The Effects of Docosahexaenoic Acid Oil and Soybean Oil on the Expression of Lipid Metabolism Related mRNA in Pigs*

B. H. Liu, Y. C. Wang, C. F. Kuo[1], W. M. Cheng, T. F. Shen and S. T. Ding**
Department of Animal Science, National Taiwan University, Taipei 106, Taiwan, ROC

ABSTRACT : To study the acute effect of dietary docosahexaenoic acid (DHA, $C_{22:6}$) on the expression of adipocyte determination and differentiation-dependent factor 1 (ADD1) mRNA in pig tissues, weaned, crossbred pigs (28 d of age) were fed with either 10% (on as-fed basis) tallow (high stearic acid), soybean oil (high linoleic acid), or high DHA algal oil for 2 d. The plasma and liver DHA reflected the composition of the diet. The adipose tissue and skeletal muscle DHA did not reflect the diet in the short term feeding. The results also showed that the diet containing 10% algal DHA oil significantly decreased the total plasma cholesterol (39%) and triacylglycerol (TG; 46%) in the pigs. Soybean oil significantly decreased plasma TG (13.7%; $p<0.05$), but did not have an effect on plasma cholesterol. The data indicate that different dietary fatty acid compositions have different effects on plasma lipids. The ADD1 mRNA was decreased ($p<0.05$) in the liver of DHA oil-treated pigs compared with the tallow-treated pigs. The diets did not have significant effect on the ADD1 mRNA in adipose tissue. Addition of algal DHA oil in the diet increased acyl CoA oxidase (ACO) mRNA concentration in the liver, suggesting that dietary DHA treatment increases peroxisomal fatty acid oxidation in the liver. However, dietary soybean oil supplementation did not affect mRNA concentrations of ADD1 or ACO in the tissues of pigs. Because ADD1 increases the expression of genes associated with lipogenesis, and ACO is able to promote fatty acid oxidation, feeding DHA oil may change the utilization of fatty acids through changing the expression of ADD1 and ACO. Therefore, feeding pigs with high DHA may lead to lower body fat deposition. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1451-1456)***

Key Words : Acyl-CoA Oxidase, Adipocyte Determination and Differentiation-dependent Factor 1, Docosahexaenoic acid, Fatty Acid, Pig

INTRODUCTION

Dietary fatty acids can be incorporated to a large extent into tissues (Sink et al., 1964; Mason and Sewell, 1967; Eder et al., 2001) and plasma lipids in pigs (Smith et al., 1996; Ding et al., 2003a). We have found changes in porcine tissue fatty acid composition, after only two wk of feeding a diet containing 15% fish oil (Ding et al., 2003a). Such a change in fatty acid composition may then modify lipid metabolism in pigs.

Several long chain fatty acids (stearic acid, oleic acid, and arachidonic acid) can stimulate porcine preadipocyte differentiation through a modification of gene expression (Ding and Mersmann, 2001; Ding et al., 2002; Ding et al., 2003b). In cultured porcine preadipocytes, docosahexaenoic acid (DHA; $C_{22:6}$) has no effect on adipocyte differentiation but reduces the lipogenic transcription factor adipocyte determination and differentiation-dependent factor 1 (ADD1) mRNA (Ding et al., 2002). This transcription factor is also known as sterol regulatory element-binding protein 1c (SREBP-1c; Kim and Spiegelman, 1996). Therefore DHA may inhibit lipogenic activity in pigs.

The ADD1 regulates the transcription of several lipogenic genes, e.g., fatty acid synthase (FAS), acetyl CoA carboxylase, and glycerol-3-phosphate acyltransferase (Brown and Goldstein, 1997) and plays a role in adipocyte differentiation (Kim and Spiegelman, 1996; Kim et al., 1998). Dietary fish oils decrease hepatic FAS through a reduction of ADD1 expression in rodents (Xu et al., 2001). Incubation of porcine preadipocytes *in vitro*, i.e., stromal-vascular cells with DHA for one day decreases the ADD1 mRNA and protein concentrations (Ding et al., 2002; Hsu and Ding, 2003). In the current study, high DHA algal oil was added to the pig diet to test the hypothesis that dietary DHA has the effect on inhibiting ADD1 mRNA in both adipose tissue and liver. Transcripts for acyl CoA oxidase (ACO), the enzyme associated with peroxisomal fatty acid oxidation was also measured in adipose tissue, liver, and skeletal muscle.

MATERIALS AND METHODS

Animals and diets

The animal protocol used in the present experiment was approved by the Animal Care and Use Committee of the National Taiwan University. Weaned, crossbred pigs (LYD: Landrace, Yorkshire, and Duroc; 28 d of age) were purchased from a commercial pig farm and transported to the National Taiwan University. They weighed 9.8±0.31 kg and were fed the control diet (tallow-containing diet. Table

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** Corresponding Author: Shih-Tong Ding. Tel: +886-2/8732-7350, Fax: +886-2/2732-4070, E-mail: sding@ntu.edu.tw
[1] Department of Food Science, Nutrition and Nutraceutical Biotechnology, Shih Chien University, Taipei 104, Taiwan, ROC.
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Table 1. Composition of experimental diet

Ingredient	Composition, % (as-fed basis)
Wheat flakes	48.75
Isolated soy protein	14.00
Whey, dried	10.00
Skimmed milk, dried	5.00
Wheat bran	10.00
Tallow, soybean oil, or DHA oil[a]	10.00
$CaHPO_4$	0.70
$CaCO_3$	0.70
Iodide salt	0.25
Vitamin premix[b]	0.30
Mineral premix[c]	0.20
Antibiotics[d]	0.10
Calculated values:	
Crude protein (%)	22.19
ME (Mcal/kg)	3.42
Calcium (%)	0.75
Phosphorus (%)	0.63

[a] Docosahexaenoic acid (DHA) oil extracted from algae and contained 44% DHA.

[b] Vitamin premix provides the following vitamins per kg diet: vitamin A, 8,000 IU; vitamin D_3, 800 IU; vitamin E, 30 IU; vitamin K_3, 1 mg; vitamin B_1, 2.0 mg; vitamin B_2, 5.0 mg; vitamin B_{12}, 25 μg. Ca-pantothenate; 12 mg, Niacin; 18 mg, Folacin; 0.4 mg, Biotin; 0.06 mg.

[c] Mineral premix provides the following minerals per kilogram of diet: Cu, 10 mg; Fe, 100 mg; Zn, 100 mg; Mn, 10 mg; Se, 0.1 mg.

[d] Antibiotics contain 22 g lincomycin and 22 g spectinomycin per kilogram.

1) for 9 d for the pigs to adapt to the diet and the environment. At 37 d of age, the pigs were fed a diet supplemented with either 10% (on as-fed basis) of tallow, soybean oil, or an algal DHA containing oil for 2 d. Eighteen pigs were allocated to 3 treatment groups with 6 pigs per treatment. The reason for choosing 10% DHA oil supplementation is because such supplementation should enrich the dietary DHA to a level similar to the addition of 40% dietary fish oil used in rodent experiments (Kim et al., 1999). The calculated protein content in the experimental diets was 22%, and the fat was 12.62% on as-fed basis. Beginning at 3 d before administration of the experimental diets, pigs were fed two meals per d, one at 07:00 and the other at 16:00. The amount of feed was provided according to the feed intake of the previous day.

After feeding the diets for 2 d, the pigs from each dietary group were selected at random and killed at 09:00, after the 07:00 feeding. Pigs were killed by electrical stunning coupled with exsanguination. Tissue samples including dorsal subcutaneous adipose tissue, Longissimus skeletal muscle, and liver were rapidly removed, wrapped in foil and frozen in liquid nitrogen to be stored at -70°C. A blood sample was obtained from the cut anterior vena cava using EDTA as anticoagulant; plasma was separated and frozen at -70°C until analysis.

Fatty acid analysis

The lipid extraction was performed by the method of Folch et al. (1957) and fatty acid composition was analyzed by using the procedure described by Hsu et al. (2004). Heptadecanoic acid (17:0), as di-17:0 L-α-phosphatidyl-choline (Sigma, St. Louis, MO) was added to each sample as an internal standard. The concentration of fatty acid methyl ester was determined by gas chromatography on a 60 m×0.53 mm I.D. Supelcowax-10 capillary column (1.00 mm film thickness; Supleco Inc., New Territories, Hong Kong), using a Varian Star 3,400cx gas chromatograph (Varian Technologies, Wakefield, RI) equipped with a hydrogen flame ionization detector. The chromatographic analysis was in duplicate and the data were averaged.

Plasma triacylglycerol and cholesterol analysis

The total plasma triacylglycerol (TG) and cholesterol were measured by using commercial kits (Merck, La Jolla, CA; Catalog number 1.14856.0001 and 1.14830.0001). All of the samples were measured in duplicate and averages were reported.

RNA analysis

Total RNA was extracted from the tissues by the guanidinium- phenol -chloroform extraction method (Chomczynski and Sacchi, 1987); modifications were described previously (McNeel and Mersmann 1999; Ying et al., 2003; Wang et al., 2004). The porcine 18S rRNA, acyl CoA oxidase (ACO), and ADD1 probe sequences were previously described (Ding et al., 1999, 2000). Polymerase chain reaction was used to generate radiolabeled cDNA probes (Yang et al., 2004). Hybridization results were quantified by phosphor-image analysis as previously described (Ding et al., 2004). The densitometric value for an individual transcript in a sample lane was normalized to the densitometric value for the 18S rRNA on the same lane.

Statistical analysis

Data were analyzed by ANOVA using a general linear model described in SAS user's guide (SAS Inst., Inc., Cary, NC) with dietary treatment as the main factor. The significant differences between treatments were tested by Duncan's New Multiple Test (SAS Institute, 2001).

RESULTS

Plasma triacylglycerol and cholesterol

Triacylglycerol and cholesterol content in the plasma were affected by the dietary DHA oil supplementation in pigs (Table 2). The pigs fed diet containing 10% algal DHA oil had significantly decreased plasma TG to 56% of that in the tallow-fed, control pigs after 2 d (Table 2). The high dietary DHA oil also decreased plasma cholesterol

Table 2. The effect of different oil supplement on porcine plasma triacylglycerol and cholesterol

Treatment	Triacylglycerol (mg/dl)	Cholesterol (mg/dl)
Tallow	62.43^{a}±3.40	95.57^{a}±3.08
DHA oil	35.31^{c}±1.80	61.94^{c}±5.15
Soybean oil	53.88^{b}±2.31	88.80^{a}±3.45

[a, b, c] Means within the same column with different superscripts differ significantly ($p<0.05$). The results are presented as the means± SEM for 5 pigs in each treatment (one lost datum).

Table 3. The effect of dietary DHA oil supplement on DHA content of various tissues in pigs[a]

	Dietary supplement		
	Tallow	DHA oil	Soybean oil
Plasma	3.93^{b}±0.57	15.41^{c}±1.73	4.83^{b}±0.39
Liver	6.83^{b}±0.60	9.28^{c}±0.63	6.41^{b}±0.70
Muscle	5.35^{b}±0.51	4.24^{b}±0.31	4.04^{b}±0.41
Adipose tissue	0.17^{b}±0.01	0.14^{b}±0.01	0.17^{b}±0.01

[a] Data are presented as DHA/total FA×100. The results are presented as the means±SEM for 5 pigs in each treatment.

[b, c] Means within the same row with different superscripts differ significantly ($p<0.05$).

concentration to 65% of the tallow-fed, control pigs. Dietary soybean oil supplementation significantly decreased plasma TG to a lesser extent (86.3% of control) than DHA. It did not have an effect on plasma cholesterol concentration ($p>0.05$) in this 2 d feeding experiment. The data indicate that different dietary fatty acid compositions have different effects on plasma TG and cholesterol concentrations.

Tissue and plasma DHA concentrations

The DHA in the plasma of the tallow-fed, soybean oil-fed, and DHA oil-fed pigs was 3.93%, 4.83%, and 15.41% of total fatty acids, respectively, indicating that the plasma DHA reflects the dietary DHA enrichment (Table 3). The DHA in the liver of the tallow-fed, soybean oil-fed, and DHA oil-fed pigs was 6.83%, 6.41% and 9.28% of total fatty acids, respectively. The enrichment of the diet with DHA oil significantly increased hepatic DHA concentration ($p<0.05$; Table 3), indicating that the hepatic DHA reflects dietary DHA oil enrichment to a great extent even after a short time treatment. The DHA was 0.17%, 0.17%, and 0.14% of total fatty acids in the adipose tissue from the tallow-fed, soybean oil-fed, and DHA-fed pigs, respectively. The DHA contents in the adipose tissue of three treatments were similar showing that the adipose tissue DHA does not reflect the dietary DHA content after the 2 d treatment ($p>0.05$). The 2 days dietary DHA oil treatment did not have an effect on DHA content in the muscle tissue either ($p>0.05$).

Transcript concentrations

The ADD1 mRNA was decreased (40%) in the liver of

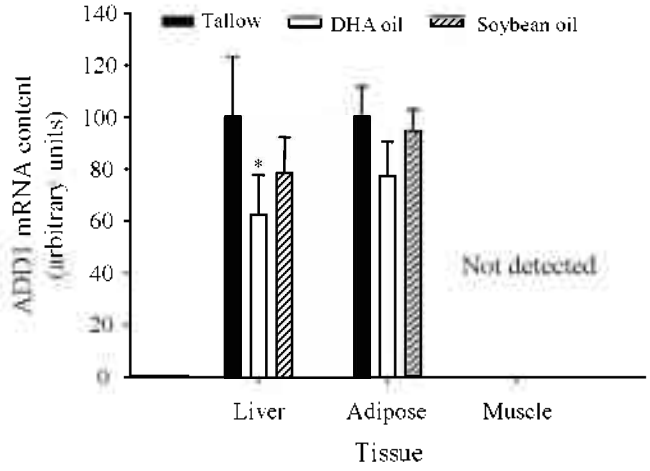

Figure 1. The effect of dietary DHA oil or soybean oil supplement on the abundance of mRNA for adipocyte determination and differentiation-dependent factor 1 (ADD1) in pig tissues. Weaned, crossbred pigs were fed either a 10% tallow, soybean oil, or an algal docosahexaenoic acid (DHA) oil diet for 2 d (6 pigs per group). At the day of sampling, the pigs from each dietary group were sacrificed 2 h after feeding. Tallow = tallow-fed pigs; Soybean oil = soybean oil-fed pigs; DHA oil = DHA oil-fed pigs. The ADD1 mRNA and 18S rRNA contents were determined by Northern analysis. The mRNA values were normalized to 18S rRNA content. The ADD1 mRNA abundances are depicted relative to the tallow-fed control. * Denotes a difference ($p<0.05$) compared with the control group.

DHA-treated pigs compared with the tallow-treated pigs (Figure 1). The numerical reduction in hepatic ADD1 mRNA in soybean oil-fed pigs was not statistically significant. The result suggests that the enrichment of liver DHA content (9%) inhibits the expression of ADD1 mRNA. There was no change in adipose tissue ADD1 mRNA after feeding DHA or soybean oil for 2 d. The ADD1 mRNA was not detected in pig skeletal muscle (Figure 1). The liver ACO mRNA concentration was increased by DHA oil treatment (Figure 2), suggesting that DHA oil treatment increases peroxisomal fatty acid oxidation in the tissue. The ACO mRNA in adipose tissue and muscle was not affected by the short term treatment with different dietary fats.

DISCUSSION

It has been reported that feeding 2% algal DHA oil to pigs for 18 d greatly modifies fatty acid composition in liver, muscle and adipose tissue, suggesting that pigs can utilize algal lipids and deposit the dietary fatty acids into these tissues (Hsu et al., 2004). Dietary fatty acids can also be incorporated into plasma lipids in pigs (Smith et al., 1996; Ding et al., 2003a; Hsu et al., 2004). In the current study with a high algal DHA oil supplementation (10%), we observed that dietary DHA oil enrichment increased plasma and hepatic DHA after 2 d of feeding (Table 3). The short

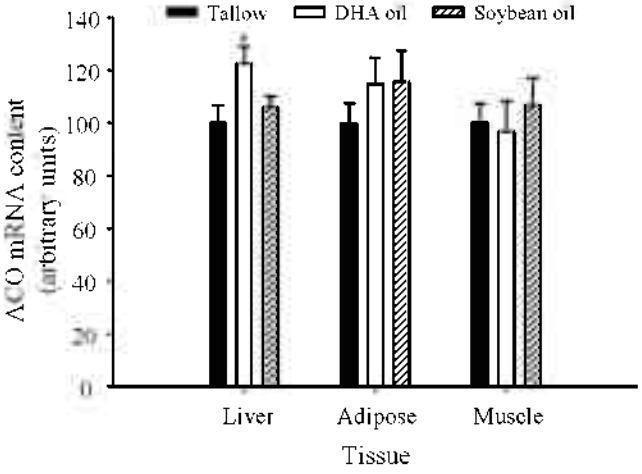

Figure 2. The effect of dietary DHA oil or soybean oil supplement on the abundance of mRNA for acyl CoA oxidase (ACO) in pig tissues. Weaned, crossbred pigs were fed either a 10% tallow, soybean oil, or an algal docosahexaenoic acid (DHA) oil diet for 2 d (6 pigs per group). The pigs were fed and the ACO mRNA was analyzed as indicated in Figure 1. The ACO mRNA abundances are depicted relative to the tallow-fed control. * Denotes a difference ($p<0.05$) compared with the control group.

term treatment, however, did not change DHA content in the muscle and adipose tissue. These data suggest that weaned piglets can digest algal oil and incorporate DHA into liver in a short-term treatment. Incorporation of DHA into skeletal muscle or adipose tissue was considerably less than into liver. Similar observations were reported after feeding a DHA enriched diet for 18 d (Hsu et al., 2004). The current study also showed that DHA was not incorporated into muscle and adipose tissue in a short time.

The transcription factor, ADD1 is expressed in the liver and adipose tissues in pigs (Ding et al., 1999, 2000) and in the livers of rodents and avian species (Gondret et al., 2001; Yen et al., 2004). The porcine ADD1 mRNA in the liver is significantly reduced by dietary DHA oil enrichment either by supplementation of algal DHA oil or fish oil (Ding et al., 2003; Hsu et al., 2004). The current study showed that while the hepatic ADD1 mRNA concentration was sensitive to short term dietary DHA oil treatment, it was not affected by the linoleic acid enrichment (soybean oil treatment), suggesting that DHA has greater potential in inhibiting the gene expression of lipogenic genes in the porcine liver than linoleic acid. The DHA oil effect on reducing the expression of hepatic ADD1 is similar to what was reported in pigs (Ding et al., 2003a; Hsu et al., 2004) and rodents (Yahagi et al., 1999; Xu et al., 1999; 2001). These results suggest that even after a short-term treatment, when the tissue accumulates enough DHA, it can reduce the mRNA of ADD1. The mechanism by which DHA oil reduces the ADD1 mRNA may be due to an increase in the mRNA degradation rate (Xu et al., 2001; Hsu and Ding, 2003). Other research indicates that dietary PUFA modify the proteolytic modification of ADD1 protein to reduce ADD1 functions (Hannah et al., 2001). The reduction of ADD1 mRNA may then reduce the body lipogenic activity which would reduce overall TG deposition. In the current study, the plasma TG was reduced by the addition of dietary DHA oil (Table 2), indicating a possible reduction in body TG synthesis. Alternatively, the reduction of plasma TG in the DHA oil treated pigs might be due to an increase of chylomicron TG clearance (Park and Harris, 2003). Horton et al. (1999) demonstrated that a subtype of ADD1 could regulate the function of low density lipoprotein receptor and modify the metabolism of very low density lipoprotein in the blood. An increase in LDL uptake and/or a reduction in VLDL would explain the reduced the plasma cholesterol concentration in the DHA-treated pigs.

Whereas hepatic ADD1 mRNA is reduced by dietary DHA oil treatment, ADD1 mRNA in the adipose tissue is not affected (Ding et al., 2003a; Hsu et al., 2004). However, ADD1 mRNA concentration is decreased by acute DHA treatment for one day in differentiating porcine stromal/vascular cells in culture (Ding et al., 2002; Hsu and Ding, 2003), suggesting that the expression of ADD1 in either hepatocytes or adipocytes can be regulated by DHA. The current study found that the DHA content in the adipose tissue of DHA oil-treated pigs was just 0.14% of the total fatty acids (Table 3). This relatively low DHA deposition in the adipose tissue might be the major reason for the inability of dietary DHA oil enrichment to regulate the expression of ADD1 in porcine adipose tissue. Furthermore, most of the DHA is expected to be in phospholipids in membranes and perhaps in these compartments DHA is not accessible to affect the expression of ADD1.

The ACO is the rate-limiting enzyme for peroxisomal fatty acid β-oxidation. Its expression is regulated by peroxisome proliferator-activated receptor-α (PPARα; Bell et al., 1998; Desvergne and Wahli, 1999). The DHA has been demonstrated to have high affinity for PPARα, therefore it potentially is a good ligand for PPARα (Grigorios et al., 1997; Kliewer et al., 1997). Activation of PPARα by DHA could increase the activity of PPARα and then increase the expression of ACO. The current study showed that high DHA accumulated in the livers of DHA-treated pigs, and this was accompanied by elevated abundance of the ACO mRNA, suggesting a greater activity of fatty acid oxidation in the liver of DHA treated pigs. Similar observations were reported previously in pigs fed high DHA diets for 2 wk (Ding et al., 2003a). Taken together, these data suggest that dietary DHA treatment may decrease the expression of genes related to fat synthesis and increase the expression of genes related to peroxisomal fatty acid oxidation in the porcine liver, therefore change the overall lipid metabolism in pigs.

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