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Journal of Nutritional Biochemistry 22 (2011) 1047–1054

Journal of Nutritional Biochemistry

Dietary conjugated linoleic acid alters long chain polyunsaturated fatty acid metabolism in brain and liver of neonatal pigs

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Received 13 March 2010; received in revised form 20 July 2010; accepted 9 September 2010

Abstract

Effects of dietary conjugated linoleic acid (CLA, 1% mixed isomers) on n-6 long-chain polyunsaturated fatty acid (LCPUFA) oxidation and biosynthesis were investigated in liver and brain tissues of neonatal piglets. Fatty acid β-oxidation was measured in tissue homogenates using [1-^{14}C]linoleic acid (LA) and -arachidonic acid (ARA) substrates, while fatty acid desaturation and elongation were traced using [U-^{13}C]LA and GC-MS. Dietary CLA had no effect on fatty acid β-oxidation, but significantly decreased n-6 LCPUFA biosynthesis by inhibition of LA elongation and desaturation. Differences were noted between our ^{13}C tracer assessment of desaturation/elongation and simple precursor-product indices computed from fatty acid composition data, indicating that caution should be exercised when employing the later. The inhibitory effects of CLA on elongation/desaturation were more pronounced in pigs fed a low fat diet (3% fat) than a high fat diet (25% fat). Direct elongation of linoleic acid to C20:2n-6 via the alternate elongation pathway might play an important role in n-6 LCPUFA synthesis because more than 40% of the synthetic products of [U-^{13}C]LA accumulated in [^{13}C]20:2n-6. Overall, the data show that dietary CLA shifted the distribution of the synthetic products of [U-^{13}C]LA between elongation and desaturation in liver and decreased the total synthetic products of [U-^{13}C]LA in brain by inhibiting LA elongation to C20:2n-6. The impact of CLA on brain LCPUFA metabolism of the developing neonate merits consideration and further investigation.
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Keywords: Desaturase; Elongase; β-oxidation; Piglet

1. Introduction

Conjugated linoleic acid (CLA) refers to the specific group of isomers of linoleic acid (LA; *cis*-9, *cis*-12 C18:2n-6) that has conjugated diene bonds. The main dietary form of CLA, the *cis*-9, *trans*-11 isomer, naturally occurs in ruminant animals as a product of biohydrogenation by rumen bacteria and is found in beef and dairy products [1,2], while the *trans*-10 *cis*-12 isomer also is present in foods and supplements derived via chemical hydrogenation. Since the discovery of CLA's anticarcinogenic activity in 1987 [3], numerous studies have examined the biological effects of CLA. Recent research with various CLA isomers has identified several potential attributes including anti-atherosclerotic effects [4], anti-diabetic effects [5], enhancement of bone formation [6], reduction of body fat mass [7], and modification of lipid metabolism [8,9]. Particularly for the latter, research focusing on CLA has increased dramatically due to the problem of worldwide obesity, metabolic syndrome and dyslipidemia. However, CLA, especially the *trans*-10, *cis*-12 isomer, has been shown to increase hepatic triglyceride, cholesterol, cholesterol ester, and free fatty acid accumulation [10] and inhibit Δ6-desaturases and cyclooxygenase [11]. These effects could induce nonalcoholic fatty liver disease and developmental metabolic disorders [1,12,13].

So far, little CLA research has addressed neonatal nutrition and development. This important area should not be overlooked, especially because studies have shown that childhood obesity has increased 3-fold in the last 20 years [14]. The potential positive biological effects of CLA must be weighed against potential disruption of LCPUFA metabolism because CLA also may interfere with LA fatty acid desaturation and elongation pathways involved in the synthesis of arachidonic acid (ARA; C20:4n-6). Linoleic acid is a dietary essential fatty acid that is crucial to neonatal development. It is necessary for the growth and development of the brain, retina [15,16], and other body tissues dependent on ARA, the central n-6 eicosanoid precursor synthesized from LA via elongation-desaturation. Because the profiles of CLA isomers were found to be similar to LA [17,18], CLA may compete for the enzymes involved in the canonical and alternate elongation/desaturation (Fig. 1) pathways [19] and may alter LCPUFA synthesis. Subsequently, eicosanoid and docosanoid production may be reduced due to decreased LCPUFA substrates. In addition, studies have documented that CLA crosses the human placenta to the

Abbreviations: ACO, acyl-CoA oxidase; ARA, arachidonic acid; CLA, conjugated linoleic acid; CPT I, carnitine palmitoyltransferase I; DHA, docosahexaenoic acid; D5D, Δ^5-desaturase; D6D, Δ^6-desaturase; D8D, Δ^8-desaturase; Elovl-A, fatty acid elongase-very-long-alternate; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acid.

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doi:10.1016/j.jnutbio.2010.09.002

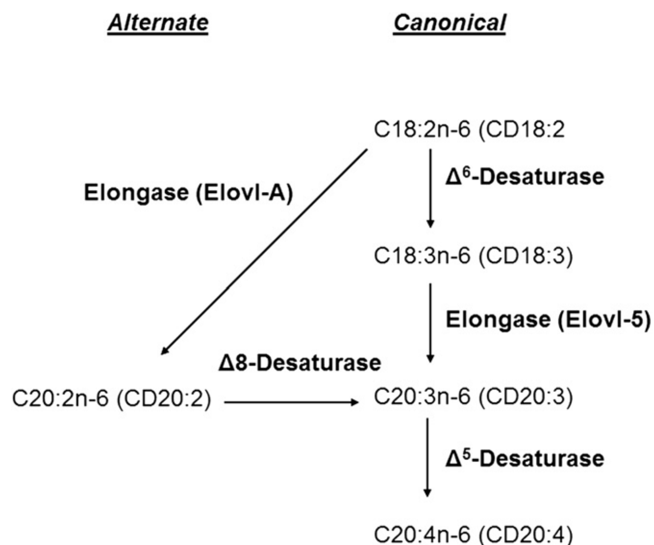

Fig. 1. Elongation/desaturation of linoleic acid and the conjugated dienes (CD) of linoleic acid, illustrating the alternate pathway [38] catalyzed by sequential elongation (via Elovl-A) and desaturation (via D8D) in comparison to the established pathway catalyzed by D6D, Elovl-5 and D5D.

developing fetus [20,21], is present in small amounts in human milk [22], and is incorporated into infant plasma lipids [23]. Therefore, evaluating the effects of CLA on LCPUFA metabolism in neonates is relevant to neonatal development and health.

The purpose of this study was to examine whether CLA affects LCPUFA oxidation as well as LA desaturation-elongation in the biosynthesis of LCPUFA. To this end, LCPUFA β-oxidation and biosynthesis were examined in liver and brain tissues of neonatal piglets fed diets with or without supplementation of CLA. The effects of dietary CLA on LCPUFA concentrations in the brain and liver tissues were evaluated also.

2. Materials and methods

2.1. Chemicals

The [U-^{13}C]LA was purchased from Spectra Gases (Columbia, MD). The [1-^{14}C]LA and ARA were purchased from American Radiolabeled Chemicals, INC (St. Louis, MO). All of other chemicals were purchased from Sigma Chemicals (St. Louis, MO).

2.2. Animals and treatments

All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Animals and dietary treatments and fatty acid composition (Table 1) were the same as described previously [24]. Briefly, a total of 24 neonatal piglets (1 day of age) from 7 sows were randomly assigned to 4 treatments (6 piglets for each treatment) according to a 2×2 factorial design with two levels of dietary fat (3 versus 25%) supplemented with either 1% LA or 1% CLA. The CLA contained 47% of *cis*-9, *trans*-11 and 53% of *trans*-10, *cis*-12 isomers supplied as fatty acid methyl esters (BASF, Mount Olive, NJ). Sunflower oil methyl esters were synthesized and added to diets (1%) without supplementation of CLA to match the methyl ester concentrations in the diets supplemented with CLA. After 12 to 16 h of training, all of the piglets routinely consumed one of the four liquid diets for 16 days, specifically: 1) low fat, containing 3% fat without CLA (LF-CLA); 2) low fat supplemented with 1% CLA (LF+CLA); 3) high fat, containing 25% fat without CLA (HF-CLA); or 4) high fat supplemented with 1% CLA (HF+CLA). The fat used in the diets was a blend of 44% palm olein oil, 19.5% soy oil, 19.5% coconut oil, 14.5% high oleic sunflower oil and 2.5% single–cell-oils (Mead Johnson Nutritionals, Evansville, IN). All diets met or exceeded piglet nutrient requirements as established by the National Research Council [25]. Piglet housing and feeding systems were described previously [26]. At the end of the experiment, piglets were euthanized via electrocution followed by exsanguination. The liver and brain tissue samples were collected immediately for metabolism measurements.

2.3. Tissue homogenate preparation

One gram of tissue (liver and brain) from each pig was homogenized in a buffer (220 mM mannitol, 70 mM sucrose, 2 mM HEPES, and 0.1 mM EDTA; pH 7.2 at 0°C) using a 7 mL glass Pyrex hand homogenizer with 3 complete top to bottom strokes. The tissue to buffer ratio was 1:7 for fatty acid oxidation and 1:4 for measurements of fatty acid elongation measurements. Protein concentrations were measured using the biuret method [27].

2.4. In vitro fatty acid β-oxidation

Tissue homogenates were incubated with 1 mmol/L concentrations each of LA and ARA, and each of the fatty acid substrates contained 0.5 μCi of [1-^{14}C]LA or [1-^{14}C]ARA, respectively (ARC, St. Louis, MO). Radiolabeled fatty acids were complexed with bovine serum albumin (5:1 molar ratio) and added to the reaction buffer. The reaction buffer contained: 50 mM sucrose, 150 mM Tris-HCl, 20 mM K_2PO_4, 10 mM $MgCl_2$-$6H_2O$, 2 mM EDTA, 1 mM L-carnitine, 10 mM ATP, 2 mM NAD, 0.2 mM CoA, and 0.1 mM malate. Incubations for each fatty acid and each tissue were conducted in duplicate within 25 mL Erlenmeyer flasks in the final volume of 3 mL. After the addition of 0.3 mL of tissue homogenate (~45 mg tissue), flasks were sealed with serum stoppers and incubated in a 37°C shaking water bath for 30 min. Reactions were terminated by injection of 0.5 mL of 3 mM $HClO_4$. Radioactivity accumulation in CO_2 and acid soluble products was measured [28] using a liquid scintillation counter (LS-6500 IC, Beckman Instruments, Fullerton, CA).

2.5. In vitro fatty acid elongation

The incubation procedure followed the method described by Li et al. [29] with slight modification. In brief, 0.3 mL homogenate (~55 mg wet tissue) were added to an incubation buffer containing a final concentration of 150 mM KH_2PO_4, 10 mM $MgCl_2$, 1.5 mM dithioerythritol, 2 mM β-NADH, 2 mM NADPH, 10 mM ATP, 0.46 mM CoA, 0.4 mM malonyl-CoA and 1.5 mM [U-^{13}C]LA; pH 7.4 at 37°C, in a final volume of 1 mL. All incubations were conducted in duplicate with [U-^{13}C]LA (Spectra Gases, Columbia MD) bound to BSA (5:1) in the presence of 10 μM rotenone and 50 μM of antimycin to inhibit oxidation. The incubation was initiated by adding [U-^{13}C]LA after 5 min of pre-incubation and terminated after 60 min by the addition of 0.1 mL 3 M HCl. A set of blank incubations for each tissue homogenate also was conducted and terminated after 5 min of pre-incubation by addition of 0.1 mL 3 M HCl. After termination, tubes were filled with nitrogen gas, capped tightly, and stored at −20°C for later fatty acid analysis.

2.6. Lipid extraction and fatty acid derivatization

Total lipids were extracted using the method described by Radin et al. [30] with slight modification [29]. An internal standard of 185 nmol of heptadecanoic acid (C17:0) dissolved in 0.1 mL of alcohol was added to each sample. Two mL of hexane and isopropanol (4:1) were added, followed by the addition of 1.0 mL saturated NaCl solution. Tubes were vortexed and then centrifuged for 10 min at 1000×g. The supernatant was transferred to a clean 20 mL test tube. The bottom layer was extracted three more times with 2.0 mL hexane. Supernatants were combined and evaporated to dryness under N_2. Total fatty acids in the extracted lipids were methylated following

Table 1
Analyzed fatty acid composition of the low (3%) and high (25%) fat formulas with or without supplemental conjugated linoleic acid (CLA; 1% mixed isomer) [a]

Fatty acid	Low fat		High fat	
	-CLA	+CLA	-CLA	+CLA
	−−*g/100 g diet*−−			
14:0	0.24	0.26	2.14	2.35
16:0	0.88	0.93	7.67	7.21
16:1 (n-7)	0.02	0.02	0.08	0.05
18:0	0.17	0.19	1.22	1.04
18:1, *cis*	1.17	1.23	11.21	10.25
18:2 (n-6)	1.81	1.10	8.36	7.22
CLA, *cis*-9, *trans*-11	0.01	0.49	0.02	0.56
CLA, *trans*-10, *cis*-12	0.01	0.55	0.02	0.62
18:3 (n-3)	0.12	0.15	0.92	0.92
20:0	0.01	0.01	0.16	0.11
20:1 (n-9)	<0.01	<0.01	0.05	0.04
20:2 (n-6)	ND	0.02	0.02	0.04
20:3 (n-6)	ND	ND	0.04	0.02
20:4 (n-6)	ND	0.02	0.37	0.26
20:5 (n-3)	<0.01	<0.01	0.04	0.03
22:0	<0.01	0.01	0.08	0.06
22:6 (n-3)	ND	ND	0.22	ND

ND indicates that these fatty acids were not detected.
[a] Derived with permission from reference [24].

alkali treatment and determined as fatty acid methyl esters [31]. For the [^{13}C]labeled fatty acid analysis, the samples were saponified [29] and pentafluorobenzyl (PFB) esters were then prepared [32]. The PFB esters were dissolved in 200 μL hexane for GC-MS analysis.

2.7. Gas Chromatography/Mass Spectrometry analysis

Fatty acid derivatives were separated on a HP-23 capillary column (cis/trans FAME CR), 30 m×0.25 mm, film thickness 0.3 μm (Agilent Technologies, Wilmington, DE). Mass spectrometric analysis was conducted using a 6890 N model gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with an Agilent Technologies 5973N mass spectrometric detector. For electron ionization (EI) analysis, the temperature was programmed from 50 to 100°C at 10°C/min, then to 200°C at 4°C/min, held for 2 min, and finally to 220°C at 4°C/min, held for 12 min. The average helium velocity was 36 cm/sec and the split ratio was 100:1. One μL of the methyl ester was manually injected and the total fatty acid amounts were determined by the areas of the total ions for each fatty acid. For chemical ionization (CI) analysis, the temperature was programmed from 150 to 280°C at 4°C/min and held for 6 min. The average helium velocity was 38 cm/sec and the split ratio was 50:1. One μL of the PFB ester was manually injected. The [^{12}C]fatty acid and [^{13}C]fatty acid ratio was determined by the areas of [M] and [M+18] isotopomers for each fatty acid. The amount of [^{13}C]fatty acid was calculated by multiplying this ratio by the total fatty acid amount as determined by the EI analysis.

2.8. Statistical analysis

All data were analyzed using the general linear model procedure of the Statistical Analysis System according to a 2×2 factorial design (SAS Institute). The Tukey test option was used for assessing treatment differences when the interaction term was significant. Differences between tissues (liver verses brain), and fatty acids (LA verses ARA) were analyzed using a Paired Student's *t* test and the TTEST procedure of SAS. Data are presented as least square means±SEM and results were considered significantly different when $P<.05$.

3. Results

3.1. Fatty acid β-oxidation

The total β-oxidation rate of LA in brain tissue was 27% greater than that of ARA ($P<.01$), and the oxidation rate in liver exceeded that of brain ($P<.01$; Fig. 2). Neither CLA nor fat level altered fatty acid β-oxidation rates by liver (fat level $P\geq.54$, CLA $P\geq.45$) or brain (fat level $P\geq.70$, CLA $P\geq.71$); nor did dietary treatments affect total β-oxidation rates of either fatty acid.

3.2. Fatty acid elongation and desaturation

The elongation/desaturation of LA was assessed by measuring accumulation rates of ^{13}C-fatty acids in liver and brain homogenates incubated with [U-^{13}C]LA (Fig. 3). In liver tissue (left panels) the accumulation rates of [^{13}C]20:3n-6 and [^{13}C]ARA were reduced by 61 and 37 %, respectively, in pigs fed CLA regardless of dietary fat level. Dietary CLA also tended to reduce the accumulation of [^{13}C] 20:2n-6 ($P=.06$) and increase the accumulation of [^{13}C]18:3n-6 ($P=.07$). The increased rate of accumulation of [^{13}C]18:3n-6 in CLA-fed pigs was more apparent in those fed the low fat diet than in those fed the high diet fat. Dietary fat level also had a large influence on the accumulation of [^{13}C]18:3n-6 and [^{13}C]20:2n-6. The accumulation of [^{13}C]18:3n-6 was 32.5% lower in pigs fed the low fat diet, while [^{13}C]20:2n-6 accumulation was decreased 64% in pigs fed the high fat diet. In brain tissue (right panels), CLA decreased accumulation rates of [^{13}C]20:2n-6 in both low and high fat diets as well as the accumulation of [^{13}C]20:3n-6 in the high fat CLA diet. However, there were no effects of CLA on the accumulation of [^{13}C] 18:3n-6 and [^{13}C]ARA in either low or high fat diets, or in the accumulation of [^{13}C]20:3n-6 in pigs fed the low fat diet. The accumulation of [^{13}C]ARA was increased by 29% in pigs fed the low fat diet, but there were no dietary fat effects on the accumulation of other [^{13}C]LCPUFA.

Although dietary CLA decreased [^{13}C]20:3n-6 and [^{13}C]20:4n-6 accumulation rates, there was no effect on total [^{13}C]LCPUFA accumulation in liver tissue (Fig. 4, top panel). In contrast to liver, the decrease in [^{13}C]20:2n-6 and [^{13}C]20:3n-6 accumulation rates caused by dietary CLA resulted in a reduction (47%) in the total accumulation of [^{13}C]LCPUFA in brain (Fig. 4, bottom panel).

3.3. Fatty acid concentrations in tissues

Both fat content and composition of the diet altered concentrations of various PUFA in liver and brain tissues (Table 2). Consistent with [^{13}C]fatty acid accumulation rates in liver tissue, dietary CLA decreased C20:3n-6 and ARA concentrations by 36 and 26% respectively, but had no effect on concentrations of C18:3n-6, C20:2n-6 or DHA. While elevated dietary fat increased hepatic fatty acid concentrations of LA (35%), C18:3n-6 (95%), ARA (64%) and DHA (418%), the C20:2n-6 concentration was decreased by 50%. In brain tissue, dietary CLA tended to decrease ARA concentration ($P<.057$), but had no effects on LA, C18:3n-6, C20:2n-6, C20:3n-6 or DHA concentrations. High dietary fat increased DHA (49%) and moderately increased ARA (20%; $P=.05$) concentrations, but decreased C20:2n-6 concentration by 44%. There were no effects of dietary fat level on any other PUFA. The concentrations of CLA isomers were not evaluated statistically due to limited tissue (~55 mg) used in the incubation reactions, but the concentrations of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (respectively) detected in CLA-fed pigs averaged (μmol/g wet tissue) 0.044 and 0.020 in the liver of pigs fed high-fat diets and 0.17 and 0.026 in those fed low-fat diets; whereas in brain, isomer concentrations averaged 0.073 and 0.034 in pigs fed high-fat and 0.094 and 0.031 in pigs fed low fat.

3.4. Indices of desaturase and elongase activity

The indices of D6D, D5D and elongase activities were calculated as the ratio of product and precursor using either [^{13}C]LCPUFA accumulated in the medium during one-hour incubation periods with [^{13}C]LA (Table 3) or LCPUFA measured in the tissues (Table 4) for each enzyme. Specifically, the ratio of C18:3n-6/LA was

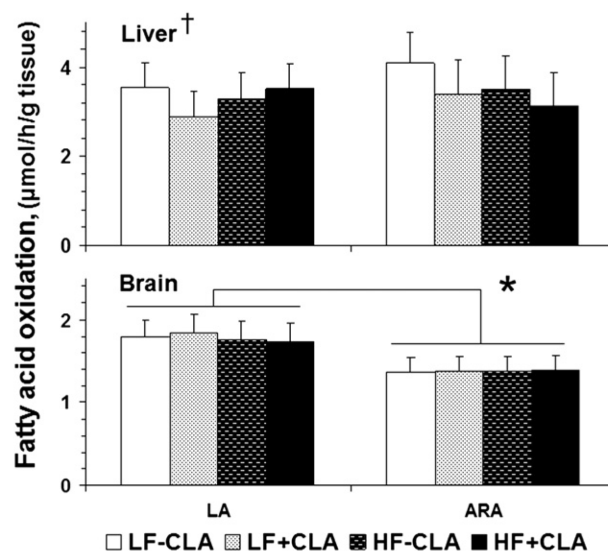

Fig. 2. Fatty acid oxidation in tissues of piglets either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer). Liver (top panel) and brain (bottom panel) homogenates were incubated with [1-^{14}C]linoleic acid (LA) or arachidonic acid (ARA) and isotope accumulation in CO_2 and acid soluble products were measured as described in the materials and methods. Values are least squares means plus SEM, n=6. †, Liver > brain ($P<.01$). *, LA > ARA ($P<.01$).

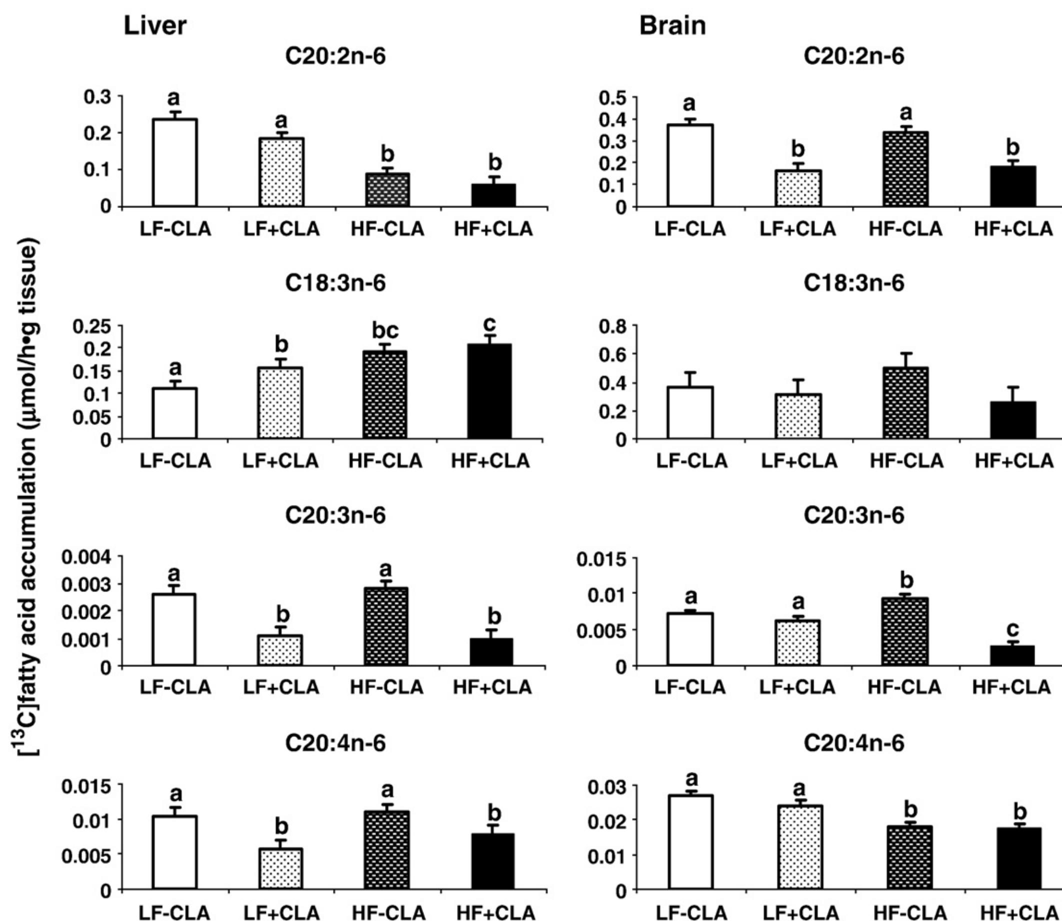

Fig. 3. Accumulation of [^{13}C]fatty acids in tissues of piglets fed either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer). Liver (left panels) and brain (right panels) homogenates were incubated with [U-^{13}C]linoleic acid and isotope accumulation in various fatty acids was measured by GC-MS as described in the materials and methods. Values are least square means plus SEM, n=6. [abc] Bars lacking a common superscript differ (P<.05).

calculated as an index for D6D, the ratio of ARA/C20:3n-6 for D5D, the ratio of C20:3n-6/C20:2n-6 for Δ8-desaturase, the ratio of C20:3n-6/C18:3n-6 for elongase (Elovl-5) and the ratio of C20:2n-6/LA for elongase (Elovl-A). In connection with the indices calculated from [^{13}C]LCPUFA, dietary CLA increased D5D activity index by 100%, decreased Elovl-5 index by 75% and reduced Δ8-desaturase activity index by 46% when measured in liver tissue from piglets fed the low fat diet. No effects of dietary CLA on D6D and Elovl-A activity indices were detected (P>.05). However, high dietary fat significantly increased hepatic D6D (50%) and Δ8-desaturase activity index (162%), but decreased the Elovl-5 index by 37% and the Elovl-A index by 65%. There was no effect of dietary fat on the hepatic D5D index. In contrast with liver, when assessed in brain tissue, dietary CLA increased the D5D index by 3.5 fold in pigs fed the high fat diet and reduced the Elovl-5 (39%) and Elovl-A (51%) indices. Dietary CLA also increased the Δ8-desaturase index in pigs fed the low fat diet, but the index decreased in brain of pigs fed the high fat diet. There were no effects of dietary CLA on brain D6D index and no effects of dietary fat on D6D, Δ8-desaturase and Elovl-A indices. By comparison, the indices calculated from LCPUFA concentrations in tissues were comparable with those obtained from [^{13}C]LCPUFA for Δ8-desaturase, Elovl-5 and Elovl-A in liver tissue, and for D6D and Δ8-desaturase in brain tissue, but different from those obtained from [^{13}C]LCPUFA for D6D and D5D in liver tissue, and for Elovl-5 and Elovl-A in brain tissue.

4. Discussion

In the present study, we measured fatty acid oxidation in vitro using liver and brain tissue homogenates from neonatal piglets fed CLA, examining both [1-^{14}C]LA and [1-^{14}C]ARA substrates. Dietary CLA had no effects on LA and ARA β-oxidation in these tissues. The lack of response to dietary CLA appeared not to be due to the enzyme affinity for chain length and/or saturation status of the fatty acids because a similar result was observed when [1-^{14}C]palmitic acid was used as substrate [24]. Some studies reported that CLA increases CPT I specific activity in adult rats and mice in both liver homogenates and isolated mitochondria [33,34] and palmitoyl-CoA oxidation in mice [35]. However, no effects of CLA on hepatic CPT I and ACO activities in hamsters were reported by others [36,37] showing that the effects of CLA on fatty acid oxidation might vary among species [38]. Thus, the lack of response may be attributable to the porcine species, the tissues studied, or the age and/or physiological status of the animals which merit further investigation.

Linoleic acid is converted to C18:3, C20:3, and ARA fatty acids respectively by the desaturation/elongation pathway [Fig. 1; 17,39]. Conversion occurs by sequential Δ^6-desaturation, elongation (Elovl-5) and Δ^5-desaturation reactions. All of these reactions are requisite for the biosynthesis of ARA from LA [40,41]. Besides the D6D/Elovl-5 pathway, an alternate elongation/desaturation pathway (Fig. 1) was reported also for LA conversion to C20:2n-6, C20:3n-6 and ARA. This

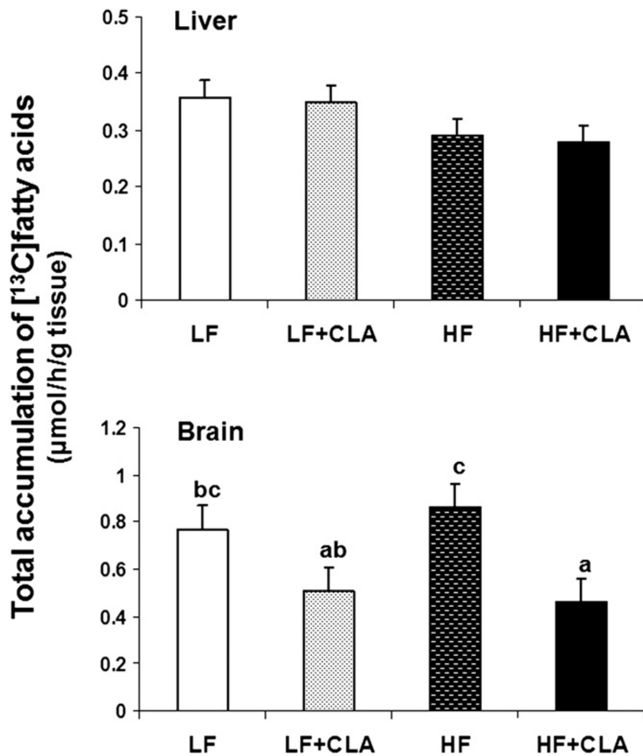

Fig. 4. Total accumulation of [^{13}C]fatty acids in tissues of piglets fed either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer). Liver (top panel) and brain (bottom panel) homogenates were incubated with [U-^{13}C]linoleic acid and total isotope accumulation in fatty acid products was measured by GC/MS as described in the materials and methods. Values are least square means plus SEM, n=6. [abc] Bars lacking a common superscript differ (P<.05).

pathway first involves elongation catalyzed by a specific C18-Δ^9-fatty acid elongase (referred to as Elovl-A in Fig. 1) which has been cloned and identified functionally in marine microalga [17,42]. The elongation is followed by Δ^8- and Δ^5-desaturation reactions. When Δ^6-desaturation is inhibited, elongation (via Elovl-A) could take place as suggested by Chuang et al. [19,43]. Some studies indicated that CLA interferes with desaturation of LA and eicosanoid production in mouse [44] and rat liver [45]. It also was found that *trans*-10, *cis*-12 CLA suppresses Δ^6- and Δ^5-desaturation in hepG2 cells [46] and in yeast transformed with human Elovl-5 and fungal Δ^5-desaturase genes [47,48], as well as in the liver of growing mice [49]. However, the effects of CLA on desaturation were based on the concentrations of the elongated and desaturated metabolites of LA or the fatty acid desaturation and elongation indices calculated as ratio of product to precursor fatty acid in the tissues, which may be influenced by the endogenous metabolism and dietary concentrations of LCPUFA. The use of these product/precursor indices has yielded divergent results in the literature [17,44]. In the present study, we examined the desaturation and elongation pathway in tissue homogenates by measuring the accumulation rates of [^{13}C]18:3n-6, [^{13}C]20:2n-6, [^{13}C]20:3n-6 and [^{13}C]ARA derived directly from [U-^{13}C]LA. The results demonstrated that dietary CLA significantly affected LA metabolism via the desaturation/elongation pathways in both liver and brain tissues.

The accumulation rates of [^{13}C]20:3n-6 were significantly decreased in both liver and brain tissues of piglets fed CLA diets, suggesting that dietary CLA inhibits LA elongation via Elovl-5 (Figs. 1 and 3). This result might be associated directly with an inhibition of the elongase enzymes because CLA also significantly decreased [^{13}C]20:2n-6 accumulation in brain tissue, the product of the alternate Elovl-A elongation pathway (Figs. 1 and 3). The decrease was not significant in liver tissue (P=.06), but the accumulation of [^{13}C]20:2n-6 was reduced by 25% in the piglets fed CLA diets compared to the piglets fed control diets. These results provide direct evidence of the inhibitory effect of dietary CLA on elongation of LA in this animal model, which agrees with previous reports [47,50]. In addition to elongation, CLA reduced the accumulation of [^{13}C]ARA by 37% (P<.03) in liver, but only by 10% (P=.06) in brain. These results indicate that dietary CLA significantly inhibited [^{13}C]20:2n-6 or [^{13}C]20:3n-6 desaturation in liver but not in brain. The difference between the two tissues appeared to be associated with the dietary LCPUFA, because the decrease of [^{13}C]20:3n-6 by CLA in brain was much more pronounced in the high fat diet than in the low fat diet. Moreover, the [^{13}C]20:3n-6 and [^{13}C]ARA accumulations were significantly higher in brain of piglets fed the low fat diet. Brain tissue was sensitive to dietary n-3 LCPUFA deprivation, by which brain DHA concentration was decreased and ARA concentration was increased [51]. Because C18:3n-3 concentration in the low fat diet was only 0.13%, 7 times lower than that in high fat diet [0.92%; Table 1], the DHA concentration in brain was reduced by 30% in our study (Table 2). Thus, the increase in [^{13}C]20:3n-6 and [^{13}C]ARA accumulation in brain may be due to reduced inhibition of elongase and desaturase due to reductions in brain DHA [40,52].

Of particular interest in this study is the role of the alternate elongation pathway (Fig. 1), which had a great impact on LCPUFA synthesis because more than 40% of the [^{13}C]LCPUFA accumulation was in [^{13}C]20:2n-6 (Fig. 3). In liver, when the desaturation rate of [^{13}C]18:2n-6 to [^{13}C]18:3n-6 increased, its elongation rate to [^{13}C]20:2n-6 reciprocally decreased. A significant linear regression was obtained between the accumulations of the two fatty acids (Fig. 5). The distributions between the Elovl-A and Δ6-desaturation were impacted significantly by dietary fat level, possibly due to the LCPUFA concentrations in the diet and the impacts appeared to be tissue specific. [^{13}C]LA tended to be converted to [^{13}C]20:2n-6 by elongation in animals fed a low fat diet, while it tended to be converted to [^{13}C]18:3n-6 by desaturation in pigs fed the high fat diet. Dietary

Table 2
Polyunsaturated fatty acids in liver and brain of piglets fed either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer) [1]

	Diet				SEM	Significance, P value		
	LF-CLA	LF+CLA	HF-CLA	HF+CLA		FL [2]	CLA	FL×CLA
	μmol fatty acid/g wet tissue							
Liver								
C18:2n-6 (LA) [3]	50.40^a	46.75^a	68.42^b	62.67^b	4.17	0.001	0.27	0.80
C18:3n-6	0.28^a	0.36^a	0.60^b	0.65^b	0.05	0.001	0.22	0.89
C20:2n-6	1.31^a	1.53^a	0.72^b	0.68^b	0.14	0.001	0.54	0.37
C20:3n-6	1.09^b	0.48^a	0.99^b	0.86^{ab}	0.13	0.330	0.01	0.09
C20:4n-6 (ARA)	17.00^b	9.52^a	23.37^b	20.23^b	2.25	0.001	0.03	0.35
C22:6n-3 (DHA)	2.11^a	2.39^a	11.04^b	12.31^b	0.71	0.001	0.31	0.47
Brain								
C18:2n-6 (LA) [3]	50.40	54.22	52.42	49.72	4.59	0.790	0.90	0.48
C18:3n-6	0.52	0.64	0.59	0.59	0.06	0.940	0.37	0.33
C20:2n-6	2.09^a	2.39^a	1.49^b	1.20^b	0.21	0.001	0.97	0.20
C20:3n-6	1.59	1.66	1.76	1.39	0.14	0.730	0.31	0.15
C20:4n-6 (ARA)	32.86^{ab}	28.66^a	37.20^b	32.97^{ab}	2.09	0.052	0.06	0.99
C22:6n-3 (DHA)	28.94^a	26.78^a	43.64^b	38.11^b	2.30	0.001	0.11	0.47

Approximate CLA concentrations are reported in the text.
[abc]Least squares means within a row lacking a common superscript differ (P<.05).
[1] Tabulated values are least square means. Piglets were fed for 16 days.
[2] FL=Dietary fat level.
[3] The concentration of LA does not include the conjugated dienes of linoleic acid (CLA; *c*9, *t*11 and *t*10, *c*12 isomers).

Table 3
Desaturase and elongase activity indices calculated by tracing flux of [^{13}C]LA to various [^{13}C]LCPUFA in the liver and brain tissues of piglets fed either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer) [1]

	Diet				SEM	Significance, *P* value		
	LF-CLA	LF+CLA	HF-CLA	HF+CLA		FL [2]	CLA	FL×CLA
Liver								
C18:3/C18:2	0.073^{a}	0.104^{ab}	0.127^{bc}	0.139^{c}	0.012	0.001	0.075	0.40
C20:4/C20:3†	4.15^{a}	8.48^{b}	4.10^{a}	8.33^{b}	1.06	0.93	0.001	0.96
C20:3/C20:2	0.011^{ab}	0.007^{a}	0.031^{c}	0.017^{b}	0.003	0.001	0.001	0.05
C20:3/C18:3	0.024^{c}	0.008^{a}	0.015^{b}	0.005^{a}	0.002	0.006	0.001	0.15
C20:2/C18:2	0.156^{c}	0.122^{b}	0.057^{a}	0.040^{a}	0.013	0.001	0.061	0.51
C20:3/C18:2*	0.0017^{b}	0.0007^{a}	0.0018^{b}	0.0007^{a}	0.0002	0.87	0.001	0.67
C20:4/C18:3*	0.097^{b}	0.056^{a}	0.060^{a}	0.040^{a}	0.008	0.004	0.002	0.24
(C18:3+C20:3)/C18:2†	0.074^{a}	0.105^{ab}	0.129^{bc}	0.140^{c}	0.012	0.001	0.091	0.40
Brain								
C18:3/C18:2	0.244	0.209	0.333	0.174	0.001	0.70	0.19	0.39
C20:4/C20:3†	3.89^{b}	4.04^{b}	1.98^{a}	6.85^{c}	0.16	0.010	0.001	0.001
C20:3/C20:2	0.019^{a}	0.037^{c}	0.028^{b}	0.015^{a}	0.002	0.001	0.27	0.001
C20:3/C18:3	0.025^{b}	0.021^{ab}	0.027^{b}	0.011^{a}	0.004	0.31	0.019	0.15
C20:2/C18:2	0.246^{b}	0.109^{a}	0.224^{b}	0.120^{a}	0.020	0.81	0.001	0.44
C20:3/C18:2*	0.0047^{b}	0.0041^{b}	0.0061^{c}	0.0017^{a}	0.0004	0.28	0.001	0.001
C20:4/C18:3*	0.098	0.086	0.055	0.074	0.014	0.057	0.82	0.26
(C18:3+C20:3)/C18:2†	0.2484	0.213	0.340	0.176	0.071	0.71	0.18	0.38

^{abc}Least squares means within a row lacking a common superscript differ. *P*<.05.
[1] Tabulated values are least square means. Piglets were fed for 16 days. C18:2 refers to linoleic acid only.
[2] FL=Dietary fat level.
* Indicators used by Agatha et al. [17]. The ratio C20:3 n6/C18:2 n6 is used for Δ-6 desaturase and C20:4 n6/C18:3 n6 for Δ-5 desaturase.
† Desaturation index used by Thijssen et al. [54]. The ratio (C18:3 n6+C20:3 n6)/C18:2 n6 is used for Δ-6-desaturase and C20:4 n6/C20:3 n6 for Δ-5-desaturase.

supplementation of CLA changed the distribution between the Elovl-A and Δ6-desaturation in liver, but had no impact on the total synthetic flux through the whole elongation/desaturation pathway (Fig. 4). This suggests that the conversion of C20:2n-6 to C20:3n-6 and/or to ARA catalyzed by sequential desaturation might be playing a more important role in controlling the rate of the elongation/desaturation pathway in the liver. Conversely, dietary fat level had less impact on the distribution between Elovl-A elongation and Δ6-desaturation in the brain. The n-6 LCPUFA synthesis pathway in brain of neonatal animals is specific for LA elongation and the direct elongation product from the alternate metabolic pathway is fully converted to AA [53]. Because more than 95% of the total synthetic flux from [U-^{13}C]LA was found in [^{13}C]18:3n-6 and [^{13}C]20:2n-6 (Fig. 3), the reduction of [^{13}C]20:2n-6 resulted in a significant decrease in the activity of the elongation/desaturation pathway in the brain.

With regard to the indices of desaturation and elongation, a similar pattern was obtained from [^{13}C]LCPUFA generated from liver tissue incubation with [^{13}C]LA (Table 3) and LCPUFA measured in the tissue (Table 3) for Δ^{6}-desaturation, Δ^{8}-desaturation, Elovl-5 elongation and Elovl-A elongation index but not for Δ^{5}-desaturation index. Dietary CLA significantly increased Δ^{5}-desauration index calculated from [^{13}C]LCPUFA but had no influence on the index calculated from tissue LCPUFA. The difference between the two indices appeared to be associated with the dietary LCPUFA. The high fat diet contained a

Table 4
Desaturase and elongase activity indices calculated from LCPUFA measured in liver and brain tissues from piglets fed either a low fat (LF, 3%) or high fat (HF, 25%) diet with or without the inclusion of conjugated linoleic acid (CLA; 1% mixed isomer) [1]

	Diet				SEM	Significance, *P* value		
	LF-CLA	LF+CLA	HF-CLA	HF+CLA		FL [2]	CLA	FL×CLA
Liver								
C18:3/C18:2	0.0067^{a}	0.0076^{b}	0.0087^{b}	0.0104^{c}	0.0006	0.001	0.005	0.86
C20:4/C20:3†	15.98^{a}	22.14^{ab}	24.44^{ab}	25.83^{b}	3.05	0.06	0.231	0.44
C20:3/C20:2	0.84^{c}	0.37^{b}	1.33^{a}	1.23^{a}	0.12	0.001	0.032	0.14
C20:3/C18:3	3.84^{b}	1.49^{a}	1.71^{a}	1.30^{a}	0.28	0.001	0.001	0.003
C20:2/C18:2	0.026^{b}	0.034^{c}	0.011^{a}	0.011^{a}	0.003	0.001	0.120	0.15
C20:3/C18:2*	0.022^{b}	0.011^{a}	0.014^{a}	0.014^{a}	0.002	0.25	0.016	0.026
C20:4/C18:3*	60.18^{b}	27.73^{a}	40.87^{a}	32.46^{a}	5.41	0.19	0.001	0.038
(C18:3+C20:3)/C18:2†	0.027^{b}	0.019^{a}	0.023^{a}	0.024^{a}	0.002	0.86	0.13	0.046
Brain								
C18:3/C18:2	0.011	0.012	0.011	0.012	0.001	0.90	0.64	0.89
C20:4/C20:3†	21.13^{ab}	17.70^{a}	21.45^{ab}	23.67^{b}	0.943	0.003	0.53	0.007
C20:3/C20:2	0.077^{a}	0.073^{a}	1.25^{b}	1.20^{b}	0.087	0.001	0.63	0.99
C20:3/C18:3	3.04	2.80	3.03	2.51	0.29	0.61	0.21	0.65
C20:2/C18:2	0.044^{ab}	0.045^{b}	0.030^{ab}	0.025^{a}	0.005	0.005	0.73	0.55
C20:3/C18:2*	0.033	0.031	0.034	0.029	0.003	0.82	0.25	0.54
C20:4/C18:3*	63.91	48.62	64.29	59.38	5.77	0.35	0.095	0.38
(C18:3+C20:3)/C18:2†	0.044	0.043	0.045	0.041	0.003	0.88	0.39	0.56

^{abc}Least squares means within a row lacking a common superscript differ. *P*<.05.
[1] Tabulated values are least square means. Piglets were fed for 16 days. C18:2 refers to linoleic acid only.
[2] FL=Dietary fat level.
* Indicators used by Agatha et al. [17]. The ratio C20:3 n6/C18:2 n6 is used for Δ-6 desaturase, C20:4n6/C18:3n6 for Δ-5-desaturase.
† Desaturation index used by Thijssen et al. [54]. The ratio (C18:3 n6+C20:3 n6)/C18:2 n6 is used for Δ-6-desaturase and C20:4 n6/C20:3 n6 for Δ-5-desaturase.

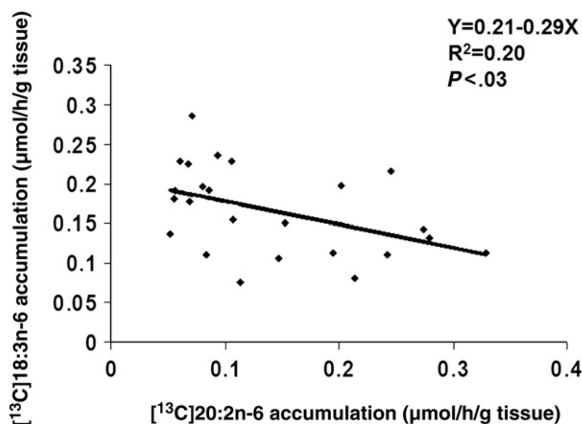

Fig. 5. Reciprocal relationship between the elongation of [^{13}C]18:2n-6 to [^{13}C]20:2n-6 and its desaturation to [^{13}C]18:3n-6 in liver.

higher amount of ARA and had a great impact on the tissue ARA concentration. The concentration of ARA measured in liver was 1.64-fold higher from high fat diet than low fat diet (Table 2). Thus, the increase of ARA from the high fat diet might attenuate the effect of CLA which resulted in a similar desaturation index. In addition, the indices calculated from [^{13}C]LCPUFA generated from brain tissue incubation with [^{13}C]LA were different from those calculated from LCPUFA measured in the brain tissue for Δ^5-desauration index and the elongases. As previously discussed, brain tissue is sensitive to dietary LCPUFA – perhaps alterations in brain concentrations of DHA and ARA were responsible for the observed changes in the indices.

Furthermore, we compared our indices to the indices used by other researchers (Table 3 & 4). We found the indices, dependant on the product(s) and precursor(s) used for the ratio calculation, varied greatly from the different reports [17,54]. In general, caution is needed when such indices are used to evaluate the activity of desaturation/elongation. The external LCPUFA supply (dietary) and internal tissue-specific metabolism may have a great impact on the indices calculated as the ratio of product and precursor in the pathway.

In conclusion, we found dietary CLA to have no effect on LA or ARA oxidation in either liver or brain of neonatal piglets. However, it does impact n-6 LCPUFA synthesis by inhibition of LA elongation and desaturation. The inhibitory effects of CLA are different in liver and brain tissues, which is associated with the dietary fat level and the corresponding concentrations of PUFA in the dietary fat. The alternative Elovl-A elongation pathway (Fig. 4) may play an important role in LCPUFA synthesis. Supplementation of CLA or LCPUFA in the diet inhibits LA elongation especially through the alternative elongation pathway. In addition, desaturase and elongase activity indices calculated as the ratio of product and precursor in the pathway are influenced by dietary LCPUFA and its metabolism in the tissues.

Acknowledgments

This project was supported by National Research Initiative Grants 2005 35200-16174 (to JO) and 2007-35206-17897 (to XL) from the USDA National Institute for Food and Agriculture.

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