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Fatty acids modulate porcine adipocyte differentiation and transcripts for transcription factors and adipocyte-characteristic proteins☆

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Abstract

Porcine stromal-vascular cells (S/V cells) differentiate into adipocytes in vitro when presented with appropriate hormones and growth factors. Porcine S/V cells were differentiated in vitro in serum-free media with or without fatty acids to determine the effect of fatty acids on differentiation and on transcripts for peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), lipoprotein lipase (LPL) and adipocyte fatty acid binding protein (aP2). Differentiation was measured by Oil Red O staining and transcript concentrations were measured by Northern analysis using porcine riboprobes. Addition of 100 μM oleic acid (C18:1) for 5 days increased differentiation and the mRNA levels for PPARγ, C/EBPα, LPL and aP2. Other medium- and long-chain fatty acids were less active. Adipocyte differentiation and transcript concentrations for PPARγ, C/EBPα, LPL and aP2 were increased by C18:1 in a dose-related manner. Differentiation was greater at 10 days than at 5 days than at 1 day, and C18:1 increased differentiation at each time. Transcript concentrations were increased by C18:1 at 1 and 5 days, but not at 10 days. These results suggest that the main effect of C18:1 is on regulating gene expression (an acute or drug-like effect) rather than changing the membrane fluidity as a result of changing membrane fatty acid composition (a chronic or nutrient-like effect). Taken together, these results indicate that selected fatty acids modulate porcine adipocyte differentiation and transcripts for adipocyte differentiation-related proteins such as PPARγ, C/EBPα, LPL and aP2.

Keywords: Porcine; Adipocytes; Fatty acids; PPARγ; C/EBPα; Adipocyte fatty acid binding protein; Lipoprotein lipase; Adipocyte differentiation

1. Introduction

The transcription factors, peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα), activate adipocyte differentiation by mediating expression of lipogenic genes [1,2]. Ectopic expression of C/EBPα or PPARγ in fibroblasts initiates differentiation of fibroblasts into adipocytes [3,4]. Lipoprotein lipase (LPL) and adipocyte fatty acid binding protein (aP2) are early and late adipocyte differentiation markers, respectively [5]. Their expression levels are representative of the lipogenic condition of the cell.

The expression of PPARγ is regulated by both hormonal and nutritional factors; for example, expression of PPARγ1 and γ2 is down-regulated by fasting and insulin-dependent diabetes [6,7]. Long-chain fatty acids (FA) are potential ligands for PPARγ [4,8]. These FA increase differentiation of clonal cells into adipocytes and increase the mRNA concentrations for LPL and aP2 [9,10].

Dietary FA have multiple cellular functions. They are oxidized as an energy source by many cell types. They are readily incorporated into phospholipids of organelle membranes and into triacylglycerol for energy storage including porcine adipocyte phospholipids and triacylglycerol [11–13]. Not only are dietary FA incorporated into membranes and storage depots of the adipocyte, but cellular nonesterified FA also potentially regulate expression of specific genes.

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We have established that PPARγ and aP2 transcripts are specifically expressed in porcine adipose tissues and that C/EBPα and LPL transcripts are predominantly expressed in adipose tissues. The transcript concentrations for these genes increase in porcine stromal-vascular (S/V) cells during differentiation in vitro and in porcine adipose tissue as it differentiates in vivo [14]. The purpose of this study was to determine the effects of individual FA on the differentiation of porcine S/V cells and on the expression of transcripts for the transcription factors PPARγ and C/EBPα, and the adipocyte-characteristic proteins aP2 and LPL.

2. Materials and methods

2.1. Animals and S/V cell culture

Seven- to 9-day-old crossbred pigs were purchased from the Texas Department of Criminal Justice (Huntsville, TX, USA). Pigs were removed from the sow, transferred to the USDA/ARS Children's Nutrition Research Center (Houston, TX, USA) and killed by captive bolt pistol coupled with exsanguination. The Baylor College of Medicine Animal Care and Use Committee approved the animal protocol. The back fat was removed aseptically and used for isolation of S/V cells using the techniques previously described [14]. In short, adipose tissue slices were digested by 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 75 min at 37°C, the isolated S/V cells were pelleted at 800 $\times$ *g* for 10 min and then washed three times by resuspension coupled with centrifugation using DMEM/F12 (1:1; Gibco-BRL-catalogue #12400; Life Technologies, Gaithersburg, MD, USA). The washed S/V cells were resuspended in DMEM/F12 containing 10% fetal bovine serum and were then plated at a concentration of $6 \times 10^4/cm^2$. After 24 h of incubation at 37°C in 5% CO_2 in air, the medium was replaced with a serum-free differentiation medium (DMEM/F12 containing 100 nM bovine insulin, 50 ng hydrocortisone/ml and 10 μg transferrin/ml). The medium was changed every 3 days. Culture of porcine S/V cells was detailed previously [14]. For FA effects, the differentiation medium was selectively supplemented with an individual FA bound to bovine serum albumin; the same amount of albumin, without FA, was added to the control medium. At the termination of the experiments, selected plates were used to determine adipocyte differentiation using Oil Red O staining [15]. The Oil Red O-stained cells were counted and used as an estimate for the level of adipocyte differentiation. Cells containing any Oil Red O-stained droplets were counted as differentiated. This methodology does not distinguish the extent of differentiation in individual cells. Total RNA was extracted from other plates by the guanidinium–phenol–chloroform extraction method [16]; details were described previously [17,18].

2.2. Fatty acid treatments

Fatty acids including lauric acid (C12:0), palmitic acid (C16:0); stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidonic acid (C20:4) and docosahexaenoic acid (C22:6) were dissolved in chloroform : methanol (2:1, v/v) for dispersal and handling of the small quantities. The solvents were removed completely under nitrogen gas and the FA were dissolved in differentiation medium containing 10% bovine serum albumin (fatty acid-free bovine serum albumin, Catalogue #3320-80, Intergen Co., Purchase, NY, USA). This medium was diluted with differentiation medium to achieve the desired FA concentrations. The final concentration of albumin was 1% in all culture media including the controls. Experiment 1 used 100 μM (final concentration) of each individual FA added to differentiation medium for 5 days. Experiment 2 examined the effects of concentration (0, 12.5, 25 and 50 μM) of selected FA (C18:1, C18:2, C18:3, C20:4 and C22:6) for 5 days on porcine S/V cells. Experiment 3 measured the effect of 0, 50, 100 and 300 μM of C18:1 on porcine S/V cells for 5 days. Experiment 4 examined the effect of a normal (5.6 mM) and elevated (16.7 mM) glucose concentration on the effects of C18:1 at 0, 50, 100 or 300 μM for 5 days. Experiment 5 measured the effect of 0, 100 and 200 μM of C18:1 on porcine S/V cells for 1, 5 or 10 days.

2.3. Transcript analysis

The mRNA concentrations of PPARγ, C/EBPα, aP2 and LPL were quantified using Northern blot analysis as previously described [14]. The sequences of the porcine RNA probes were published in GenBank (PPARγ, AF103946; C/EBPα, AF103944; aP2, AF102872; LPL, AF102859; 18S, AF102857). In short, 20 μg total RNA from each sample was electrophoresed, blotted to a nylon membrane and hybridized with a radiolabeled riboprobe synthesized by the Strip-EZ T7 kit (Ambion, Austin, TX, USA). Each RNA sample was represented on two different membranes. All membranes for a given experiment were hybridized at the same time using the same riboprobe. Relative abundance of each mRNA was determined by phosphor-image analysis (Storm 860 instrument with the ImageQuant software program, Molecular Dynamics, Sunnyvale, CA, USA). The density value for each sample was normalized to the density value for the 18S ribosomal RNA in the same sample.

2.4. Statistical analysis

A single batch of S/V cells was used to conduct a replicate of an individual experiment. Experiment 1 represents three replicates, whereas Experiments 2 and 5 represent two rep-

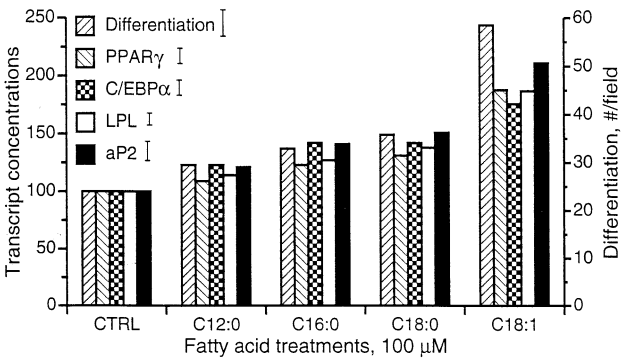

Figure 1. Effect of individual fatty acids on porcine stromal-vascular (S/V) cell differentiation (Experiment 1). The S/V cells were isolated from pig subcutaneous fat and cultured in DMEM/F12 (1:1) plus 10% fetal bovine serum for 24 h. Then the medium was replaced with a serum-free differentiation medium (DMEM/F12 containing 100 nM bovine insulin, 50 ng hydrocortisone/ml and 10 μg transferrin/ml) $\pm$ 100 μM FA in 1% bovine serum albumin for 5 days. Differentiation was determined by Oil Red O staining. Total RNA was extracted to measure transcript concentrations by Northern analysis. All transcript concentrations were normalized by 18S ribosomal RNA and expressed as percentage of control (=100%). Data are means from three experiments, each with cells isolated from a different pig. Data were analyzed by repeated measure analysis of variance. The pooled standard deviation for each variable is indicated in the upper left corner. For differentiation, C12:0 and C16:0 did not differ from the control ($P > .1$), C18:0 tended to be greater than the control ($P < .1$) and C18:1 was greater than the control ($P < .05$). For PPARγ and aP2 transcripts, only C18:1 tended to be greater than the control ($P < .1$), whereas for C/EBPα and for LPL transcripts, only C18:1 was greater than the control ($P < .05$).

licates each and Experiment 3 plus 4 represents either 6 or 7 replicates for each variable. Each replicate used S/V cells isolated from a different pig. For Experiments 1, 2, and 5, repeated measures analysis of variance and Tukey-Kramer post hoc tests were performed using the InStat statistical software program (Graph Pad, San Diego, CA, USA). The InStat program was also used to determine correlations for Experiment 1 and the regression analysis for Experiment 3 plus 4.

3. Results

3.1. Experiment 1

After 5-day exposure to 100 μM FA, only C18:1 significantly ($P < .05$) increased adipocyte differentiation measured by Oil Red O staining (Fig. 1). Differentiation was numerically increased by C12:0 and C16:0 ($P > .1$) and tended to be increased by C18:0 ($P < .1$). Transcript concentrations for PPARγ and aP2 tended to be increased by C18:1 ($P < .1$), whereas those for C/EBPα and LPL were increased ($P < .05$). Other FA (C12:0, C16:0 and C18:0) increased transcript concentrations numerically, but not significantly ($P > .1$). After incubation for several days with 100 μM C18:2, C18:3, C20:4 or C22:6, many cells detached and were lost. Therefore, no data are presented for these FA.

3.2. Experiment 2

Because the porcine S/V cells did not tolerate 100 μM of C18:2, C18:3, C20:4 or C22:6 for 5 days, the concentrations of these FA were reduced. The S/V cells were treated with each of these FA at 0, 12.5 or 25 μM for 5 days; adipocyte differentiation and expression level of the transcripts for PPARγ, C/EBPα, LPL and aP2 were measured. There were no significant treatment effects (data not indicated). Fifty micromoles of either C18:1 or C18:2 for 5 days also had no effect on differentiation or transcript concentrations.

3.3. Experiment 3 plus 4

Several concentrations of C18:1 (i.e., 50, 100 and 300 μM) were tested for effects on S/V cell differentiation and transcript concentrations (Experiment 3, data not indicated).

Glucose is a major energy and reducing-equivalent source for the adipocyte, and when dietary fats are at low concentration, glucose is a source of carbon for de novo FA synthesis. The standard medium we use to grow and differentiate porcine S/V cells contains 300 mg/dl glucose (16.7 mM). We reduced the concentration to 100 mg/dl glucose (5.6 mM), which is approximately equivalent to a normal fasting blood sugar concentration. There was no effect of glucose concentration on S/V cell differentiation or on transcript concentrations after 5 days in the absence or presence of 50, 100 or 300 μM C18:1 (Experiment 4, data not indicated). Consequently, the data for the two glucose concentrations (Experiment 4) were combined. These data were then combined with those from Experiment 3 (Fig. 2 shows combined Experiments 3 and 4)). After 5-day incubation, adipocyte differentiation and each of the transcript concentrations were increased with increasing concentration of C18:1.

3.4. Experiment 5

In addition to the 5-day exposure to FA, both shorter and longer times of treatments were investigated. For cells with no added C18:1, differentiation (measured by number of Oil Red O-stained cells/field) increased from 4 to 19 to 29 at 1, 5 and 10 days, respectively. Addition of 100 or 200 μM C18:1 increased differentiation of S/V cells at 1, 5 and 10 days (Fig. 3). However, the greatest percentage increase in differentiation by C18:1 compared with the control was after 1 day (164% or 223% increase with 100 or 200 μM C18:1, respectively). The increase in the percentage of differentiation compared to the control after addition of 100 or

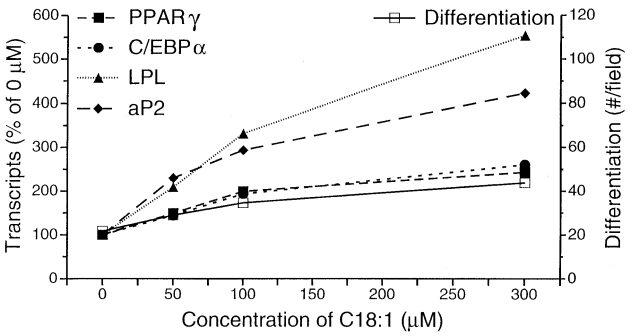

Figure 2. Concentration dependence of C18:1 on porcine stromal-vascular (S/V) cell differentiation (Experiment 3 plus 4). The S/V cells were isolated from pig subcutaneous fat and cultured as indicated in Fig. 1. The fatty acid treatments were 0, 50, 100 or 300 μM for 5 days. Differentiation was determined by Oil Red O staining. Cells were extracted for total RNA to measure transcript concentrations by Northern analysis. All transcript concentrations were normalized by 18S ribosomal RNA and expressed as percentage of control (=100%). Data were analyzed by regression analysis. The data are means from six or seven experiments, each with cells isolated from a different pig. The S.D. of residuals for the regression were 11.3, 108, 101, 385 and 298 for differentiation, PPARγ, C/EBPα, LPL and aP2, respectively. The slopes were all positively linear with P values of $<$.01, .04, .01, .03 and .05 for differentiation, PPARγ, C/EBPα, LPL and aP2, respectively. The runs test for deviation from linearity indicated P values of .03, .71, .16, .06 and .09 for differentiation, PPARγ, C/EBPα, LPL and aP2, respectively. Although nonlinearity is suggested for some variables, an analysis of variance comparing deviations from linearity with scatter among replicates indicated no departure from linearity (all $P > .64$).

200 μM C18:1 at 5 days (20% or 71% increase, respectively) and at 10 days (25% or 45% increase, respectively) was progressively smaller. Transcript concentrations were increased statistically ($P < .05$) or numerically ($P < .2$) after 1- or 5-day exposure to C18:1. After 10 days of treatment with C18:1, transcript concentrations for PPARγ, C/EBPα, LPL and aP2 were no different than control values. The major finding was that compared with the control with no added FA, the acute effects (1 day) of C18:1 on differentiation were greater than the chronic responses (5 days $>$ 10 days).

4. Discussion

4.1. Fatty acids and adipocyte differentiation

The PPARγ–RXRα heterodimer becomes activated when an appropriate ligand is bound. Long-chain FA are possible ligands for the PPARγ–RXRα heterodimer and many of the effects of FA on adipocyte differentiation appear to be mediated by PPARγ [4,8,19,20]. Our current data show that C18:1 stimulated porcine S/V cell differentiation and increased PPARγ, C/EBPα, LPL and aP2 transcript concentrations to a greater extent than saturated long-chain FA (Fig. 1). The effect of C18:1 on porcine S/V cell differentiation in vitro was dose-dependent (Fig. 2).

One caveat regarding these findings is that cell number was not determined, so that differentiation was expressed as Oil Red O-stained cells per field. If the cell number was different in the absence or presence of different fatty acids, it could modify the interpretation of the results. Subjective observation indicated no cell loss when the medium contained C12:0, C16:0, C18:0 or C18:1. In several subsequent experiments (unpublished data), subjective observation also indicated no obvious cell loss when the medium contained C18:1. The number of cells per field was quantified in two experiments (unpublished data); the control number was 211 $\pm$ 18 and the C18:1-treated number was 261 $\pm$ 2 with P = .18. We have no evidence that loss of cells is a confounding variable to interpretation of the effects of long-chain saturated and monounsaturated FA in this serum-free system, but have not rigorously excluded it.

The hypothesis that FA are acting as ligands for PPARγ during differentiation is bolstered by the observation of measurable PPARγ mRNA in S/V cells at the beginning of differentiation [14]. The concentration of this transcript increases 2.5 times as differentiation progresses. The PPARγ1 and 2 proteins are present in adipose tissue less than half-way through gestation, and the PPARγ1 protein is detectable in porcine S/V cells isolated from young postnatal pigs, whereas the PPARγ2 protein is detectable after culture for one or more days [21]. Thus, there is PPARγ protein present in the isolated porcine S/V cells so that addition of an appropriate ligand for PPARγ should cause activation of this transcription factor and induce differentiation. Furthermore, the C/EBPβ and C/EBPα proteins are present in porcine S/V cells after 1 day of culture [22]. Differentiation is probably continued and accelerated because the PPARγ and C/EBPα transcript concentrations increase [14]; the PPARγ and C/EBPα proteins have not been measured under exactly the same culture conditions [21,22], so that one can only speculate that the proteins also increase during differentiation.

Incubation of porcine S/V cells in serum-free medium plus a high concentration (100 μM) of long-chain polyunsaturated fatty acids (PUFA) for 5 days led to considerable cell loss from the plates. In contrast, many long-chain FA (30 to 300 μM of C16:0, C18:0, C18:1, C18:2 and C18:3) significantly increase adipocyte differentiation in rodent-derived clonal cells [4,5,9,10]. These FA also increase the mRNA concentrations for aP2 and LPL, with the most efficacious FA being C18:3 in Ob1771 and 3T3-F442A cells. The differences observed between porcine S/V cells and rodent-derived clonal cells suggest that there may be species differences in the regulation of gene expression and adipocyte differentiation by individual FA. However, the stimulatory effects of PUFA on clonal cell adipocyte differentiation are usually demonstrated in the presence of serum, whereas our studies were in serum-free medium because serum inhibits differentiation of porcine S/V cells [15].

All results, including FA specificity to increase differen-

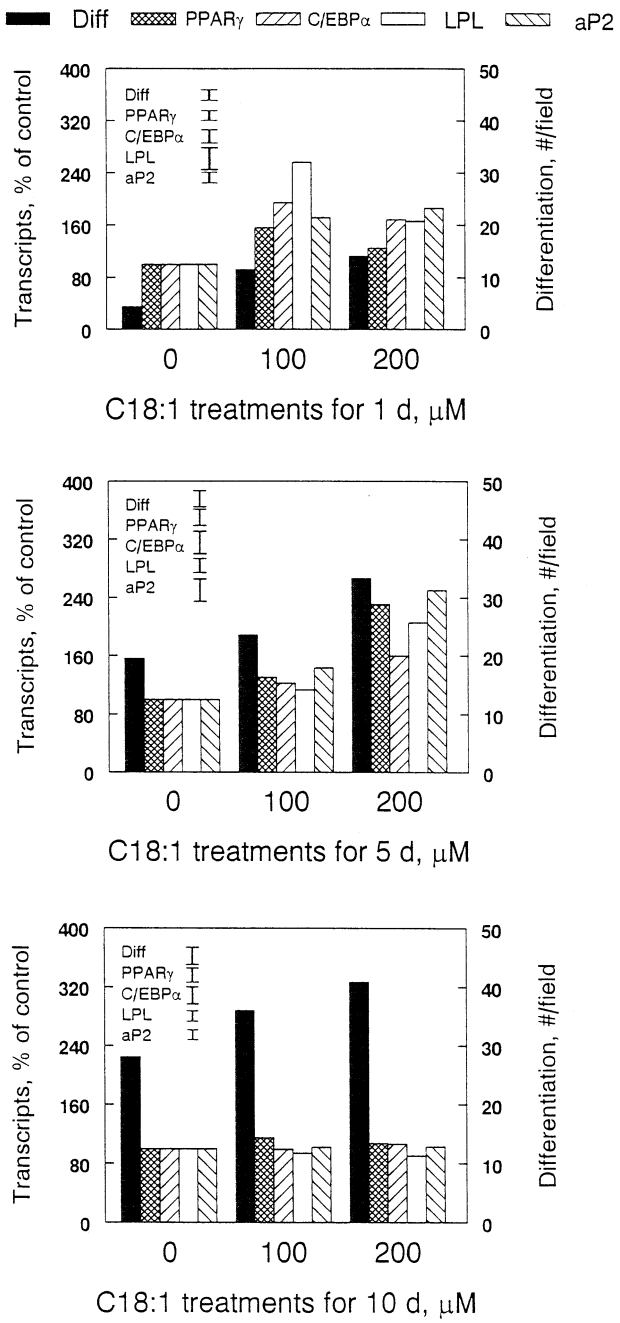

Figure 3. Chronology of the effect of C18:1 on porcine stromal-vascular (S/V) cell differentiation (Experiment 5). The S/V cells were isolated from pig subcutaneous fat and cultured as indicated in Fig. 1. Fatty acid was added at 0, 50, 100 or 300 μM in 1% bovine serum albumin for 1, 5 or 10 days. Differentiation was determined by Oil Red O staining. Cells were extracted for total RNA to measure transcript concentrations by Northern analysis. All the transcript concentrations were normalized by 18S ribosomal RNA and expressed as percentage of control (=100%). Diff is the abbreviation for adipocyte differentiation. The data represent means from two experiments, each with cells isolated from a different pig. The pooled standard deviation for each variable is listed on the upper left corner. Data were analyzed by repeated measure analysis of variance. For 1-day treatments, C18:1 significantly increased adipocyte differentiation and mRNA concentrations for C/EBPα and aP2 ($P < .05$) and increased mRNA concentrations for PPARγ and LPL, numerically ($P < .17$). For 5-day treatments, C18:1 significantly increased adipocyte differentiation and mRNA concentrations for LPL and aP2 ($P < .05$) and increased mRNA concentrations for PPARγ, numerically ($P < .17$). For 10-day treatments, C18:1 numerically increased adipocyte differentiation ($P < .18$), but had no effect on the transcripts measured ($P > .2$).

tiation and transcript concentrations, as well as the cell loss in the presence of PUFA strictly apply to porcine S/V cells cultured in the serum-free medium used in these experiments. They may not extrapolate to other culture conditions, other cell types or to conditions in vivo.

The cause of the major loss of porcine S/V cells observed after several days of culture in the presence of C18:2, C18:3, C20:4 and C22:6 in serum-free medium is not known. Speculatively, several explanations may be proposed. First, the PUFA may be more effective detergents than C18:1 or the saturated FA, causing disruption of cell integrity. Second, incorporation of large amounts of an individual PUFA into cell membranes may modify membrane fluidity and disrupt attachment to the plate. Third, PUFA oxidation products may be responsible for the effects. Fourth, a metabolite(s) of the PUFA may be the effector for cell loss (e.g., an eicosanoid). Fifth, apoptosis may be stimulated.

4.1.1. Glucose effect

In porcine adipose tissue slices, the amount of glucose incorporated into total lipid increases with pig age; that is, increasing adipocyte hypertrophy [23]. Concomitantly, the proportion of glucose incorporated into glyceride-glycerol decreases and the proportion of glucose incorporated into glyceride-FA increases. In undifferentiated porcine S/V cells in culture, glucose is incorporated into glyceride-FA at a low but measurable rate, whereas after differentiation, the rate is increased some thirtyfold [24]. Thus, both undifferentiated and differentiated porcine adipocytes can use glucose for de novo FA biosynthesis, albeit at different rates. To establish a cell culture condition to study the effects of FA on the differentiation process, the medium glucose concentration should be sufficient to provide oxidative substrates and perhaps the precursors for de novo synthesis of long-chain FA. Under these conditions, much of the added FA should be available to act as a potential ligand for PPARγ. The FA and glucose interaction study indicated that C18:1 increased adipocyte differentiation and transcript concentrations for PPARγ, C/EBPα, LPL and aP2, but the glucose concentrations (5.6 mM vs. 16.7 mM) had no effect, even when no exogenous FA was added. These results suggest that the primary effect of C18:1 on porcine adipocyte differentiation is not through provision of substrates for oxidation or for lipid synthesis, but through regulation of genes involved in adipogenesis. In contrast to our observation, differentiation of 3T3-L1 cells is decreased by higher-medium glucose (25 mM vs. 5 mM) in the presence of 100 nM insulin [25].

Short- versus long-term incubation with FA

As reported previously [14], porcine S/V cells differentiated into adipocytes upon stimulation by insulin, hydrocortisone, and transferrin. After exposure to differentiation medium for 4 days, the percentage of differentiation was approximately 12% and increased to 35% by 10 days. The results of Experiment 5 (Fig. 3) indicate increased adipocyte differentiation with time in culture and a further increase upon addition of 100 or 200 μM C18:1 to the medium. The increase in adipocyte differentiation was reflected in the increase in the transcript concentrations for PPARγ, C/EBPα, LPL and aP2 at 1 and 5 days of treatment with C18:1, but not at 10 days. Overall, short-term exposure to C18:1 produced a greater effect than long-term exposure. These results suggest that the main effect of C18:1 was to regulate gene expression (an acute or drug-like effect) rather than to change membrane fluidity as a result of changing membrane fatty acid composition (a chronic or nutrient-like effect).

4.2. Specific transcripts

4.2.1. PPARγ

The PPARγ is a key player in rodent adipocyte differentiation, controlling the expression of genes with a PPAR response element (PPRE) in their promoter region [4,26]. Some of the genes (e.g., LPL, fatty acid synthase, acyl coenzyme A synthase and aP2) are involved in fatty acid uptake and accumulation in adipocytes [27–29]. Pooling of all data in Experiment 1 indicated that PPARγ transcript concentration was positively correlated ($r = .964$, $P < .01$) with porcine adipocyte differentiation. This suggests that PPARγ is involved in porcine adipocyte differentiation. We found that C18:1 increased the transcript concentration for PPARγ in a dose-dependent fashion (Fig. 2). The data suggest that C18:1 may not only activate PPARγ as a ligand [8], but may also increase the PPARγ mRNA concentration. Perhaps, in porcine S/V cells, C18:1 is a potent activating ligand for PPARγ, and activation of the PPARγ–RXRα heterodimer increases its expression through autoactivation or through activation of other genes (e.g., adipocyte differentiation and determination factor 1 or C/EBPα), which could increase the expression level of PPARγ [30].

4.2.2. C/EBPα

The C/EBPα is a key element in regulation of adipocyte differentiation [31–33]. It has been well established that the PPARγ–RXRα dimer, activated by a bound ligand, induces transcription followed by translation of C/EBPα [1,34]. Overexpression of C/EBPα initiates the differentiation of 3T3-L1 cells in vitro [35]. The expression level of porcine C/EBPα transcripts is increased as porcine adipocyte differentiation increases [14], and it has been suggested that pig adipocyte differentiation is regulated mainly by the C/EBPα protein [36]. We demonstrated that C18:1 increased the steady-state mRNA concentration for C/EBPα. There was a positive correlation between C/EBPα and PPARγ (Experiment 1; $r = .95$, $P < .01$) transcript concentrations, so that the increase of C/EBPα transcript concentration upon treatment with C18:1 may result from control of PPARγ by C18:1.

4.2.3. LPL

Lipoprotein lipase (EC 3.1.1.34) is an enzyme that hydrolyzes triacylglycerol in lipoproteins and enables tissues to take up FA. It is significantly involved in regulating lipid metabolism in adipose and muscle tissues [37]. Transcript concentrations for LPL were highly expressed in porcine adipose tissues and the transcript concentration was positively associated with adipocyte differentiation [14]. The current results indicate that C18:1 increased the transcript concentration for LPL in porcine S/V cells. Pooling of all data in Experiment 1 indicated that the transcript concentration for LPL was positively correlated with that of PPARγ ($r = .94$, $P < .01$), suggesting that the expression of LPL mRNA is associated with the expression of PPARγ. There is a PPRE in the promoter region of the LPL gene [27], suggesting that the FA effect on LPL mRNA concentration is through the PPARγ gene.

4.2.4. aP2

aP2 is expressed late during adipocyte differentiation and has been postulated to play important roles in lipid metabolism. Concentration of aP2 mRNA is associated with porcine adipocyte differentiation in vitro [14]. Experiment 1 indicated that many long-chain FA at 100 μM increased the aP2 transcript concentrations numerically with significant stimulation by C18:1. This result is similar to what has been reported in rodent-derived clonal cell lines [5,9,10,38]. Also, in pigs fed a high-fat compared with a low-fat diet, the aP2 was increased [39]. There is a PPRE in the 5′ promoter region of the aP2 gene [40], so that one mechanism for the up-regulation of the aP2 mRNA by FA may be through PPARγ. In porcine S/V cells treated with C18:1, the transcript concentrations for aP2 increased concomitantly with PPARγ, suggesting that the up-regulation of the mRNA for aP2 was by PPARγ.

In summary, our results demonstrate that C18:1 increases adipocyte differentiation and the mRNA concentrations for PPARγ, C/EBPα, LPL and aP2 in porcine S/V cells in a dose-dependent fashion. Data suggest that the function of FA is not only to activate PPARγ but also to increase the transcript concentrations for PPARγ. The increase of steady-state mRNA for PPARγ, C/EBPα, LPL and aP2 could result from an initial increase in transcription and translation of PPARγ. This increase presumably would then cause an increase in transcription and translation of C/EBPα, and the two transcription factors would produce an increase in LPL and aP2 transcript concentrations. An alternative mechanism may be a decrease in mRNA degradation by C18:1 treatment [41]. Regardless of the ultimate mechanism and series of events, C18:1 stimulated transcript

concentrations for PPARγ, C/EBPα, LPL and aP2 in porcine S/V cells to a greater extent than did the other long-chain FA tested. The acute effects of FA were greater than the chronic effects, particularly on transcript concentrations. Our results suggest there may be species differences in the effect of individual FA on adipocyte differentiation. It remains to be determined whether this specificity resides in the affinity of FA as ligands for species-specific PPARγ or in some other mechanism.

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