its $n-6$ corresponding fatty acid DPA (22:5 $n-6$) were very markedly enriched by the diet. This could be expected considering the presence of several $n-3$ fatty acid precursors in shark liver oil [26]. Docosahexaenoic acid assumes different roles in sperm functions. Its presence is strongly correlated to optimal motility [27] and fertility [28] and its deficiency leads to a loss of sperm motility and appearance of morphological abnormalities [29]. Supplementation with $n-3$ fatty acids-rich oil was also shown to improve sperm velocity [6]. However this effect was not observed in another study [30].

Our data show for the first time that oral shark liver oil can improve motility and velocity parameters in boar sperm together with an increase in $n-6$ and $n-3$ PUFA ratio and with an increase in saturated alkyl-Gro content in sperm. These data indicate that both PUFA and alkyl-Gro may be involved in beneficial effects on boar sperm functions. This might be of interest for improving semen performance and the outcome of artificial insemination [31].

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