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ORIGINAL ARTICLE

Fucoxanthin Enhances Chain Elongation and Desaturation of Alpha-Linolenic Acid in HepG2 Cells

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Abstract Dietary fucoxanthin (FX), a carotenoid compound from brown algae, was found to increase docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) in the liver of mice. DHA and ARA are known to be biosynthesized from the respective precursor α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LNA, 18:2n-6), through desaturation and chain elongation. We examined the effect of FX on the fatty acid metabolism in HepG2 cells (Hepatocellular carcinoma, human). In the first experiment, cells were co-treated with ALA (100 μM) and FX (0–100 μM) or vehicle for 48 h. FX increased eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), DHA at concentrations of ≥50 μM. To clarify the change in the metabolism of polyunsaturated fatty acid (PUFA), in the second experiment, cells were co-treated with universally-[^{13}C]-labeled (U-[^{13}C]-) ALA (100 μM) and FX (100 μM) for 0.5, 3, 6, 24 and 48 h. [^{13}C] labeled-EPA, DPA and DHA content in HepG2 cells were all increased by FX after 48 h treatment. Furthermore, estimated delta-5 desaturase (D5D) but not delta-6 desaturase (D6D) activity index was increased at 48 h. These results suggested that FX may enhance the conversion of ALA to longer chain n-3 PUFA through increasing D5D activity in the liver.

Keywords Fucoxanthin · Delta-5 desaturase · Elongation · ALA · EPA · DHA · n-3 PUFA

Abbreviations

ACOX-1	Acyl-CoA oxidase-1
ARA	Arachidonic acid
ALA	α-Linolenic acid
BSA	Bovine serum albumin
D5D	Delta-5 desaturase
D6D	Delta-6 desaturase
DHA	Docosahexaenoic acid
DGLA	Dihomo-γ-linolenic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
ETA	Eicosatetraenoic acid
FAME	Fatty acid methyl ester
FBS	Fetal bovine serum
FX	Fucoxanthin
GLA	γ-Linolenic acid
LNA	Linoleic acid
LXRα	Liver X receptor α
MUFA	Monounsaturated fatty acid
OLA	Oleic acid
PPARα	Peroxisome proliferator-activated receptor α
PUFA	Polyunsaturated fatty acid
SCD1	Stearoyl-CoA desaturase 1
SDA	Stearidonic acid

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SREBP Sterol regulatory element binding protein
U-[^{13}C]- Universally [^{13}C]-labeled

Introduction

The health benefits of long-chain n-3 polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) typically found in marine foods, are well documented. These include reducing plasma triglycerides, lowering blood pressure, decreasing the thrombotic tendency, improving vascular endothelial function and ameliorating insulin sensitivity, and overall, an increased intake reduces the risk of metabolic syndrome and cardiovascular disease [1, 2]. In addition, evidence supporting the roles of EPA and DHA in cognitive functions as well as neurological and psychiatric disorders have also been reviewed recently [3].

In addition to obtaining EPA and DHA directly from dietary intake, mammals, including humans, are equipped with enzymes for metabolizing α-linolenic acid (ALA) to its longer chain and more unsaturated forms including EPA, docosapentaenoic acid (DPA) and DHA as summarized in Fig. 1 (modified from [4]). The delta-6 desaturase (D6D) and delta-5 desaturase (D5D) catalyze the rate-limiting steps in conversion of ALA into longer chain n-3 PUFA in humans. Supplemental ALA raises blood EPA and DPA levels but have little changes in DHA status in humans [5] and rats [6], suggesting that the synthesis of DHA from ALA is inefficient. On the other hand, D5D and D6D activity index can be estimated from the fatty acid product-to-precursor ratios and are widely used in epidemiological studies. The estimated

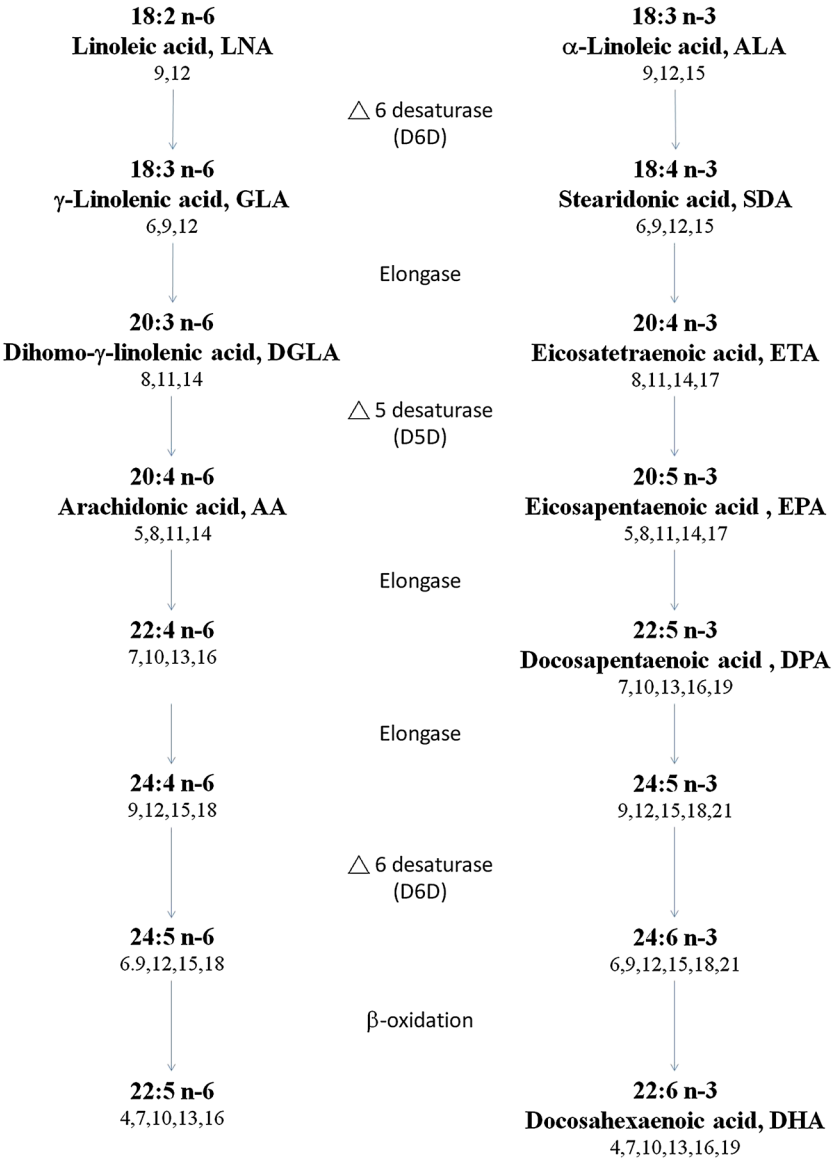


Fig. 1 Conversion of linoleic and alpha-linolenic acid to long-chain n-6 and n-3 PUFA by the action of delta-6 desaturase, delta-5 desaturase and elongases

D5D activity index was found to be inversely associated, while the D6D activity index directly associated with the risk of type II diabetes [7, 8]. The D5D activity index has also been shown to be inversely associated with plasma triglyceride level [9], obesity and insulin resistance [10].

Fucoxanthin (FX), a marine carotenoid, has been reported to have many health benefits, such as anti-oxidant, anti-obesity and anti-cancer activities [11]. A few studies observed that dietary FX enriches hepatic EPA, DHA and arachidonic acid (ARA) content in rodents [12–15]. In contrast, a study using rat hepatoma BRL-3A cells observed a suppression of EPA from ALA by fucoxanthin and fucoxanthinol [16]. As HepG2 cells were more commonly used in the studies of longer chains PUFA conversions [17, 18], we traced universally-[^{13}C]-labeled (U-[^{13}C]-) ALA metabolism in this cell line to examine the effect of FX. At ALA and FX concentrations that are higher than those previously reported [16], our results agree with those obtained from rodent studies [12–15] that FX enhanced conversion of ALA to EPA and DHA. This may be related to increased D5D, but not D6D activities.

Materials and Methods

Materials

FX was purified from *Hincksia mitchellae* (Harvey) P. C. Silva brown algae as described previously [19] and dissolved in absolute ethanol as a stock. Preparation of U-[^{13}C]-ALA was modified following a previous publication [20]. Mixed fatty acid methyl ester (FAME) standards were obtained from Supelco (Bellefonte, PA, USA). Natural ALA and fatty acid-free bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Cell culture medium and serum were purchased from Gibco (Grand Island, NY, USA). All other chemicals and reagents were of analytical grade.

Albumin-Bound ALA

Preparation of BSA-bound natural ALA or U-[^{13}C]-ALA were modified from the method of Su [21] by adding 0.2 N $NaOH_{(aq)}$ to the fatty acids on an equimolar basis and then adding the neutralized fatty acid to an appropriate volume of 0.33 mM fatty acid-free BSA dissolved in HEPES buffer (25 mM, pH 7.0). The vials were flushed with nitrogen and orbital shaking at room temperature for 5 h and filtered through a 0.22-μm syringe filter. A fatty acid salt/BSA complex in a molar ratio of 3:1 was obtained.

Cell Culture

HepG2 cells (Human hepatoma cell line) were obtained from the Bioresource Collection and Research Center (BCRC number RM60025, Hsinchu City, Taiwan) and cultured in DMEM supplemented with 10 % fetal bovine serum (FBS). Cells were grown in 75-cm^2 tissue culture flasks and incubated at 37 °C in a 5 % CO_2 humidified incubator. At 80 % confluence, cells were harvested and seeded into a 6-well plate at a density of 2×10^6 cells/well in 2 mL growth medium for 3 days of incubation and then used for the experiments. In the first experiment, the medium was removed and replaced with serum-free DMEM supplemented with vehicle (BSA and ethanol) or BSA-bound ALA (100 μM) plus FX (0, 25, 50, 75 or 100 μM) for 48 h. These treatments did not significantly alter cell viability (data not shown), as indicated by the MTT assay. The cells were harvested and stored at −80 °C before lipid extraction and fatty acid analysis. In the second experiment, the medium was removed and replaced with a vehicle (BSA and ethanol), BSA-bound U-[^{13}C]-ALA (100 μM) or BSA-bound U-[^{13}C]-ALA (100 μM) plus FX (100 μM) for 0.5, 3, 6, 24 or 48 h. The cells were collected and stored at −80 °C before further analysis.

Lipid Extraction, Methylation and Analysis

Treated cells were trypsinized, washed with PBS three times and re-suspended in 0.8 mL PBS. 3 mL of methanol/chloroform (2:1, by vol) and internal control (C13:0 for the first experiment; C17:0 and C23:0 for the second experiment) were added to cell suspension and sonicated for 20 min and incubated at 4 °C overnight. Then 1 mL chloroform and 1 mL 0.7 % $NaCl_{(aq)}$ were added, this was then vortexed, and centrifuged at 2000 rpm for 5 min. The chloroform layers were collected and dried under $N_{2(gas)}$. Then 1 mL methanol/dichloromethane (3:1, by vol) was added, vortexed, and 200 μL acetyl chloride was added with slowly vortex. The tubes were tightly closed and subjected to transesterification at 75 °C for 1 h. After cooling down to room temperature, 4 mL 7 % $K_2CO_{3(aq)}$ was added to the tubes to stop the reaction and neutralize the mixture. Two milliliters of hexane was added to extract the FAME. The tubes were briefly vortexed and then centrifuged at 2000 rpm for 5 min. The top layer was collected, dried under $N_{2(gas)}$, re-dissolved in 100 μL hexane and transferred to a sample vial for GC analysis. For the first experiment, GC- FID (Agilent, Santa Clara, CA, USA) was used for fatty acid composition analysis. For the second experiment, triple Quadrupole GC–MS (Agilent Technologies, Santa Clara, CA) was used for identification and quantitation of [^{13}C] labeled-fatty acid methyl esters. All the ALA metabolites containing the labeled fatty acid chain were detected specifically by MS monitoring the expected mass for the naturally abundant species + 18, in all cases this was at the same retention time as the native fatty acid methyl ester but separated based on mass.

Statistics

Data are expressed as means ± SD. Statistical analysis was performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). For all statistical analyses, data were transformed logarithmically if the variances were non-homogeneous. One-way ANOVA and Duncan's multiple range tests were used to compare differences between treatments in the first experiment. Significances of effects of time and FX on U-[^{13}C]-ALA conversions in Exp 2 were analyzed by two-way ANOVA. Student's *t* test was used to compare ALA and ALA + FX treatment in the second experiment when a significant interaction of time × FX resulted. Statistical significance was defined as $p < 0.05$.

Results

Exp 1: Fatty Acid Composition of Cells Co-treated with ALA and FX

To investigate the effect of FX on the metabolism of ALA, HepG2 cells were treated with 100 μM ALA supplemented with 0–100 μM FX for 48 h. Results were shown in Table 1. Cells treated with "ALA only" accumulated various n-3 fatty acids including EPA and DPA, which were non-detectable in cells treated with vehicle. Co-treatment with ≥50 μM FX further increased levels of EPA, DPA and DHA. The level of EPA in the ALA + 75 μM FX-treated cells was 2.5-fold that of the ALA-treated cells (96.29 ± 13.00 *vs*. 39.15 ± 6.49 nmol/well). The level of DPA in the ALA + 75 μM FX-treated cells was 2.0-fold that of the ALA-treated cells (27.40 ± 3.16 *vs*. 13.86 ± 1.89 nmol/well). The level of DHA in the ALA + 75 μM FX-treated cells was two times that of the ALA-treated cells (28.98 ± 4.90 *vs*. 14.76 ± 1.83 nmol/well). The accumulation of stearidonic acid (SDA, 18:4n-3) was significantly higher with the co-treatment of ≥75 μM FX. Noticeably, the amount of ARA was also increased by the co-treatment of 75 and 100 μM FX.

Exp. 2 Metabolites of U-[^{13}C]-ALA in Cells Co-treated with FX

To further examine the effect of FX on the elongation and desaturation of ALA in the HepG2 cells, cells were co-treated with 100 μM U-[^{13}C]-ALA and 100 μM FX for 0–48 h and n-3 fatty acids labeled with 18 ^{13}C atoms were tracked. Results of two-way ANOVA indicated that both incubation time and FX significantly increased [^{13}C] labeled EPA, DPA, 24:5 n-3 and DHA levels ($p < 0.05$) but the interactions of time × FX were also significant ($p < 0.05$). For the remaining [^{13}C] labeled fatty acids, only the time effect were significant ($p < 0.05$). For the first 6 h, there was a rapid increase of U-[^{13}C]-ALA in the cells (Fig. 2a) indicating the uptake of the fatty acid. The level

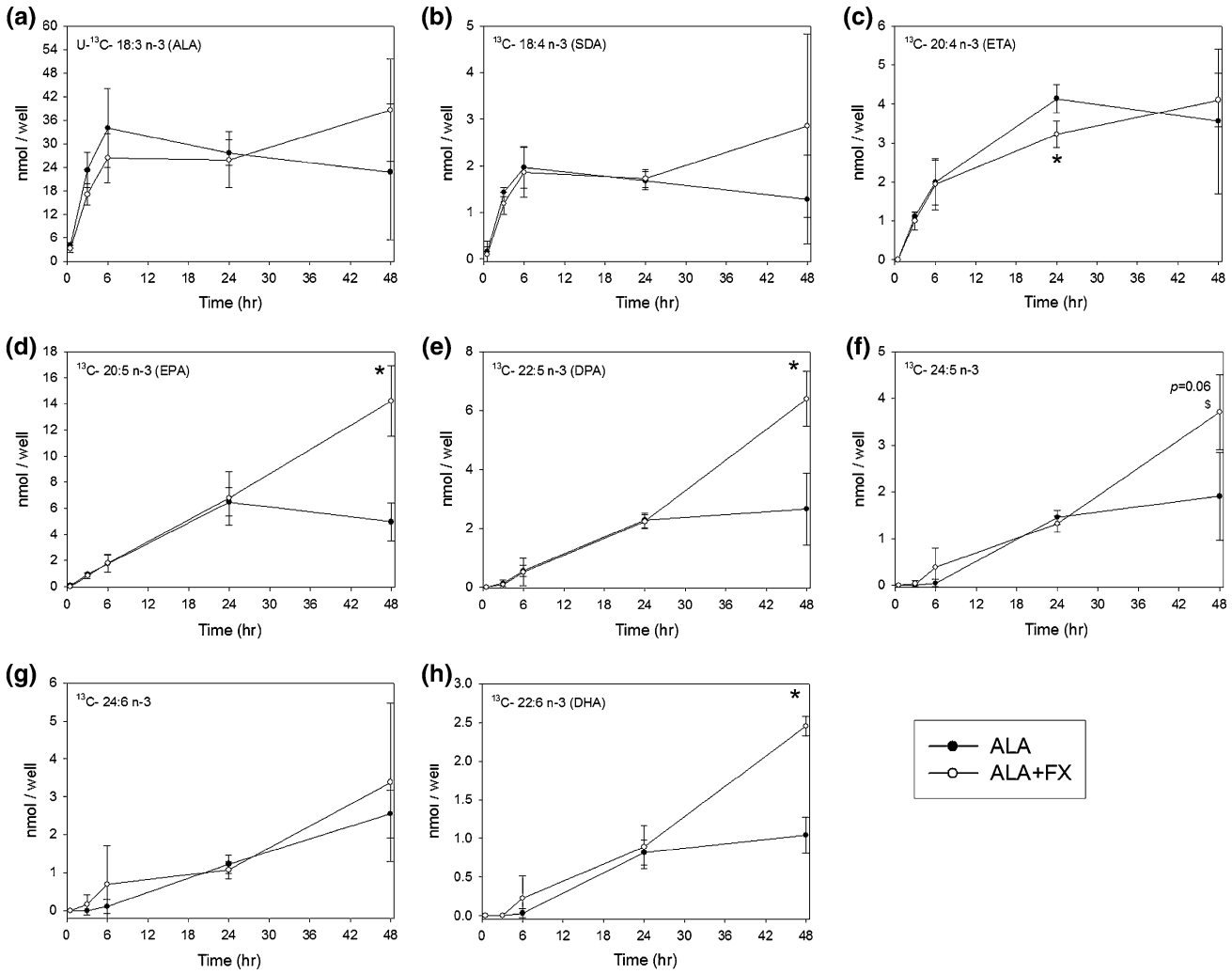
Table 1 Fatty acid composition (nmol/well) of HepG2 cells treated with ALA and various concentrations of FX for 48 h

FX (μM)	−	−	25	50	75	100
ALA (100 μM)	−	+	+	+	+	+
Total saturated	297.76 ± 106.49^{c}	452.53 ± 59.12^{ab}	341.89 ± 54.96^{bc}	504.58 ± 30.89^{a}	543.34 ± 41.28^{a}	490.45 ± 67.10^{a}
Total MUFA	483.72 ± 176.06^{ab}	552.78 ± 63.72^{a}	329.52 ± 70.77^{b}	503.65 ± 56.18^{ab}	561.54 ± 86.02^{a}	536.54 ± 117.50^{a}
18:1n-9 OLA	146.51 ± 53.42^{ab}	175.72 ± 16.82^{a}	89.87 ± 18.39^{b}	145.31 ± 15.48^{ab}	164.94 ± 27.41^{a}	156.54 ± 37.78^{a}
Total n-9	231.99 ± 82.85^{a}	228.63 ± 18.28^{a}	131.54 ± 25.82^{b}	205.37 ± 17.05^{ab}	231.98 ± 33.67^{a}	223.01 ± 48.85^{a}
18:2n-6 LNA	38.37 ± 11.58^{a}	22.73 ± 0.93^{bc}	14.48 ± 1.04^{c}	23.54 ± 1.38^{bc}	26.87 ± 0.39^{b}	27.06 ± 3.96^{b}
20:3n-6 DGLA	2.61 ± 0.63^{a}	2.74 ± 0.45^{a}	2.90 ± 0.27^{a}	3.88 ± 0.30^{a}	3.68 ± 0.94^{a}	3.39 ± 0.92^{a}
20:4n-6 ARA	14.08 ± 7.24^{b}	13.93 ± 1.61^{b}	15.24 ± 3.60^{ab}	21.05 ± 0.70^{ab}	23.54 ± 4.98^{a}	22.79 ± 4.75^{a}
Total n-6 PUFA	54.19 ± 19.93^{a}	39.40 ± 1.98^{ab}	31.66 ± 6.13^{b}	48.47 ± 0.90^{ab}	54.09 ± 5.51^{a}	53.23 ± 8.33^{a}
18:3n-3ALA	n.d.	43.59 ± 10.06^{a}	23.62 ± 3.86^{b}	46.05 ± 13.36^{a}	50.54 ± 11.66^{a}	33.33 ± 9.77^{ab}
18:4n-3 SDA	n.d.	11.62 ± 1.25^{bc}	7.55 ± 0.48^{c}	14.98 ± 1.72^{ab}	17.94 ± 0.48^{a}	16.99 ± 4.58^{a}
20:4n-3 ETA	n.d.	26.15 ± 4.60^{a}	14.94 ± 2.67^{b}	23.85 ± 5.65^{a}	25.37 ± 2.33^{a}	16.60 ± 3.16^{b}
20:5n-3 EPA	n.d.	39.15 ± 6.49^{b}	49.15 ± 9.34^{b}	86.53 ± 3.12^{a}	96.29 ± 13.00^{a}	84.60 ± 14.27^{a}
22:5n-3 DPA	n.d.	13.86 ± 1.89^{b}	11.81 ± 0.77^{b}	23.86 ± 3.23^{a}	27.40 ± 3.16^{a}	23.38 ± 1.19^{a}
22:6n-3 DHA	4.04 ± 2.32^{c}	14.76 ± 1.83^{b}	14.11 ± 3.15^{b}	25.96 ± 1.78^{a}	28.98 ± 4.90^{a}	26.15 ± 5.10^{a}
Total n-3 PUFA	4.04 ± 2.32^{d}	149.13 ± 25.26^{c}	121.18 ± 15.76^{c}	221.24 ± 25.65^{ab}	246.52 ± 17.13^{a}	201.05 ± 33.96^{b}

Values are means ± SD ($n = 3$). Values not sharing a common superscript letter are significantly different from one another ($p < 0.05$) by one-way ANOVA and Duncan's multiple range test

Data presented as μg/well, % and nmol% are provided in the online supplemental data

n.d. non-detectable



p values	ALA	SDA	ETA	EPA	DPA	24:5 n-3	24:6 n-3	DHA
Time	<.0001#	0.0014#	<.0001#	<.0001#	<.0001#	<.0001#	<.0001#	<.0001#
FX	0.9697	0.3789	0.6918	0.0006#	0.0013#	0.0150#	0.3327	<.0001#
Time*FX	0.1576	0.2255	0.5289	<.0001#	<.0001#	0.0040#	0.7880	<.0001#

Fig. 2 The effect of fucoxanthin on U-[^{13}C]-ALA metabolism in HepG2 cells. HepG2 were co-treated with fucoxanthin (FX, 100 μM) and U-^{13}C-ALA (100 μM) for 0.5, 3, 6, 24 and 48 h. Fatty acid metabolites of the U-^{13}C-ALA were analyzed by GC–MS. The values represent means and SD ($n = 3$). Significance of the effect of time and FX was analyzed by two-way ANOVA and p values shown in the table. *Hash* denotes significant effect by either factor at $p < 0.05$. While the interaction (time × FX) is significant, the difference between ALA and ALA + FX was further analyzed by Student's t test. *Asterisk* denotes $p < 0.05$. *Dollar* denotes $0.05 < p < 0.1$

remained from 6 to 48 h. Profiles of changes in U-[^{13}C]-SDA, the first metabolite, through the 48 h were similar to that of U-[^{13}C]-ALA. Levels of other metabolites, however, kept increasing until 24 h. At 48 h, the amounts of EPA, DPA and DHA labeled with 18 ^{13}C atoms were significantly higher in cells co-treated with FX compared to cells treated with U-[^{13}C]-ALA only (Fig. 2d, e, h). FX did not affect the amount of U-[^{13}C]-ALA, SDA, and 24:6n-3 at all time points (Fig. 2a, b, g). Noticeably, the amount of [^{13}C] EPA was highest among that of all metabolites. This agrees with the results of Exp 1 (Table 1).

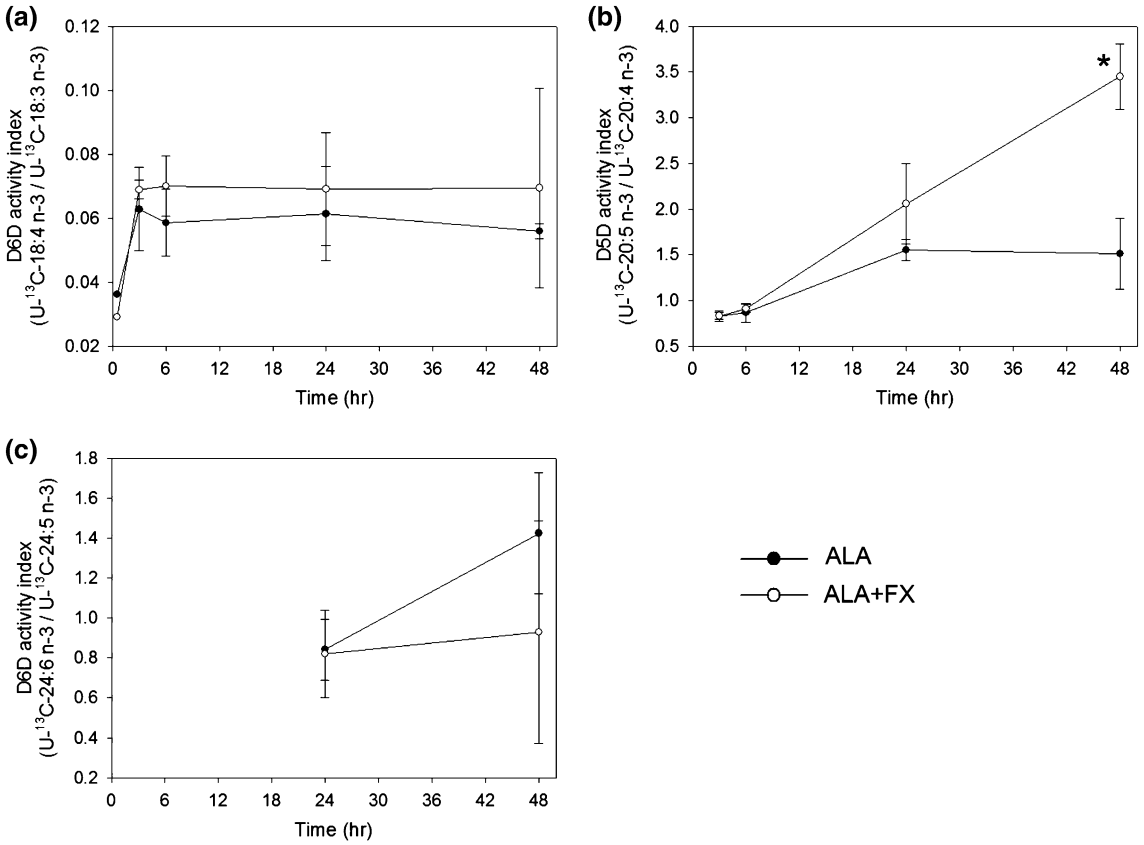
Desaturase Activity Index Estimated from Exp 2

Desaturase activity indexes- calculated as FA product-to-precursor ratios- have been used as an estimate of the enzymatic conversion. D5D activity was commonly calculated as the ratio of ARA to dihomo-γ-linolenic acid (DGLA, 20:3n-6),

whereas the D6D activity was usually estimated as the ratio of γ-linolenic acid (GLA, 18:3n-6) to linoleic acid (LNA, 18:2n-6). As U-[^{13}C]-ALA was used as the tracer, D5D activity index was calculated as the ratio of [^{13}C]-20:5n-3/[^{13}C]-20:4n-3 obtained in Exp 2. The effects of time, FX treatment as well as their interaction were all significant on this D5D activity index ($p < 0.0001$). As shown in Fig. 3b, the D5D activity index was significantly higher in the FX co-treated cells at 48 h compared to those treated with U-[^{13}C]-ALA only. D6D activity indexes were calculated as the ratio of U-[^{13}C]-18:4n-3/U-[^{13}C]-18:3n-3 and [^{13}C]-24:6n-3/[^{13}C]-24:5n-3, respectively. None were significantly affected by time and FX as shown in the results of two-way ANOVA (Fig. 3a, c). These results indicated that co-treatment with FX significantly increased the estimated D5D activity index but did not change the D6D activity index after 48 h of treatment.

Discussion

Tsukui *et al.* [15] first reported that 0.2 % FX in the diet significantly increased hepatic DHA in KK-A^{y} obesity/type II diabetes prone mice. They subsequently confirmed that 0.05 % FX in the diet also significantly increased



p values	C18:4/C18:3 (D6D)	C20:5/C20:4 (D5D)	C24:6/C24:5 (D6D)
Time	0.1939	<.0001$^{\#}$	0.0016$^{\#}$
FX	0.5274	<.0001$^{\#}$	0.1883
Time*FX	0.9682	<.0001$^{\#}$	0.6047

Fig. 3 The effect of fucoxanthin on D6D and D5D activity index in HepG2 cells co-treated with U-[^{13}C]-ALA for 0.5–48 h. Data of Fig. 2 were used to calculate the estimated Delta-6 desaturase (D6D) activity index as U-[^{13}C]-18:4n-3/U-[^{13}C]-18:3n-3 (**a**) and [^{13}C]-24:6n-3/[^{13}C]-24:5n-3 (**c**). The estimated Delta-5 desaturase (D5D) activity index was calculated as [^{13}C]-20:5n-3/-[^{13}C]-20:4n-3. The values represent means and SD ($n = 3$). Significance of the effect of Time and FX was analyzed by two-way ANOVA and p values shown in the table. *Hash* denotes significant effect by either factor at $p < 0.05$. While the interaction (Time × FX) is significant, the difference between ALA and ALA + FX was further analyzed by Student's t test. *Asterisk* denotes a significant difference between two treatments, $p < 0.05$

hepatic DHA and ARA in C57/BL mice [13]. Our data of the present study supported these finding that fucoxanthin increased EPA, DPA and DHA in HepG2 cells treated with ALA. We further demonstrated enhanced conversion of ALA to EPA, DPA and DHA in cells co-treated with FX by isotope tracking. A previous study by Aki *et al*. aimed to delineate the mechanism using cultured rat hepatoma BRL-3A cells, however, they found decreases in EPA and no changes in DHA [16]. Compared to our study, Aki *et al*. used lower concentrations of not only ALA (50 μM) but also FX (10 μM). Based on our results of Exp 1, higher concentrations of FX are required to observe significant differences in levels of EPA, DPA and DHA (Table 1). Moreover, they incubated cells for 8 and 24 h [16]. Our results of Exp 2 indicate that enhancing effects of FX on the conversion of ALA to EPA, DPA and DHA were observed at 48 h of incubation and not at 24 h. More studies using other cell lines or liver microsomes would be of interest.

Increases in n-3 fatty acids in response to ALA treatment of HepG2 cells in our study are similar to those reported by Portolesi *et al*. [17], although we measured the total fatty acids of cells while they measured the phospholipid fraction. In both studies, increases in ALA in response to ALA treatment reached a plateau at 6 h after treatment. The increase was highest in EPA, followed by DPA and to a lower extent in DHA. Moreover, co-treatment with FX and ALA further enriched EPA, DPA and DHA (Table 1). The conversion of ALA to EPA involves D6D, elongase and D5D reactions sequentially. The slight but significant increase in ARA by FX and ALA co-treatment (Table 1) suggested that FX might enhance the desaturation and elongation pathway.

In the second experiment, we used stable isotope-labeled ALA as a tracer to specifically monitor the influence of FX on ALA metabolism in HepG2 cells. Indeed, the isotope tracer labeled [^{13}C]-EPA, DPA and DHA content were all significantly higher by 48 h of co-treatment of FX and labeled ALA, whilst [^{13}C]-ETA level was slightly lower at 24 h (Fig. 2). The U-[^{13}C]-ALA level remained steady through 6–48 h while labeled DHA, EPA, and DPA significantly increased, further suggesting the higher incorporation of ALA in the cells treated by FX. Furthermore, the estimated D5D activity index but not D6D activity index was significantly elevated by FX (Fig. 3). D5D catalyzes the conversion of ETA to EPA and DGLA to ARA. These results suggest D5D might play a major role in the FX enhanced conversion of n-3 PUFA in the HepG2 cells. The elevated D5D index might also account for the increased ARA in FX co-treated cells in the experiment 1.

Evidence of n-3 and n-6 fatty acid antagonism has come from tissue compositional studies as well as from radioisotope studies. In particular, desaturation of ALA to SDA was inhibited by LNA and, conversely, LNA conversion to GLA was inhibited by ALA [22]. In addition, the affinity of D6D for ALA is two to three times that of LNA [23]. As the FBS-containing growth medium used for culturing cells provided LNA (5.82 %) and ARA (8.99 %) and less ALA (0.5 %), control cells (vehicle only) contained much more n-6 fatty acids than n-3 fatty acids. Decreases in ARA in cells treated only with ALA (100 μM) might be a result of the antagonism. However, FX co-treatment still increased ARA, suggesting the enhancement of the conversion pathway.

The rapid accumulation of U-[^{13}C]-ALA in cells for the first 3 h indicated that the uptake was faster than the removal by the D6D reaction (Fig. 2a). A rapid increase in D6D activity index from 0 to 3 h coincided with less accumulation between 3 and 6 h. That the level did not increase from 6 to 48 h suggests a balance between uptake and removal. Similarly, U-[^{13}C]-SDA level remained relatively constant from 6 to 48 h indicating a balance between the production by D6D and the removal by elongase. In contrast, the [^{13}C]-ETA level still increase between 6 to 24 h, indicating a faster production by elongase than the removal by the D5D reaction. This also coincided with the increase in the D5D activity index between 6 to 24 h. For cells treated with only U-[^{13}C]-ALA, further [^{13}C]-labeled metabolites increased before but not after 24 h, suggest the sequential elevation of D6D and D5D might be accounted for. In contrast, the [^{13}C]-EPA, DPA and DHA further increased between 24–48 h and were associated with an elevated D5D activity index at 48 h in FX co-treated cells. The enhancing effect of FX on ALA conversion thus need relatively longer time, implying transcriptional regulation might be involved.

The expressions of D5D and D6D are known to be regulated by sterol regulatory element binding protein-1c (SRBP-1c) and peroxisome proliferator-activated receptor α (PPARα) at the transcriptional level [24, 25]. PPARα agonists, such as fenofibrate and WY-14643, up-regulate expressions of these desaturases which is dependent on PPARα [24–26]. Liver X receptor α (LXRα) agonist, T090137, also up-regulates expressions of these desaturases through the induction of SREBP-1c [26]. Moreover, the last step of ALA to DHA conversion, C24:6n-3 to DHA, involved a peroxisomal *β*-oxidation which has been known to be regulated by PPARα. Woo *et al*. (2010) reported that FX supplementation significantly increased mRNA of PPARα and its target gene acyl-CoA oxidase-1 (ACOX-1) in high fat diet-fed C57BL/6 N mice [27]. Beppu (2012) reported increased SREBP-1 and SREBP-2 protein in the liver of KK-A^{y} mice fed the FX-containing diet [28]. However, Ha *et al*. (2013) found a significant decrease in SREBP-1c mRNA expression in the liver of rats fed a high fat diet supplemented with 0.2 % fucoxanthin [29]. Whether FX modulates the ALA conversion

enzyme through these transcription factors needs further investigation.

In conclusion, we confirm that FX promotes the ALA conversion to EPA, DPA and DHA in HepG2 cells in this study. Most of EPA and DHA for human consumption are from small fatty marine fish which is at risk of being overfished and recent research has been toward more sustainable sources [30]. FX plus ALA might be an alternative way to increase blood and tissue DHA levels and further clinical investigation is needed.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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