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Effect of polyunsaturated fatty acids on the expression of transcription factor adipocyte determination and differentiation-dependent factor 1 and of lipogenic and fatty acid oxidation enzymes in porcine differentiating adipocytes

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Polyunsaturated fatty acids (FA) regulate genes involved in lipid metabolism. The effects of polyunsaturated FA on the transcription factor adipocyte determination and differentiation-dependent factor (ADD) 1 and fatty acid synthase (FAS) mRNA in differentiating porcine adipocytes were measured using a stromal vascular cell culture system. Porcine stromal vascular cells were isolated from subcutaneous adipose tissues and plated in Dulbecco's modified Eagle's medium (DMEM)–nutrient mixture F-12 Ham (F-12) plus fetal bovine serum (100 ml/l) for 24 h. Then cells were differentiated in DMEM–F12 plus insulin, hydrocortisone and transferrin without or with polyunsaturated FA at 6·25, 25·00 or 100·00 μM. The ADD1 mRNA was decreased by 100·00 μM-arachidonic acid, 6·25 to 100·00 μM-docosahexaenoic acid or *cis*-9,*trans*-11-conjugated linoleic acid. The polyunsaturated FA reduced the transcription rate of FAS, but not of ADD1. All three polyunsaturated FA accelerated degradation of ADD1 and FAS mRNA to reduce the abundance of ADD1 and FAS mRNA. Results also showed that polyunsaturated FA inhibit the ADD1 expression, not only of mRNA concentration, but also of mature ADD1 protein concentration, suggesting an overall reduction of ADD1 function by polyunsaturated FA. Our present experiments demonstrate that polyunsaturated FA regulate the gene expression of ADD1 and enzymes involved in lipid metabolism in porcine adipocytes.

Acyl-CoA oxidase: Adipocyte differentiation: Adipocyte determination and differentiation-dependent factor 1: Arachidonic acid: Conjugated linoleic acid: Docosahexaenoic acid: Fatty acid synthase: Polyunsaturated fatty acids: Porcine

Dietary polyunsaturated fatty acids (FA) modulate FA composition in many tissues (Otten *et al.* 1993) and reduce body fat deposition (Hill *et al.* 1993; Couet *et al.* 1997; Baillie *et al.* 1999). They can affect body fat deposition through the following two main mechanisms. First, polyunsaturated FA increase expression of genes that are involved in thermogenesis or in FA oxidation, such as acyl-CoA oxidase (ACO), uncoupling protein-3 (Baillie *et al.* 1999) and carnitine palmitoyltransferase I (Brandt *et al.* 1998). Second, polyunsaturated FA decrease expression of lipogenic genes, such as acetyl-CoA carboxylase, fatty acid synthase (FAS), and glycerol-3-phosphate acyltransferase (Jump & Clarke, 1999; Yahagi *et al.* 1999). The proteins involved in FA oxidation are regulated by peroxisome proliferator-activated receptor α, a transcription factor that stimulates gene expression of ACO and carnitine palmitoyltransferase I (Osumi *et al.* 1987; Bell *et al.* 1998; Pineda Torra *et al.* 1999). On the other hand, lipogenic enzymes are regulated by the transcription factor adipocyte determination and differentiation-dependent factor (ADD) 1/sterol regulatory element binding protein 1 (Horton *et al.* 1999; Shimomura *et al.* 1999).

ADD1 is a transcription factor that belongs to the basic helix–loop–helix leucine zipper family. It is synthesized as a 125 kDa precursor protein bound to the endoplasmic reticulum. When cleaved by a sterol-dependent sequential two-step proteolytic process, the N terminal of ADD1 is released from the membrane into the nucleus as a 68 kDa mature transcription factor (Brown & Goldstein, 1997). It is named ADD1 because it promotes adipocyte differentiation in rats (Tontonoz *et al.* 1993). The proteolytic release of mature ADD1 is mainly regulated by sterols (Wang *et al.* 1994) and polyunsaturated FA (Yahagi *et al.* 1999). Besides being regulated by proteolytic release,

Abbreviations: AA, arachidonic acid; ACO, acyl-CoA oxidase; ADD, adipocyte determination and differentiation-dependent factor; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DMEM, Dulbecco's modified Eagle's medium; F-12, nutrient mixture F-12 Ham; FA, fatty acid; FAS, fatty acid synthase; S/V, stromal vascular.

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abundance of ADD1 mRNA was decreased by polyunsaturated FA in the rodent liver by accelerating the degradation rate of ADD1 mRNA (Xu *et al.* 1999, 2001).

In rabbits, ADD1 is high in both liver and adipose tissue where FA synthesis is high (Gondret *et al.* 2001). The same group also showed that ADD1 was high in chicken liver where FA synthesis is high. In pigs, *de novo* FA synthesis takes place primarily in adipose tissues. The ADD1 mRNA is highly expressed in the adipose tissue and to a lesser extent in the liver (Ding *et al.* 1999, 2000). We hypothesized that in porcine adipocytes, polyunsaturated FA reduce the gene expression of ADD1 by accelerating the degradation rate of its mRNA, and in turn suppress the expression of lipogenic enzymes (such as FAS) or stimulate enzymes of FA oxidation such as ACO.

Materials and methods

Cell culture

Based our past experience, the subcutaneous adipose tissues from younger pigs contain more stromal vascular (S/V) cells that also have greater potential for differentiating into adipocytes than those from the older pigs. Therefore, 7-d-old crossbred piglets were killed, and subcutaneous adipose tissue was removed from the dorsal cervical and thoracic regions under sterile conditions for preparing S/V cells. Adipose tissue was sliced into thin pieces (0·66 mm) and digested with collagenase in sterile Krebs Ringer bicarbonate buffer supplemented with 5·6 mM-glucose, 50 U penicillin/ml and 50 μg streptomycin/ml. After digestion for 90 min at 37°C, the isolated S/V cells were pelleted at 800 ***g*** for 10 min and then washed three times by resuspension coupled with centrifugation using Dulbecco's modified Eagle's medium (DMEM)–nutrient mixture F-12 Ham (F-12) (1:1, v/v; Invitrogen Gibco Catalogue 12400-54, New York, NY, USA). The washed S/V cells were resuspended in DMEM–F-12 containing fetal bovine serum (100 ml/l) and plated at a concentration of 4×10^2 cells/mm^2. The S/V cells were then cultured at 37°C in air containing 5 % CO_2 for 24 h to let the cells attach to the plate.

Expt 1: the effect of various concentration of polyunsaturated fatty acids on the expression of adipocyte determination and differentiation-dependent factor 1

After 24 h in DMEM–F-12 with fetal bovine serum, the S/V cells were cultured in the differentiation medium (DMEM–F12 with 100 nM-insulin, 50 ng hydrocortisone/ml, 10 μg transferrin/ml and FA-free bovine serum albumin (10 ml/l)) containing 0·00, 6·25, 25·00 or 100·00 μM-docosahexaenoic acid (DHA), conjugated linoleic acid (CLA) or arachidonic acid (AA) for 24 h (three plates per treatment). These polyunsaturated FA were chosen for their inhibitory effect on gene expression of ADD1 (Ding *et al.* 2002*b*). They were purchased from Matreya, Inc. (Pleasant Gap, PA, USA). The CLA contained 980 g *cis*-9,*trans*-11-CLA/kg. FA were carried by binding with bovine serum albumin in DMEM–F-12 medium, following the procedures stated by Ding & Mersmann (2001) and Ding *et al.* (2002*b*). The control group was treated without FA but with the same bovine serum albumin (10 ml/l) as in other FA-treated groups. Total RNA was extracted from treated plates by the guanidinium–phenol–chloroform extraction method (Chomczynski & Sacchi, 1987). The mRNA expression of ADD1 was determined by Northern analysis (Ding *et al.* 1999) to determine the lowest effective concentration of each polyunsaturated FA on regulating the expression of ADD1.

Expt 2: nuclear run-on assay

The nuclear run-on assay was modified from the procedure previously described (Chen *et al.* 1995). After FA treatment for 24 h, S/V cells were scraped into 5 ml PBS (pH 7·4), centrifuged at 800 ***g*** for 5 min, and the pellet resuspended in 0·8 ml lysis buffer (0·14 M-NaCl, 1·5 mM-$MgCl_2$, 10 mM-Tris-HCl pH 8·6, PEG-*p*-isooctylphenyl ether (5 ml/l)). The suspension was incubated on ice for 5 min, nuclei were pelleted at 3000 ***g*** for 10 min and then resuspended in storage buffer (50 mM-Tris-HCl pH 8·0, with 5 mM-$MgCl_2$, 10 mM-β-mercaptoethanol and glycerol (200 g/l)). The nuclear number was determined by haemocytometry. The samples were stored at −80°C until further analysis. Run-on transcription reactions were carried out by incubating 2×10^6 nuclei with 0·5 mM each of ATP, CTP and GTP (Amersham Pharmacia, Little Chalfont Buckinghamshire, UK), plus 50 μg tRNA (Sigma, St. Louis, MO, USA), 100 U RnasOUT™ (Amersham Pharmacia) and 3·70 MBq [α-^{32}P]UTP (Amersham Pharmacia) in 25 mM-Tris-HCl pH 8·0, 2·5 mM-dithiothreitol, 60 mM-$MgCl_2$, 60 mM-$(NH_4)_2SO_4$ and glycerol (100 g/l) in a volume of 200 μl at room temperature for 30 min. After digestion with 5 U DNase I (Amersham Pharmacia) at room temperature for 30 min, nuclei were lysed upon the addition of 1·6 ml lysis buffer, 40 μl proteinase K (20 mg/ml; Amersham Pharmacia) and 200 μl SDS (100 ml/l) and then incubated at 55°C for 30 min. The labelled RNA was extracted by guanidinium–phenol–chloroform, precipitated by adding isopropanol and dissolved in 50 μl water. Nascent RNA for ADD1, FAS, ACO and 18S were hybridized with their specific cDNA by slot blot hybridization. An empty vector included in each assay served as a negative control to avoid non-specific hybridization. The blots were quantified with the same procedure described in Northern analysis.

Expt 3: mRNA stability

In order to clarify whether polyunsaturated FA affected ADD1 expression by modifying transcripts decay, pig S/V cells were plated for 24 h, then treated with differentiation medium containing 100 nM-insulin, 50 ng hydrocortisone/ml, 10 μg transferrin/ml (no serum or albumin) for 24 h, and then changed to differentiation medium containing bovine serum albumin (10 ml/l) (control), and without or with 100·00 μM-AA, 25·00 μM-CLA or 2·005 μM-DHA. After 4 h of the FA treatments, actinomycin D (5 μg/ml) was added to the culture media to stop transcription. After adding actinomycin D, total RNA was extracted at 0, 2, 4, 6 and 8 h to determine the mRNA degradation

rate of ADD1 and FAS. To determine the mRNA degradation rates, the ADD1 and FAS mRNA concentrations were determined at each time point by Northern analysis. The degradation rate was calculated from the slope of the plot of time *v.* mRNA concentration, adjusted for 18S concentration.

The mRNA concentrations of ADD1, FAS, ACO and 18S were quantified using Northern blot analysis as previously described (Ding *et al.* 1999). The source of the probes is stated in Ding *et al.* (1999) and Ding *et al.* (2000). Total RNA (20 μg) from each treatment was electrophoresed, blotted on a nylon membrane and then hybridized with the [α-^{32}P]dCTP (Amersham Pharmacia)-labelled cDNA probe synthesized by polymerase chain reaction or random prime labelling kit (readiprime™II; Amersham Pharmacia). Abundance of each mRNA was determined by a phosphoimager (BAS-1500; Fujifilm, Tokyo, Japan) and quantification software program. The mRNA abundance of each sample was normalized to the density value of the 18S ribosomal RNA in the same sample.

Expt 4: Western analysis for adipocyte determination and differentiation-dependent factor 1

The proteins from pig S/V cell nuclei were extracted to determine the protein level of ADD1 by Western analysis following the procedure described by Ding *et al.* (2002*a*,*b*). In short, 20 μg protein was applied to a SDS–polyacrylamide gel with acrylamide (750 g/l) and electrophoresed for 1 h at 60 V and then 2 h at 80 V. Following electrophoresis, the gel was equilibrated in the transfer buffer (192 mM-glycine, 25 mM-Tris base and methanol (100 ml/l)) for 10 min and then transferred to polyvinylidene fluoride transfer membrane (Amersham Pharmacia). After transfer, the membrane was used to hybridize with an ADD1 (sterol regulatory element binding protein 1) monoclonal antibody, which was produced and purified from hybridoma cells (ATCC, CRL2121; Ding *et al*. 2002). Detection was carried out by the enhanced chemiluminescence Western blotting detection system kit (Amersham Pharmacia).

Statistical analysis

These experiments were a randomized complete block design. A single pool of S/V cells was used to conduct a replicate of an individual experiment and each experiment was replicated four times. ANOVA and Duncan's new multiple range test was performed by using SAS statistical software (Statistical Analysis Systems, Cary, NC, USA).

Results

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on gene expression

The ADD1 transcripts were significantly suppressed by AA, CLA and DHA in pig S/V cell culture ($P<0{\cdot}05$; Fig. 1(A)). Concentrations of 100·00, 6·25 and 6·25 μM for AA, CLA and DHA respectively inhibited the expression of ADD1. Among these three FA, 100 μM-DHA had the strongest effect, and it suppressed ADD1 mRNA abundance by approximately 70 %. In contrast to ADD1, ACO transcripts were significantly increased by AA, CLA and DHA in pig S/V cells ($P<0{\cdot}05$; Fig. 1(B)). The lowest concentrations of AA, CLA or DHA that increased the expression of ACO were 25·00, 6·25 and 6·25 μM respectively. Among these three polyunsaturated FA, 100·00 μM-DHA had the strongest effect, and it increased ACO abundance by approximately 100 %.

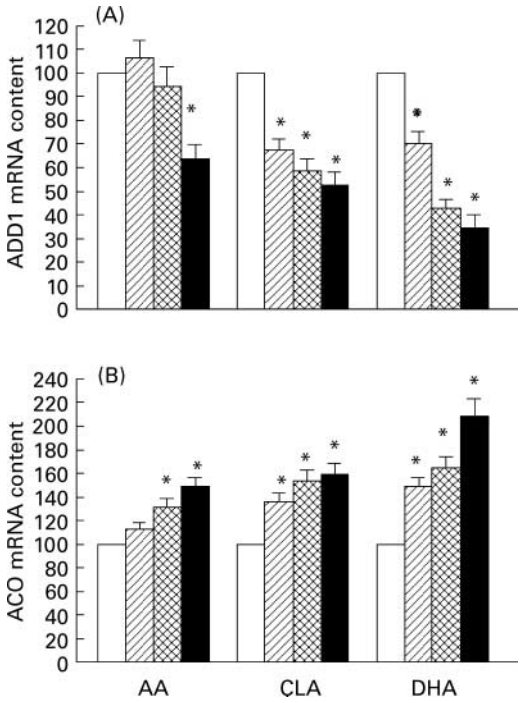

Fig. 1. Polyunsaturated fatty acids coordinately inhibit adipocyte differentiation determination-dependent factor (ADD) 1 and induce acyl-CoA oxidase (ACO) mRNA expression in differentiating pig stromal vascular cells culture. Values are means with their standard deviations shown by vertical bars (n 3 replicates per treatment). Pig stromal vascular cells were cultured in Dulbecco's modified Eagle's medium–nutrient mixture F-12 Ham with fetal bovine serum (100 ml/l) for 24 h to confluence. The medium was then replaced with serum-free medium containing insulin, hydrocortisone and transferrin (differentiation medium) without or with various concentrations of the indicated fatty acid (0·00 □, 6·25 (▨), 25·00 (▩) or 100·00 (■) μM-arachidonic acid (AA), conjugated linoleic acid (CLA) or docosahexaenoic acid (DHA) for 24 h. Total RNA was extracted from cultured cells. The ADD1, ACO and 18S rRNA were determined by Northern analysis. The ADD1 and ACO mRNA abundances are depicted relative to the control ((A), ADD1; (B), ACO). The mRNA values are normalized to 18S rRNA content. For details of procedures, see p. 508. Mean values were significantly different from those of the control group: *$P<0{\cdot}05$.

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on transcription of genes

The 0·00 μM-FA (control), 100·00 μM-AA, 25·00 μM-CLA or 25·00 μM-DHA treatments were used to determine the influence of these FA on the transcription rates of ADD1 and FAS. We found that the transcription rate of ADD1 was not affected by polyunsaturated FA (Fig. 2, Table 1), but the transcription rate of FAS was reduced by approximately 50 % by AA, CLA and DHA treatments ($P<0{\cdot}05$).

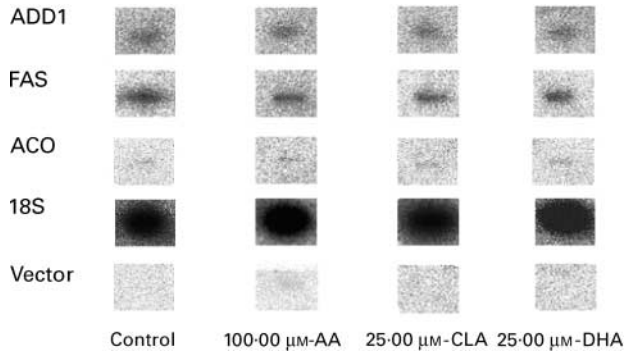

Fig. 2. Slot blotting of nuclear run-on assay in pig stromal vascular cells culture treated with polyunsaturated fatty acids. ADD, adipocyte differentiation determination-dependent factor; FAS, fatty acid synthase; ACO; acyl-CoA oxidase. Pig stromal vascular cells were cultured in Dulbecco's modified Eagle's medium–nutrient mixture F-12 Ham with fetal bovine serum (100 ml/l) for 24 h to confluence, then treated with differentiation medium for another 24 h. Nuclear run-on were conducted using nuclei isolated from pig stromal vascular cells which were treated for 24 h with differentiation medium supplemented with bovine serum albumin (10 g/l) (control), 100·00 μM-arachidonic acid (AA), 25·00 μM-conjugated linoleic acid (CLA) or 25·00 μM-docosohexaenoic acid (DHA). The 18S rRNA was used as a house-keeping normalization control, whereas vector was an empty cloning vector for all the gene fragments, used as a negative control. For details of procedures, see p. 508.

On the other hand, AA, CLA and DHA significantly increased the transcription rate of ACO ($P<0·05$; Fig. 2, Table 1).

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on transcript degradation

The degradation rates of ADD1 and FAS mRNA were significantly affected by 100·00 μM-AA, 25·00 μM-CLA and 25·00 μM-DHA treatments respectively ($P<0·05$; Fig. 3). The regression equations for the control, AA, CLA and DHA treatments were $y=-2·675x+92·45$ (r 0·9203), $y=-5·407x+93·94$ (r 0·9190), $y=-5·609x+101·91$ ($r=0·9216$) and $y=-5·946x+99·20$ (r 0·9621) respectively, where x was time and y was the ADD1 mRNA concentration. The regression equations for FAS mRNA decay for the treatments of control, AA, CLA and DHA were: $-3·695x+102·90$ (r 0·9609), $y=-6·562x+104·38$ (r 0·9712), $y=-5·789x+88·79$ (r 0·8993) and $y=-8·375x+99·28$ (r 0·8944) respectively. The slopes of the regressions for ADD1 and FAS mRNA degradation from cells treated with AA, CLA or DHA were significantly greater than those of the controls. All three polyunsaturated FA accelerated mRNA degradation of ADD1 and FAS. In addition, the polyunsaturated FA reduced the abundance of ADD1 and FAS mRNA during 12 h of treatment. All three polyunsaturated FA decreased mRNA for ADD1 and FAS through the acceleration of mRNA degradation rate.

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on nuclear adipocyte determination and differentiation-dependent factor 1 protein

Western analysis revealed that 100·00 μM-AA, 25·00 μM-CLA or 25·00 μM-DHA significantly decreased ($P<0·05$) the nuclear content of mature ADD1 protein approximately 50 % in porcine adipocyte (Fig. 4). Overall, the results indicate that polyunsaturated FA may decrease ADD1 expression at the translational level or perhaps by post-translational modification, as well as at the transcriptional level.

Discussion

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on transcript concentrations

The current study showed that AA, CLA and DHA reduced ADD1 and FAS mRNA concentrations and increased ACO mRNA concentration in porcine S/V cells. The results were similar to the findings in rodent liver (Xu *et al.* 1999; Yahagi *et al.* 1999). Furthermore, we demonstrated that much lower concentrations of CLA or DHA (6·25 to 25·00 μM) than those that were were tested by Brandt *et al.* (1998) and Ding *et al.* (2002*b*) were effective in inhibiting the expression of ADD1 and FAS and stimulating ACO gene expression. These lower concentrations for CLA and DHA are closer to the physiological concentrations of porcine blood (>10 μM when fed fish

Table 1. Effects of polyunsaturated fatty acids on genes transcription rate of pig stromal vascular cells culture†

(Mean values and standard deviations for four replicates per treatment)

	Transcription rate						
	Control	100·00 μM-AA		25·00 μM-CLA		25·00 μM-DHA	
Gene transcript	Mean	Mean	SD	Mean	SD	Mean	SD
ADD1	100	98·1	12·3	95·8	19·1	86·5	11·7
FAS	100	57·1*	8·9	55·6*	11·5	49·9*	12·6
ACO	100	142·6*	16·8	154·6*	15·3	137·2*	10·9

AA, arachidonic acid; CLA, *cis*-9, *trans*-11-conjugated linoleic acid; DHA, docosahexaenoic acid; ADD, adipocyte determination and differentiation-dependent factor; FAS, fatty acid synthase; ACO, acyl-CoA oxidase.

Mean values were significantly different from those of the control group: *$P<0·05$.

† This is a summary of the results shown in Fig. 2. Nuclear run-on was carried out to determine the effect of polyunsaturated fatty acid treatment on the transcription rate of ADD1, FAS and ACO. Data were normalized with 18S rRNA.

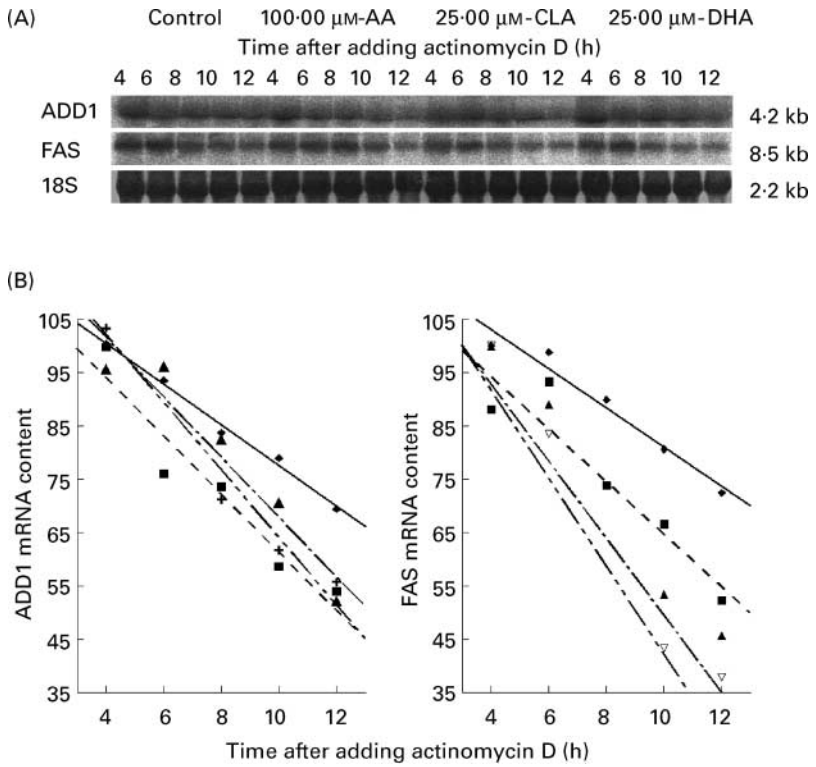

Fig. 3. Polyunsaturated fatty acids accelerated adipocyte determination and differentiation-dependent factor (ADD) 1 and fatty acid synthase (FAS) mRNA decay in pig stromal vascular cells culture. Values are means for four replicates per treatment. Pig stromal vascular cells were cultured in Dulbecco's modified Eagle's medium–nutrient mixture F-12 Ham with fetal bovine serum (100 ml/l) for 24 h to confluence, and then treated with differentiation medium for another 24 h. After 48 h culture, cells were changed to differentiation medium containing bovine serum albumin (10 g/l) (control), 100·00 μM-arachidonic acid (AA), 25·00 μM-conjugated linoleic acid (CLA) or 25·00 μM-docosohexaenoic acid (DHA). At the same time, actinomycin D (5 μg/ml) was added to the medium to stop transcription. Total RNA was extracted at 0, 2, 4, 6 and 8 h after adding actinomycin D. (A), ADD1, FAS and 18S rRNA determined by Northern analysis; (B), decay of ADD1 and FAS (◆, Control; ■, AA; ▲, CLA; ▽, DHA). The mRNA abundance is relative to 0 h of control and normalized to 18S rRNA content. For details of procedures, see p. 508. The ADD1 mRNA decay equations for control, AA, CLA, and DHA from 4 to 12 h were: $y = -2{\cdot}675x + 92{\cdot}45$ (r 0·9203), $y = -5{\cdot}407x + 93{\cdot}94$ (r 0·9190), $y = -5{\cdot}609x + 101{\cdot}91$ (r 0·9216) and $y = -5{\cdot}946x + 99{\cdot}20$ (r 0·9621) respectively. The regression equations for FAS mRNA decay were: $-3{\cdot}695x + 102{\cdot}90$ (r 0·9609), $y = -6{\cdot}562x + 104{\cdot}38$ (r 0·9712), $y = -5{\cdot}789x + 88{\cdot}79$ (r 0·8993) and $y = -8{\cdot}375x + 99{\cdot}28$ (r 0·8944) respectively. The mRNA abundance of ADD1 and FAS treated with AA, CLA and DHA was reduced significantly ($P<0{\cdot}05$) compared with control at 12 h, and the decay curves of AA, CLA and DHA were significantly ($P<0{\cdot}05$) steeper than those of the control.

oil; Ding *et al.* 2002*b*). In rodents, numerous studies have shown that polyunsaturated FA from fish oils suppressed the expression of lipogenic genes in the liver and also upregulated the expression of genes involved in lipid oxidation in the liver and muscle tissues (Baillie *et al.* 1999; Xu *et al.* 1999; Yahagi *et al.* 1999). The polyunsaturated FA, particularly *n*-3 FA, may play a role in partitioning intracellular fuel to reduce lipid accumulation (Price *et al.* 2000; Clarke, 2001). We demonstrated that 24 h exposure to DHA increased the expression of ACO, an enzyme involved in FA oxidation, and DHA was more effective than AA or CLA. These results suggest that DHA is an excellent candidate to simultaneously inhibit lipogenesis and increase FA oxidation in porcine adipocytes.

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on nuclear adipocyte determination and differentiation-dependent factor 1

The decrease of ADD1 mRNA concentration after treatment with AA, CLA or DHA was not the result of a decrease in gene transcription in porcine adipocytes, because the transcription rate measured by nuclear run-on was not affected by these treatments. Similar observations have been reported in rodent livers treated with dietary fish oil (Xu *et al.* 1999, 2001). We also observed a reduction of nuclear ADD1 mature protein by the AA, CLA and DHA treatments. This result is similar to that which was reported in the liver of rodents fed a high level of fish oil (Yahagi *et al.* 1999). The same authors also found that dietary fish oil decreases the mature hepatic ADD1 protein through a post-translational modification mechanism. Our present results indicated that both *n*-3 and *n*-6 polyunsaturated FA (DHA and AA respectively) reduced the mature nuclear ADD1 protein as well as the transcript in porcine adipocytes.

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on fatty acid synthase

The transcription rate of FAS was reduced by AA, CLA and DHA treatments in porcine adipocytes. Since it has been shown that the expression of FAS is regulated by ADD1 (Pai *et al.* 1998), the reduction of FAS transcription rate in porcine adipocytes treated with polyunsaturated FA

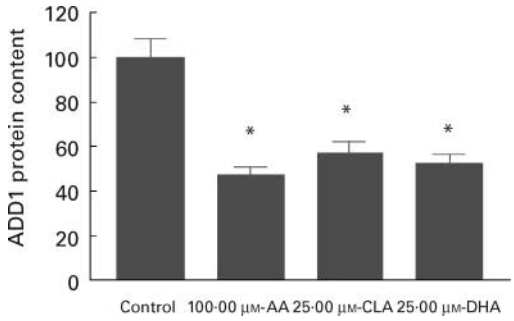

Fig. 4. Western blot analysis of adipocyte determination and differentiation-dependent factor (ADD) 1 in nuclear extracts from pig stromal vascular cells culture treated with polyunsaturated fatty acids. Values are means for four replicates per treatment. Pig stromal vascular cells were cultured in Dulbecco's modified Eagle's medium–nutrient mixture F-12 Ham with fetal bovine serum (100 ml/l) for 24 h to confluence, then treated with differentiation medium for another 24 h. Protein were extracted from nuclei isolated from pig stromal vascular cells which were treated for 24 h with differentiation medium supplemented with bovine serum albumin (10 g/l) (control), 100·00 μM-arachidonic acid (AA), 25·00 μM-conjugated linoleic acid (CLA) or 25·00 μM-docosohexaenoic acid (DHA). The protein content of the nuclear form of ADD1 relative to control is shown. For details of procedures, see p. 508. Mean values were significantly different from those of control: $^*P < 0{\cdot}05$.

may result from a lower ADD1 mRNA and protein concentrations resulting from the polyunsaturated FA treatments. We also found that AA, CLA and DHA accelerated FAS mRNA decay in porcine adipocytes. Therefore, the present results suggest that polyunsaturated FA affect FAS mRNA expression either by directly accelerating FAS mRNA decay or by indirectly reducing ADD1, which in turn decreases the transcription rate of FAS. It had been reported that in rodent livers, dietary polyunsaturated FA reduces the expression of FAS through a reduction of ADD1, but not by increasing the FAS mRNA degradation rate (Xu *et al.* 2001).

Effect of arachidonic acid, conjugated linoleic acid and docosahexaenoic acid on acyl-CoA oxidase

The current study showed that AA, CLA and DHA increased the transcription rate of ACO, indicating a function of polyunsaturated FA treatment to increase FA oxidation in pig differentiating adipocytes. Reddy & Hashimoto (2001) indicated that polyunsaturated FA may be the ligands for peroxisome proliferator-activated receptor α, which would activate peroxisome proliferator-activated receptor α to upregulate the expression of ACO. Others also found that dietary fish oil increased the expression of ACO in rodent livers and muscles and suggested that dietary polyunsaturated FA could increase the FA oxidation in these tissues (Baillie *et al.* 1999). These results all suggest that polyunsaturated FA increase FA oxidation through increase of gene expression of enzymes involved in FA oxidation.

Conclusions

From the results presented in the present paper, the mechanism by which polyunsaturated FA accelerates ADD1 and FAS mRNA decay needs to be further clarified. It may be due to the destabilizing element located at the 5′- or 3′-untranslated region, or in the open reading frame that contains an adenine–uridine- rich sequence within the mRNA (Day & Tuite, 1998). Such an element may respond to polyunsaturated FA treatments to accelerate mRNA degradation. Alternatively, a participating eicosanoid signal or phosphorylation of a specific nuclear protein may have a role in increasing mRNA degradation (Clarke & Jump, 1994; Jump, 2002). Futher speculation is that polyunsaturated FA accelerates mRNA decay through its oxidative property.

In summary, our present study demonstrated a rather low DHA concentration (6·25 μM) reduced lipogenic genes and increased FA oxidation (ACO). The present results suggest that dietary DHA might be used to manipulate FA synthesis and oxidation in pigs. Further studies are needed to clarify the mechanism by which polyunsaturated FA regulate the expression of ADD1 and FAS.

Acknowledgements

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