Prostaglandins, Leukotrienes and Essential Fatty Acids 85 (2011) 335–343

Contents lists available at SciVerse ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Heart arachidonic acid is uniquely sensitive to dietary arachidonic acid and docosahexaenoic acid content in domestic piglets ☆

Cynthia Tyburczy [a], Kumar S.D. Kothapalli [a], Woo Jung Park [a], Bryant S. Blank [b], Kathryn L. Bradford [a], J. Paul Zimmer [c], Christopher M. Butt [c], Norman Salem Jr.[c], J. Thomas Brenna [a,*]

[a] Division of Nutritional Sciences, B38 Savage Hall, Cornell University, Ithaca, NY 14853, USA
[b] College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA
[c] Martek Biosciences Corp., Columbia, MD 21045, USA

ARTICLE INFO

Article history:
Received 20 June 2011
Received in revised form
11 August 2011
Accepted 12 August 2011

Keywords:
Piglet
Arachidonic acid
Infant nutrition
Docosahexaenoic acid

ABSTRACT

This study determined the sensitivity of heart and brain arachidonic acid (ARA) and docosahexaenoic acid (DHA) to the dietary ARA level in a dose–response design with constant, high DHA in neonatal piglets. On day 3 of age, pigs were assigned to 1 of 6 dietary formulas varying in ARA/DHA as follows (% fatty acid, FA/FA): (A1) 0.1/1.0; (A2) 0.53/1.0; (A3–D3) 0.69/1.0; (A4) 1.1/1.0; (D2) 0.67/0.62; and (D1) 0.66/0.33. At necropsy (day 28) higher levels of dietary ARA were associated with increased heart and liver ARA, while brain ARA remained unaffected. Dietary ARA had no effect on tissue DHA accretion. Heart was particularly sensitive, with pigs in the intermediate groups having different ARA (A2, 18.6 ± 0.7%; A3, 19.4 ± 1.0%) and a 0.17% increase in dietary ARA resulted in a 0.84% increase in heart ARA. Further investigations are warranted to determine the clinical significance of heart ARA status in developing neonates.

1. Introduction

Arachidonic acid (20:4*n*−6; ARA) and docosahexaenoic acid (22:6*n*−3; DHA) are two long chain polyunsaturated fatty acids (LCPUFA; ≥2 double bonds, ≥20 carbon) that contribute to normal growth and development during the perinatal period. Both are natural components of breastmilk and since 2002, these LCPUFA have been added to infant formulas in the US at target levels of 0.66% and 0.33% total fatty acids (FA), respectively [1]. Mean worldwide levels of ARA and DHA in breastmilk are 0.47 ± 0.13% total FA (range: 0.24–1.0% FA) and 0.32 ± 0.22% FA (range: 0.06–1.4% FA), respectively, with individual variability in DHA attributed largely to maternal diet [2]. Inevitably, the wide variation in breastmilk LCPUFA as well as the relative inaccessibility to human infant tissue presents a major challenge for optimizing levels of ARA and DHA in formula to support neonatal development.

It is well-established that the addition of ARA and DHA to formula enhances blood LCPUFA levels equivalent to those of breastfed infants and improves visual acuity and cognitive performance compared with infants fed LCPUFA-free formula [3–6]. Functional outcome studies with infants provide clear evidence for the addition of DHA at 0.32% total FA [7,8], while higher DHA levels may offer further benefit for neural development, especially in target populations [9–11]. Optimal levels of ARA remain to be determined at any DHA intake and are based largely on mean global levels in breastmilk [1]. ARA comprises approximately 10–12% total FA in human infant central nervous tissue (e.g. cerebral cortex and retina) and appears to be influenced to a greater extent by postnatal age than dietary ARA supply [12]. Studies with animal models suggest a relative resistance of brain and retina ARA to dietary ARA intake, possibly as a mechanism for regulating its potent bioactivity [13,14]. ARA levels in the heart, liver and blood-borne pools vary with ARA intake and may compete with *n*−3 LCPUFA for tissue incorporation [3,15–22], especially in the liver [19,22]. Further, in some regions of the neonatal brain, ARA declines when DHA intake is increased [22].

The sensitivity of heart ARA to dietary ARA content has been reported a few times but has not been systematically studied [16–18,20]. In pigs [20], dietary ARA and DHA were maintained at a constant 2:1 ratio and total levels varied up to 5 times the target

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; FA, fatty acid; FAME, FA methyl ester; FR, formula-reared; LCPUFA, long chain polyunsaturated fatty acid; MR, maternal-reared

☆ This project was also supported in part by Award T32DK007158 from the National Institute of Diabetes and Digestive and Kidney Diseases of the NIH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

* Corresponding author. Tel.: +1 607 255 9182; fax: +1 607 255 1033.
E-mail address: jtb4@cornell.edu (J.T. Brenna).

0952-3278/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2011.08.005

levels in formula. While liver ARA increased with only the highest ARA intakes, heart ARA responded in a direct dose–response manner to each incremental increase in dietary ARA. Further, heart ARA more than doubled with the highest ARA level compared with pigs fed LCPUFA-free formula.

We tested the hypothesis that heart and brain ARA is sensitive to dietary ARA in a dose–response design with DHA constant at a high physiological level. We examined four levels of ARA ranging from 0.09% to 1.0% total FA against a background of 1.0% DHA. Intermediate levels were 0.53% and 0.67% total FA, where 0.53% is slightly above the worldwide ARA mean in human breastmilk and 0.67% is commonly used in infant formulas, though it is among the higher values reported for human breastmilk [2]. A DHA dose-response was also included to determine the influence of dietary DHA level on tissue ARA incorporation when ARA levels are comparable to those added in conventional formula (0.66% ARA). The range of DHA content in human milk is 0.06–1.4% [2]. A randomized, maternal-reared (MR) reference group was included for comparison. The results of these experiments indicate that the heart and liver were sensitive to dietary ARA level, but the brain showed little response.

Table 1
Nutrient composition and analysis of experimental diets.

Composition	Content
Ingredient, g/kg dry weight	
Dried skim milk	550
Oil blend	239
Calcium sodium caseinate	96
Mineral mix[a]	48
Vitamin mix[b]	48
Xanthan gum	14
Methionine, DL	5
Nutrient analysis, %kcal	
Protein	24.3
Carbohydrate	28.7
Fat	47
Caloric density (kcal/g)	4.67

[a] The mineral mix contained (g/kg): 257.8 sucrose, 615.8 dibasic calcium phosphate, 106.7 sodium chloride, 11.8 ferric citrate, 4.0 zinc carbonate, 3.4 magnesium oxide, 0.4 cupric carbonate, 0.2 manganous carbonate, 0.1 potassium iodate, and 0.01 sodium selenite.

[b] The vitamin mix contained (g/kg): 910.1 sucrose, 75.0 choline bitartrate, 6.2 vitamin E acetate (50%), 3.4 vitamin A acetate (500,000 IU/g), 1.5 vitamin D3 (100,000 IU/g), 1.3 vitamin B12 (0.1% mannitol), 0.9 pantothenic acid (d, calcium), 0.9 niacin, 0.4 biotin (1%), 0.1 riboflavin, 0.1 thiamine HCl 0.03 menadione sodium bisulfite, 0.01 folic acid, 0.01 pyridoxine HCl.

2. Materials and methods

2.1. Animals and diets

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Cornell University. On day 3 of life, domestic piglets were matched for weight and gender and assigned to one of six milk replacer diets ($n=8$ per diet). Piglets in the formula-reared (FR) groups were then transferred to the Large Animal Research and Teaching Unit, where they were housed individually in raised metal cages and maintained on a 16/8 h light/dark cycle. The seventh group was MR and remained with the dam at the swine facility for the duration of the study. Diet intake and growth performance have been reported elsewhere [23].

Milk replacer formula consisting of 60% experimental diet (Research Diets, Inc., New Brunswick, NJ) and 40% Birthright baby pig milk replacer (Ralco Nutrition, Inc., Marshall, MN) was fed to FR piglets on days 3–28 of age. The ingredient composition and nutrient analysis of the base experimental diet are presented in Table 1. A detailed nutrient composition and analysis of the Birthright baby pig milk replacer has been reported previously [24]. As fed, the milk replacer formula had a caloric density of 0.7 kcal/mL and a nutrient composition as follows (%wt/wt): protein 27.0, fat 22.1, carbohydrate 39.5, fiber 0.7 and ash 10.7. The FA compositions of the milk replacer formulas and sow milk (day 14 in lactation) are presented in Table 2. Diets varied in the ratio of ARA/DHA as follows (% FA/FA): (A1) 0.1/1.0; (A2) 0.53/1.0; (A3–D3) 0.69/1.0; (A4) 1.1/1.0; (D2) 0.67/0.62; and (D1) 0.66/0.33 (conventional infant formula). Diets A1–A4 are a dose-response for ARA against DHA constant at 1.0%, while Diets D1–D3 are a dose-response for DHA against ARA constant at 0.67%. The sow diets were a conventional pig ration consisting of corn and soybean as 96% of the total dry weight, and the PUFA content was comprised of 18:2$n-6$ (53.9 $\pm$ 0.2% FA) and 18:3$n-3$ (3.4 $\pm$ 0.1% FA). The excess 18:2 and low 18:3 apparently drove the very low sow milk DHA (0.01 $\pm$ 0.01% FA), while ARA was high compared to the human milk range (0.74 $\pm$ 0.02% FA).

2.2. Sampling

On day 28 of age, piglets were killed via an intravenous injection of Fatal Plus (1 mL/4.54 kg body weight; Vortech Pharmaceuticals, Dearborn, MI, USA) followed by exsanguination. Necropsy was performed by a team to facilitate rapid removal and weighing of organs, and all samples were flash frozen in liquid nitrogen within 10 min of cessation of heart beat. The eyes and brain were removed by the attending veterinarian using bone cutters and surgical

Table 2
FA composition of milk replacer formulas and sow milk.[a]

Diet	A1	A2	A3–D3	A4	D2	D1	MR
% total FA							
ARA	0.09	0.53	0.69	1.06	0.67	0.66	0.74 $\pm$ 0.02
DHA	1.00	1.02	1.01	1.04	0.62	0.33	0.01 $\pm$ 0.01
ARA/DHA	0.1	0.5	0.7	1.0	1.1	2.0	74.0
$\sum$SFA+MUFA	79.86	79.94	79.43	79.18	80.02	80.02	83.94 $\pm$ 3.16
18:2$n-6$	17.25	16.83	17.11	16.93	16.9	17.19	12.05 $\pm$ 2.98
$\Sigma n-6$	17.5	17.52	17.95	18.15	17.76	18	15.21 $\pm$ 3.08
18:3$n-3$	1.53	1.41	1.48	1.47	1.48	1.53	0.57 $\pm$ 0.06
$\Sigma n-3$	2.53	2.43	2.49	2.51	2.1	1.86	0.71 $\pm$ 0.04
18:2$n-6$/18:3$n-3$	11.3	12	11.6	11.5	11.4	11.3	21.1

[a] Milk from two sows taken on day 14 of lactation; MR piglets remained with the sow for the duration of the study. The PUFA content of sow diets during gestation and lactation consisted of 53.90 $\pm$ 0.17% 18:2$n-6$ and 3.44 $\pm$ 0.04% 18:3$n-3$.