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Short communication

Impact of lignans on the polyunsaturated fatty acid metabolic processing in a rainbow trout (*Oncorhynchus mykiss*) cell line

Julie Mellery[a,*], Florian Scalisi[a], Chloé Bonnineau[a,b], Patrick Kestemont[c], Xavier Rollin[a], Yvan Larondelle[a,*]

[a] Life Sciences Institute, Université catholique de Louvain, Croix du Sud, 2/L7.05.08, 1348 Louvain-la-Neuve, Belgium
[b] Irstea, UR MALY, Centre de Lyon-Villeurbanne, 5 rue de la Doua, 69616 Villeurbanne, France
[c] Unité de Recherche en Biologie Environnementale et Evolutive, Université de Namur, Rue de Bruxelles, 61, 5000 Namur, Belgium

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ABSTRACT

In the context of fish oil replacement by alternative plant-derived oils in the fish feed, the influence of lignans, which are phenolic compounds present at significant concentrations in some plant-derived oils, was investigated on the omega-3 (n-3) fatty acid metabolism of the rainbow trout liver cell line RTL-W1 enriched in alpha-linolenic acid (ALA, 18:3n-3). The major linseed oil lignans, namely secoisolariciresinol diglucoside (SDG) and secoisolariciresinol (SECO), as well as the enterolignan enterodiol (END) were evaluated. Moreover, the effects of sesame oil lignans, namely sesamin (SES) and episesamin (EPI), were also investigated and compared to those previously observed in *in vivo* and *in vitro* studies which reported favourable effects on the fatty acid bioconversion capacity of salmonids. Cells were incubated with 50 μM ALA and 50 μM of SES, EPI, SDG, SECO or END for 48 h at 19 °C. The cells incubated with ALA had significantly higher amounts of 18:4n-3, 20:4n-3 and 20:5n-3 (eicosapentaenoic acid, EPA) as compared to control cells. The supplementation with SES decreased the 18:4n-3, 20:4n-3 and EPA contents, which indicates an inhibition of the desaturation and elongation steps involved in the fatty acid bioconversion pathway. As compared to SES supplementation, EPI supplementation showed a limited impact by only reducing 18:4n-3 and 20:4n-3. Similarly, END supplementation decreased 18:4n-3 and EPA, suggesting an inhibition of the desaturation capacity. Conversely, the supplementation of linseed lignans SDG and SECO had no impact on the fatty acid bioconversion capacity of RTL-W1 liver cells. In conclusion, the present results on the sesame seed lignans SES and EPI contradict the positive effects previously reported on fish lipid metabolism. In contrast, no influence of the linseed lignans SDG and SECO was observed. This suggests that the presence of lignans in linseed oil has no impact on the fish fatty acid bioconversion capacity when the oil is included to the diet.

1. Introduction

Historically, aquaculture of high value species has relied on feed formulated with fish oil and fish meal, which are finite and limited marine resources (FAO, 2014). In order to reduce fish oil inclusion into fish feed, some plant-derived oils are considered as promising alternatives since they can replace the dietary fish oil without compromising fish growth performance (Turchini et al., 2009). However, most plant-derived oils lack omega-3 (n-3) long chain (> C20) polyunsaturated fatty acids (LC-PUFA) and their inclusion in fish feed results in a reduced content of health-promoting n-3 LC-PUFA, namely eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), in fish tissues (Turchini et al., 2009; Hixson, 2014). Unlike most plant-derived oils, linseed (*Linum usitatissimum*) oil is particularly rich in alpha-linolenic acid (ALA, 18:3n-3) (NRC, 2011). ALA is the precursor of the bioconversion pathway alternately desaturating and elongating n-3 polyunsaturated fatty acids (PUFA) to produce n-3 LC-PUFA. These steps are catalysed by the endoplasmic reticulum membrane-bound Δ5 and Δ6 fatty acid desaturases (Δ5 Fads2 and Δ6 Fads2) and fatty acid elongases 2 and 5 (Elovl2 and Elovl5). A peroxisomal chain shortening is also involved (Tocher, 2003). The inclusion of ALA-rich linseed oil into fish feed induces increased desaturation and elongation enzymatic activities, especially in salmonids, and therefore an increased fish n-3 LC-PUFA content, while not reaching the n-3 LC-PUFA content of fish fed a fish oil diet (Turchini et al., 2009; Francis et al., 2014).

Lignans are polyphenolic compounds found in high concentration in some plants (Landete, 2012). Some of them possess the capacity to affect the lipid metabolism of mammals and fish (Ashakumary et al.,

* Corresponding authors.
E-mail addresses: julie.mellery@uclouvain.be (J. Mellery), yvan.larondelle@uclouvain.be (Y. Larondelle).

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1999; Trattner et al., 2008a, 2008b; Schiller Vestergren et al., 2013). Linseed is a major source of the plant lignan secoisolariciresinol (SECO), mostly found as secoisolariciresinol diglucoside (SDG) in the seeds. Sesame seeds (*Sesamum indicum*) also contain a large amount of the lignan sesamin (SES) (Landete, 2012). During the refining of sesame oil, SES is epimerised in episesamin (EPI) (Jin and Hattori, 2011). In the gastrointestinal tract of mammals, SECO, SDG, SES and EPI are transformed into enterolignans, namely enterodiol (END) and enterolactone (ENL) (Wang, 2002; Liu et al., 2006; Jin and Hattori, 2011). Some studies previously reported that the inclusion of SES and/or EPI to the diet induced an increased β-oxidation of fatty acids in rodents (Ashakumary et al., 1999; Kushiro et al., 2002). In fish, these lignans have been shown to increase the DHA content in rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet (Trattner et al., 2008a). Moreover, the supplementation of SES and EPI to the ALA-enriched culture medium of Atlantic salmon (*Salmo salar* L.) hepatocytes increased the bioconversion of ALA into DHA (Trattner et al., 2008b) and the amounts of the 18:4n-3 and 20:4n-3 bioconversion intermediates (Schiller Vestergren et al., 2011). However, these results contrast with those reporting no major effects on the DHA content in tissues of salmonids fed diets formulated with plant-derived oils and SES (Schiller Vestergren et al., 2012, 2013). Considering linseed lignans and enterolignans, neither *in vivo* nor *in vitro* studies have been up to now carried out on their potential impact on fish lipid metabolism.

Considering on one hand the context of fish oil replacement by alternative ALA-rich plant-derived oils in the fish feed and on the other hand the dietary request for EPA and DHA-rich fish, the present study evaluated the influence of several lignans on the bioconversion of ALA into n-3 LC-PUFA. More precisely, the effects of SES, EPI, SECO, SDG and END were investigated on the n-3 PUFA elongation and desaturation capacity of the RTL-W1 cell line (Rainbow Trout Liver-Waterloo 1) (Lee et al., 1993) enriched in ALA.

2. Material and methods

2.1. Chemicals and reagents

SES (≥ 95% purity, CAS 607-80-7) was obtained from Cayman (Ann Arbor, MI, USA), EPI (99.9% purity, CAS 133-03-9) from Chromadex (Irvine, CA, USA) and SDG (99% purity, CAS 158932-33-3) from PhytoLab (Vestenbergsgreuth, Germany). SECO (≥ 95% purity, CAS 29388-59-8) and END (≥ 95% purity, CAS 80226-00-2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The foetal bovine serum (FBS) was obtained from PAA Laboratories (Pasching, Austria) and the fatty acid free-bovine serum albumin from Sigma-Aldrich. ALA, the internal standard tridecanoic acid (13:0) and pure fatty acid methyl esters for fatty acid identification and quantification were obtained from Larodan (Solna, Sweden) and Nu-Check Prep (Elysian, MN, USA).

2.2. RTL-W1 cell culture

The RTL-W1 cell line, derived from the liver of a 4 year-old male rainbow trout (Lee et al., 1993), was supplied by S. Bony (INRA, France) and thawed at passage 38. The cells were routinely grown in monolayer in 75 or 175 cm^2 flasks (Cellstar, Greiner Bio-One, Vilvoorde, Belgium) at 19 °C, as previously described (Ferain et al., 2016). The growth medium contained Leibovitz-15 (L15) medium, 5% (*v/v*) FBS and 1% (*v/v*) penicillin-streptomycin mix and was renewed every 48 or 72 h. About every 7 days, cells reaching 80% of confluence were detached using 0.125% trypsin in phosphate-buffered saline supplemented with 0.03% ethylenediaminetetraacetic acid (PBS-EDTA). Trypan Blue was used as exclusion dye for cell counting.

2.3. Incubation of RTL-W1 cells with ALA and modulators

Since the RTL-W1 growth medium is deprived of n-3 LC-PUFA precursor, namely ALA, the impact of modulators (lignans/enterolignan) on the n-3 PUFA bioconversion capacity of RTL-W1 cells was tested in the presence of ALA. Four lignans and one enterolignan were investigated as modulators: SES, EPI, SDG, SECO and END. Moreover, three conditions were implemented as control: the "CT" cells were incubated with a basic medium, the "CT ALA" cells with basic medium enriched in ALA and the "CT VS" cells with the basic medium enriched in ALA and with the lignan vehicle solvent (VS), namely dimethyl sulfoxide (DMSO).

For each incubation condition (3 controls and 5 modulators tested), four independent repetitions were performed with RTL-W1 cells between passages 53 and 56. At the beginning of each passage, cells were seeded in eight 175 cm^2 flasks in the presence of a basic medium containing L15, 2% (*v/v*) of FBS and 1% (*v/v*) of penicillin-streptomycin mix. Five days after the seeding, the basic medium was removed and cells were incubated in control or experimental medium for 48 h at 19 °C. A 48-hour incubation was chosen as it was previously applied on Atlantic salmon hepatocytes supplemented in SES and EPI (Trattner et al., 2008b; Schiller Vestergren et al., 2011). The CT cells were incubated in a control medium containing L15 medium with no phenol red, 2% (*v/v*) FBS and 1% (*v/v*) penicillin-streptomycin mix. The CT ALA cells were incubated in the control medium enriched with 50 μM ALA. ALA was supplied as a complex with fatty acid free-bovine serum albumin (4:1 mol:mol) as previously described (Best et al., 2006). The CT VS cells were incubated in the control medium enriched with 50 μM ALA and 0.15% (*v/v*) DMSO. The five experimental media were composed of the control medium enriched with 50 μM ALA, 0.15% (*v/v*) DMSO and 50 μM of SES, EPI, SDG, SECO or END. The composition of the control and experimental media is summarised in Table 1. The stock solutions of modulators were dissolved in pure DMSO at a 33.3 mM concentration. At the end of the incubation, cells were collected using 0.0625% trypsin in PBS-EDTA.

The cytotoxicity of ALA, DMSO and each of the modulators was tested with the CellTiter-Blue (Promega, Leiden, The Netherlands) and 5-carboxyfluorescein diacetate acetoxymethyl ester (Life Technologies, Carlsbad, CA, USA) assays, as previously described (Ferain et al., 2016), to determine the influence of these chemicals on the metabolic activity and membrane integrity, respectively.

2.4. Fatty acid profile analysis

After two washing steps with L15 supplemented with fatty acid free-bovine serum albumin (10 mg/l), approximately 800,000 cells were collected from each condition for lipid extraction according to a method adapted from Bligh and Dyer (1959) using chloroform:methanol:water (2:2:1.8, *v:v:v*). Tridecanoic acid (13:0) was used as internal standard. The extracted fatty acids were converted into fatty acid methyl esters as previously described (Schneider et al., 2012) and subsequently separated by gas chromatography (GC). The GC Trace (Thermo Scientific, Milan, Italy) was equipped with an RT2560 capillary column (100 m × 0.25 mm internal diameter, 0.2 μm film thickness) (Restek, Bellefonte, PA, USA), an automatic injector and a flame ionisation detector kept at a constant temperature of 255 °C. The system used hydrogen as carrier gas at an operating pressure of 200 kPa. The oven

Table 1
Comparison of the control and experimental media used for the incubation of RTL-W1 cells for 48 h at 19 °C.

	CT	CT ALA	CT VS	SES	EPI	SDG	SECO	END
Control medium	x	x	x	x	x	x	x	x
ALA 50 μM		x	x	x	x	x	x	x
DMSO 0.15%			x	x	x	x	x	x
Modulator 50 μM				x	x	x	x	x

CT: control, VS: vehicle solvent, SES: sesamin, EPI: episesamin, SDG: secoisolariciresinol diglucoside, SECO: secoisolariciresinol, END: enterodiol. The control medium included L15 medium with no phenol red, 2% (*v/v*) FBS and 1% (*v/v*) penicillin-streptomycin mix.

temperature programme was as follows: an initial temperature of 80 °C, which progressively increased at 25 °C/min up to 175 °C, a holding temperature of 175 °C during 25 min followed by an increase at 10 °C/min up to 200 °C, a holding temperature of 200 °C during 20 min followed by an increase at 10 °C/min up to 220 °C, a holding temperature of 220 °C during 5 min followed by an increase at 10 °C/min up to 235 °C and a holding temperature of 235 °C for 15 min. Each peak was identified by comparison of retention times with those for pure methyl ester standards. Data processing was performed using the ChromQuest software 5.0 (Thermo Finnigan, Milan, Italy).

2.5. Statistical analyses

Data are presented as mean ± standard error of the mean of four biological replicates ($n = 4$). The analysis of the variance (one-way ANOVA) and the Tukey *post hoc* test ($P < 0.05$) were used to determine significant differences between conditions. Prior to statistical analyses, data were transformed with square root to meet the assumptions for statistical methods. Statistical analyses were performed using the JMP software (JMP Pro 12, SAS, Cary, NC, USA).

3. Results

Cytotoxicity tests were firstly performed to determine the potential toxicity of ALA, DMSO, and the modulators during the 48-hour incubation (data not shown). The incubation with 50 μM ALA alone or with DMSO or 50 μM EPI, SDG or SECO dissolved in DMSO did not decrease the cell viability (as estimated by metabolic activity and membrane integrity) while the incubation with 50 μM ALA and 50 μM SES or END dissolved in DMSO slightly reduced the cell viability by 10%.

The fatty acid profiles of RTL-W1 cells incubated for 48 h in the three control conditions or with the five different modulators are expressed in nmol per million of incubated cells (Table 2). The most abundant saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) identified in control cells were, in decreasing amounts, 18:1n-9, 16:0 and 18:0. The amounts of these three fatty acids were not significantly affected by any of the incubation conditions. Similarly, the n-6 PUFA profile of cells was not impacted by the supplementation of ALA, DMSO and any modulator into the medium. Within CT cells, EPA, docosapentaenoic acid (DPA, 22:5n-3) and DHA were the most abundant n-3 PUFA. The addition of ALA to the medium resulted in higher amounts of ALA and of the n-3 PUFA bioconverted products 18:4n-3, 20:4n-3 and EPA in CT ALA cells. In contrast, the DPA and DHA contents were not affected by ALA addition. The modulator vehicle solvent, DMSO, did not impact the fatty acid composition of cells, as compared to the CT ALA condition. While the addition of the linseed lignans SDG and SECO to the culture medium had no impact on the fatty acid composition of cells, the addition of the other modulators to the culture medium affected the fatty acid profile of cells differently. The addition of SES significantly decreased the n-3 bioconverted product content by reducing the 18:4n-3, 20:4n-3 and EPA amounts, as compared to CT VS cells. In the presence of EPI in the culture medium, a significantly lower amount of 18:4n-3 and 20:4n-3 was observed as compared to the content recovered in CT VS cells. With lignan addition, the reduction of 18:4n-3, 20:4n-3 and EPA contents were stronger with the inclusion of SES, as compared to that of EPI. The contents of 18:4n-3 and EPA were reduced in cells incubated with END as compared to cells of the CT VS condition.

4. Discussion

The present study assessed and compared the capacity of five bioactive compounds to affect the bioconversion of ALA into more desaturated and elongated n-3 LC-PUFA in the RTL-W1 cell line. For this purpose, the influence on cellular fatty acid composition of four lignans, namely SES, EPI, SDG and SECO and of one enterolignan, namely END, were evaluated on cells enriched in ALA.

Untreated RTL-W1 cells (CT condition) were rich in SFA and MUFA, reflecting the fatty acid profile of the culture medium, in which FBS was the lipid source (Tocher et al., 1988). The addition of ALA to the culture medium induced its uptake by the RTL-W1 cells and the production of the ALA-based Δ6 desaturation product, 18:4n-3, as well as its elongation product, 20:4n-3. Moreover, ALA supplementation also increased the amount of EPA in cells, resulting from the Δ5 desaturation of 20:4n-3. Previous studies already showed that the RTL-W1 (Ferain et al., 2016), rainbow trout gonad (Tocher and Sargent, 1990), fathead minnow (*Pimephales promelas*, FHM) (Gregory et al., 2011), and Atlantic salmon (Ghioni et al., 1999) cell lines were readily able to produce 18:4n-3, 20:4n-3 and EPA from ALA. Our results confirm the ability of

Table 2
Fatty acid composition of RTL-W1 cells after a 48-hour exposure to 50 μM of plant lignans or enterodiol in the presence of 0.15% (v/v) DMSO and 50 μM ALA. Results are expressed as nmol·10^6 cells^{-1} (± SEM, $n = 4$).

	CT	CT ALA	CT VS	SES	EPI	SDG	SECO	END
16:0	9.8 ± 0.6	9.2 ± 0.74	10.3 ± 0.9	10.6 ± 0.5	10.5 ± 0.7	9.0 ± 0.3	9.5 ± 0.7	9.4 ± 0.5
18:0	9.4 ± 0.6	10.7 ± 0.89	11.1 ± 0.4	10.5 ± 0.5	11.1 ± 0.8	10.4 ± 0.2	10.8 ± 0.7	10.3 ± 0.3
18:1n-7	3.2 ± 0.3	2.8 ± 0.28	2.8 ± 0.1	2.9 ± 0.1	3.1 ± 0.2	2.8 ± 0.1	2.8 ± 0.2	2.7 ± 0.2
18:1n-9	24.4 ± 1.6	20.2 ± 1.98	21.3 ± 0.8	22.0 ± 0.7	23.3 ± 2.1	20.1 ± 0.7	20.5 ± 1.1	20.1 ± 0.9
18:2n-6	2.5 ± 0.1	2.2 ± 0.23	2.4 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	2.2 ± 0.1	2.6 ± 0.4	2.4 ± 0.1
18:3n-6	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
20:3n-6	2.1 ± 0.1	1.7 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.2	1.7 ± 0.0	1.7 ± 0.1	1.8 ± 0.1
20:4n-6	3.6 ± 0.2	3.6 ± 0.4	3.8 ± 0.1	3.7 ± 0.1	4.1 ± 0.4	3.6 ± 0.1	3.7 ± 0.2	3.6 ± 0.1
18:3n-3	0.0 ± 0.0^{b}	11.9 ± 1.20^{a}	13.0 ± 0.2^{a}	11.6 ± 0.5^{a}	13.4 ± 1.0^{a}	12.0 ± 0.2^{a}	12.3 ± 0.7^{a}	14.1 ± 0.8^{a}
18:4n-3	0.0 ± 0.0^{e}	5.4 ± 0.5^{a}	5.4 ± 0.2^{a}	1.9 ± 0.1^{d}	4.1 ± 0.3^{bc}	5.2 ± 0.1^{a}	5.2 ± 0.2^{ab}	4.0 ± 0.2^{c}
20:3n-3	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
20:4n-3	0.0 ± 0.0^{c}	0.9 ± 0.2^{ab}	1.1 ± 0.1^{a}	0.0 ± 0.0^{c}	0.4 ± 0.2^{b}	0.9 ± 0.0^{ab}	0.7 ± 0.1^{ab}	1.0 ± 0.0^{ab}
20:5n-3	1.2 ± 0.1^{d}	2.9 ± 0.3^{ab}	3.1 ± 0.1^{a}	1.7 ± 0.1^{cd}	2.5 ± 0.2^{ab}	2.9 ± 0.1^{ab}	2.8 ± 0.1^{ab}	2.2 ± 0.0^{bc}
22:5n-3	2.0 ± 0.1	2.1 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
22:6n-3	2.9 ± 0.2	2.9 ± 0.3	3.1 ± 0.1	3.1 ± 0.1	3.3 ± 0.3	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
n-6 PUFA	8.3 ± 0.5	7.6 ± 0.8	8.0 ± 0.2	8.2 ± 0.4	8.7 ± 0.8	7.5 ± 0.2	8.0 ± 0.6	7.7 ± 0.2
n-3 PUFA	6.1 ± 0.4^{c}	26.3 ± 2.8^{ab}	28.4 ± 0.6^{a}	20.3 ± 0.8^{b}	26.1 ± 1.8^{ab}	26.2 ± 0.5^{ab}	26.1 ± 1.3^{ab}	26.6 ± 1.1^{a}
n-3 BC	6.1 ± 0.4^{c}	14.4 ± 1.6^{a}	15.3 ± 0.5^{a}	8.8 ± 0.3^{b}	12.7 ± 0.8^{a}	14.2 ± 0.4^{a}	13.8 ± 0.6^{a}	12.6 ± 0.4^{a}
Total	61.8 ± 3.9^{b}	76.8 ± 7.4^{ab}	82.1 ± 3.0^{a}	75.1 ± 2.8^{ab}	83.1 ± 6.5^{a}	76.3 ± 1.7^{ab}	77.7 ± 4.3^{ab}	77.2 ± 2.3^{ab}

On the same line, mean values with no common superscript letter are significantly different ($P < 0.05$). CT: control, VS: vehicle solvent, SES: sesamin, EPI: episesamin, SDG: secoisolariciresinol diglucoside, SECO: secoisolariciresinol, END: enterodiol, n-6 PUFA: sum of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6, n-3 PUFA: sum of 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 24:5n-3, 24:6n-3 and 22:6n-3, n-3 BC: sum of the bioconverted products 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 24:5n-3, 24:6n-3 and 22:6n-3, total: sum of all identified fatty acids.

RTL-W1 cells to convert ALA into n-3 LC-PUFA through Δ6 Fads2, Elovl5 and Δ5 Fads2 enzymatic activities. In contrast, the amount of the last products of the n-3 PUFA biosynthesis pathway, namely DPA and DHA, did not increase with ALA enrichment. This reflected a poor elongation rate of EPA into DPA in ALA enriched cells. Since EPA is principally elongated by Elovl2 (Morais et al., 2009; Gregory and James, 2014), the reduced elongation may be linked to a reduced Elovl2 enzymatic activity. The negligible synthesis of DPA from ALA has already been observed in the RTL-W1 liver cells (Ferain et al., 2016) but is in contradiction with results on the FHM cell line (Gregory et al., 2011). In the present study, the addition of exogenous ALA to the culture medium did not modify the amount of DHA in CT ALA cells. In a previous study with RTL-W1 cells, even EPA supplementation (50 μM) did not induce the production of DHA (Ferain et al., 2016), suggesting the loss of several enzymes including those of the peroxisomal chain shortening. This observation is in line with the lack of peroxisomes in RTL-W1 cells observed by Malhão et al. (2013).

None of the modulators impacted the amount of SFA, MUFA and n-6 PUFA recovered in cells, as compared to the CT ALA and CT VS cells. In contrast, in isolated hepatocytes from Atlantic salmon, the addition of SES and EPI (50 μM) was reported to decrease the levels of 16:0, 18:1n-7 and 18:1n-9, expressed in relative percentages. The authors suggested an increased mitochondrial β-oxidation to explain these modifications (Trattner et al., 2008b). In rodents fed SES and EPI, mechanisms involving the peroxisome proliferator-activated receptor alpha (*Pparα*) were suggested to be responsible for the observed increase in the gene expression and activity of hepatic β-oxidation enzymes (Ashakumary et al., 1999; Kushiro et al., 2002).

4.1. Modulation by SES and EPI

The positive effects of ALA enrichment on the fatty acid metabolism of cells were reduced by the SES supplementation. Thus, SES addition appeared to reduce the desaturation rates of ALA and 20:4n-3 and the elongation rate of 18:4n-3, leading to lower amounts of 18:4n-3, 20:4n-3 and EPA in SES exposed cells than in CT VS cells. Further experiments are required to conclude whether this reduced desaturation and elongation was due to an alteration of the Δ6 Fads2, Δ5 Fads2 and Elovl5 activities, or a down-regulation of the expression of their associated genes, namely fatty acid desaturase 2 (*fads2*) and elongases 5 (*elovl5*), or a decrease of the *fads2* and *elovl5* mRNA translation rates. As observed for SES, the addition of the lignan EPI also reduced the ALA desaturation rate and the 18:4n-3 elongation rate, leading to lower amounts of 18:4n-3 and 20:4n-3 in EPI exposed cells than in CT VS cells. While the high similarity between the SES and EPI structures (Jin and Hattori, 2011) could explain similar mechanisms of action between the two modulators, the effects of SES addition were significantly more important as compared to the effects of EPI addition. Indeed, the amounts of 18:4n-3, 20:4n-3 and EPA were much lower in cells incubated with SES as compared to EPI. Differences between SES and EPI effects have previously been observed in the literature. For instance, in rats, EPI was more efficient than SES in increasing the hepatic fatty acid β-oxidation due to the higher EPI bioavailability in the liver (Kushiro et al., 2002). Moreover, the incubation of Atlantic salmon isolated hepatocytes with a medium enriched in ALA and EPI up-regulated the *pparα* expression, unlike a SES and EPI equimolar-mixture supplementation, which appeared without effect on the *pparα* expression (Schiller Vestergren et al., 2011). Conversely, the DNA synthesis inhibition in human leukaemia cells was more relevant with SES than EPI (Ju et al., 2001).

In contrast to our results, SES and EPI were found to stimulate the n-3 PUFA biosynthesis pathway in a primary culture of Atlantic salmon hepatocytes (Trattner et al., 2008b; Schiller Vestergren et al., 2011). In one hand, Trattner et al. (2008b) pointed out that the incubation of cells with an equimolar-mixture of SES and EPI (50 μM) during 48 h increased the bioconversion of ALA into DHA in a primary culture of Atlantic salmon hepatocytes. On the other hand, Schiller Vestergren et al. (2011) demonstrated that the incubation of cells with an equimolar-mixture of SES and EPI or EPI alone (50 μM) increased the amounts of 18:4n-3 and 20:4n-3 in a primary culture of Atlantic salmon hepatocytes. The differences in the cellular fatty acid composition between the studies performed with primary cultures of hepatocytes and the present study on RTL-W1 cells may partially explain the contrasting results obtained. More precisely, the cells used in the present study had a fatty acid profile containing much more ALA and much less n-3 LC-PUFA, as compared to the hepatocyte primary cultures (about 16% *versus* 3% for ALA and 4% *versus* 30% for DHA respectively, expressed in percentage of total identified fatty acids). Indeed, in both previous studies, Atlantic salmon were fed a fish oil-based commercial diet prior to the isolation of hepatocytes. In contrast, the RTL-W1 cells were cultivated with 2% of FBS and had therefore a very limited access to n-3 LC-PUFA. Moreover, the culture medium used for both Atlantic salmon hepatocyte studies contained 7 μM of ALA whereas 50 μM of ALA were added to the culture media in the present study. Such differences in fatty acid composition are very likely to affect the membrane composition, which is known to modify the carrier-mediated transport and the activity of membrane-bound enzymes (Stubhaug et al., 2005), such as the desaturases and elongases involved in the n-3 PUFA biosynthesis pathway. In addition, the membrane composition variation may have influenced the lignan cellular uptake and potentially lead to different effects if those are not linearly linked to the actual modulator intra-cellular concentrations.

4.2. Modulation by SDG and SECO

The present study was the first one focusing on the impact of SDG and SECO on the n-3 fatty acid metabolism. SDG is the major lignan identified in linseed, which is the richest source of plant lignans (Landete, 2012). The present study demonstrated no apparent effect of linseed lignans on the lipid metabolism of RTL-W1 cells incubated with 50 μM ALA. This suggests that the presence of lignans, found in significant concentrations in linseed oil, is not a part of the explanation of the increased fatty acid bioconversion capacity observed in fish fed a linseed oil diet as compared to those fed a fish oil diet. Moreover, the present study suggests that linseed lignans have no detrimental effect on the fish fatty acid bioconversion capacity. Other linseed oil compounds may interact with the fish n-3 PUFA bioconversion capacity. For example, the study of Schiller Vestergren et al. (2013) highlighted that the polar fraction of linseed oil may affect the metabolism of PUFA in rainbow trout since higher EPA and lower ALA proportions were found in white muscle of fish fed a diet based on purified linseed oil with removed polar fraction, as compared to fish fed a classical linseed oil-based diet.

In humans and rats, SDG and SECO are known to reach the liver mainly as END and ENL since linseed lignans are rapidly metabolised by gastrointestinal bacteria before being absorbed by enterocytes (Wang, 2002; Liu et al., 2006). In fish, the transformation of SDG and SECO by gastrointestinal bacteria is unknown. Concerning the capacity of lignans to enter in RTL-W1 cells and therefore to induce a potential effect inside the cells, the lignan uptake by fish hepatocytes has never been investigated. Moreover, the absorption rate probably varies according to the lignan structure. Additional experiments are required to evaluate the actual uptake of these compounds by the RTL-W1 cells.

4.3. Modulation by END

As for linseed lignans, the present study is the first one to assess the impact of an enterolignan on the n-3 fatty acid metabolism in fish. In the present study, the END supplementation induced a negative effect on the fatty acid metabolism, as highlighted by the decreased 18:4n-3 and EPA contents in cells as compared to the CT VS cells. Enterolignans are one of the major phytoestrogen classes, which can act as either

oestrogen agonists or antagonists, depending on the biological level of oestradiol (Wang, 2002). In the human adipose tissue, oestrogens are known to down-regulate the expression of genes encoding the Δ5- and Δ9-desaturases (Lundholm et al., 2008). END may have altered the RTL-W1 cell desaturation capacity through its oestrogenic activity. In humans and rats, both sesame seed and linseed lignans are considered as efficient enterolignan precursors (Peñalvo et al., 2005; Liu et al., 2006; Jin and Hattori, 2011). Therefore, the impact of END on the liver cells may reflect the *in vivo* influence of dietary SES, EPI, SDG and SECO on salmonid hepatic lipid metabolism. However, the existence of enterolignans in the salmonid gastrointestinal tract is still unknown.

5. Conclusion

The present study on the RTL-W1 cell line indicated that the ALA supplemented to the culture medium was readily absorbed and bioconverted into 18:4n-3, 20:4n-3 and EPA. The SES addition to the culture medium decreased the amount of 18:4n-3, 20:4n-3 and EPA, which indicates an inhibition of the desaturation and elongation steps involved in the transformation of ALA into EPA. EPI addition showed a limited impact as compared to SES addition, and only reduced the amount of 18:4n-3 and 20:4n-3 in cells. The negative impact of SES and EPI contrasts with the previous literature reporting a positive effect of sesame seed lignans on the fish fatty acid bioconversion capacity. The addition of the linseed lignans SDG and SECO to the culture medium had no impact on the ALA bioconversion capacity of RTL-W1 cells. In a context of dietary fish oil replacement by ALA-rich plant-derived oils, the present results suggest that lignans of linseed oil are not involved in the high fatty acid bioconversion capacity observed in fish fed a linseed oil diet.

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