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Regulatory Role of Sirt1 on the Gene Expression of Fatty Acid-Binding Protein 3 in Cultured Porcine Adipocytes

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ABSTRACT

To investigate whether Sirt1 could modulate fatty acid-binding protein 3 (FABP3), we treated porcine adipocytes either with the Sirt1 inhibitor nicotinamide (NAM), with the Sirt1 activator resveratrol (RES), or by knockdown of Sirt1 by Sirt1-siRNA. NAM or knockdown with Sirt1-siRNA significantly inhibited Sirt1 mRNA expression, while increasing FABP3 mRNA levels. RES or RES + Sirt1-siRNA treatments further proved that Sirt1 negatively regulated FABP3 gene expression in adipocytes. We also found a similar Sirt1 regulation pattern for PPARγ to that of FABP3 in adipocytes. Furthermore, NAM/RES + PPARγ-siRNA treatments showed that Sirt1 may regulate the FABP3 gene expression partly through the PPARγ-mediated signals. In summary, Sirt1 regulates the expression of FABP3 gene in adipocytes, and PPARγ apparently plays an important role in this process. J. Cell. Biochem. 107: 984–991, 2009.

KEY WORDS: FABP3; NICOTINAMIDE; RESVERATROL; RNA INTERFERENCE; PIG; PPARγ; Sirt1

Fatty acid-binding proteins (FABPs), belonging to the superfamily of lipid-binding proteins [Zimmerman and Veerkamp, 1998], play crucial roles in intracellular fatty acid transport by binding, targeting and transporting long-chain fatty acids to their correct metabolic sites [Chmurzynska, 2006]. Numerous functions have been proposed, including cellular uptake and transport of fatty acids (FAs), targeting of FAs to specific metabolic pathways, participation in gene expression regulation and cell growth [Haunerland and Spener, 2004; Szczerbal et al., 2007]. At this point in time, nine tissue-specific cytoplasmic FABPs have been identified and their tertiary structures have proven to be highly conserved [Chmurzynska, 2006; Furuhashi and Hotamisligil, 2008; Storch and Corsico, 2008]. Among these, FABP3, also known as heart FABP (H-FABP), is a major cytosolic fatty acid-binding factor found initially in heart and skeletal muscle. FABP3 is involved in fatty acid uptake and metabolism in these muscles as demonstrated by studies with FABP3-deficient mice [Erol et al., 2004; Murphy et al., 2004; Binas, 2005]. FABP3 has also been isolated from a wide range of additional tissues including brain, renal cortex, lung, testis, aorta, adrenal gland, mammary gland, placenta and ovary [Chmurzynska, 2006]. FABP3 has also been recently identified in adipose tissue [Chen et al., 2006] and in porcine subcutaneous and intramuscular adipocytes [Gardan et al., 2007; Li et al., 2007].

FABP3 is generally considered to be involved in the regulation of fatty acid utilization in muscle [Glatz et al., 2003]. However, a recent report suggests an additional regulatory role for FABP3 during adipogenesis of stromal-vascular cells derived from porcine adipose tissue [Li et al., 2007]. FABP3 gene polymorphisms affect intramuscular fat content and backfat thickness in pigs [Gerbens et al., 1999]. Furthermore, FABP3 has been shown to exhibit a parallel pattern of upregulation similar to that of another fatty acid-binding protein, aP2, during adipose tissue development [Li et al., 2007]. Similar to ap2, the moderate expression and upregulation of FABP3 during in vivo adipose tissue development and in vitro adipogenic differentiation indicate that FABP3 may play an important role in adipocyte function [Li et al., 2007]. However, the mechanism underlying the regulation of FABP3 in adipocytes remains poorly understood.

Sirt1, a NAD-dependent histone deacetylase, has been correlated with cell proliferation and differentiation [Langley et al., 2002], apoptosis [Motta et al., 2004; Kitamura et al., 2005], and metabolism [Picard et al., 2004; Rodgers et al., 2005]. Recent studies have shown that Sirt1 may play a key modulatory role in animal fat metabolism and muscle development [Picard and Guarente, 2005; Rodgers, 2005]. Sirt1 is involved in adipogenesis and appears to regulate lipid metabolism and ap2 gene expression in adipocytes by repressing

Abbreviations used: DMEM/F12, Dulbecco's modified eagle medium/HamF12; NAM, nicotinamide; PPARγ, peroxisome proliferator-activated receptor γ; RES, resveratrol; RNAi, RNA interference; siRNA, small interfering RNA.

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PPARγ [Picard et al., 2004; Picard and Guarente, 2005]. However, whether Sirt1 is involved in the regulation of FABP3 gene expression in adipocytes is unclear.

Therefore, in the current study, we treated porcine adipocytes with the general Sirt1 inhibitor nicotinamide (NAM), by knockdown of Sirt1 by Sirt1-specific siRNA or with the Sirt1 activator resveratrol (RES) to examine whether Sirt1 plays any role in regulating FABP3 gene expression in porcine adipocytes. In addition, the roles of PPARγ in the regulation process of FABP3 by Sirt1 were also studied by knockdown of PPARγ with PPARγ-specific siRNA. This work has the potential to increase our understanding of the role of Sirt1 and FABP3 in adipocytes fat metabolism and their potential as targets for controlling fat storage and obesity.

MATERIALS AND METHODS

ANIMALS AND CELL CULTURE

All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. Pigs from 5 to 7 d of age were overdosed with sodium thiopental and exsanguinated. Subcutaneous adipose tissue was removed and the porcine preadipocytes were prepared by previously published methods [Shan et al., 2008]. Briefly, adipose tissue was cut with scissors into approximately 1 mm sections under sterile condition and digested with collagenase type I for 60 min, at 37°C in a shaking water bath. The solution was then filtered and the preadipocytes were isolated. The cells were washed with Dubelco's modified Eagle medium/HamF12 (DMEM/F12) (Gibco Laboratories, Grand Island, NY, USA), centrifuged and resuspended in plating medium (DMEM/F12 + 10% FBS), stained with Rappaports stain and counted on a hemocytometer. The porcine preadipocytes were seeded on 6-well (35-mm) tissue culture plates at a density of 2×10^4 cells/cm^2 in complete media (DMEM/F12 + 10% FBS + 100 U penicillin + 100 U streptomycin) and cultured at 37°C under a humidified atmosphere of 95% air and 5% CO_2. Media were changed every 3 d. After 10 d of lipid filling when the adipocytes achieved about fully differentiated, cultures were washed with DMEM/F12, and then used for NAM or RES treatment experiments. For nicotinamide (NAM) experiments, porcine adipocytes were incubated on d 10 in DMEM/F12 (without FBS and phenol red) with 50 and 100 μM of NAM for 48 h (analyzed on 12 d) for dose–response experiments, and the optimal concentration of 100 μM of NAM for 24 h (analyzed on 11 d) and 48 h (analyzed on 12 d) for time-response experiments. For resveratrol (RES) experiments, porcine adipocytes were incubated on d 10 in DMEM/F12 (without FBS and phenol red) with the optimal concentration of 50 μM of RES for 48 h (analyzed on 12 d). Gene expression was determined by real-time quantitative PCR.

RNA INTERFERENCE (RNAi)

Based on our previous cloned complete sequence of porcine Sirt1 (GenBank Accession No. EU030283), 4 potential small interference (siRNA) target sites were determined using the Qiagen small interfering RNA (siRNA) design program. These were confirmed by BLAST for specificity. Oligonucleotides to produce plasmid-based siRNA were cloned into pSilencer^{TM} 4.1-CMV neo plasmids (Ambion, Austin, TX, USA) and all constructs were confirmed by sequencing. The most effective target sequence (AAGAGATGG-CATTTATGCACG) of porcine Sirt1 for RNAi (Sirt1-siRNA) was screened out and the RNAi conditions were optimized. Transfections were performed on d 2 using Lipofectamine^{TM} 2000 (Invitrogen) according to the manufacturer's protocol. Briefly, 1 d before transfection, cells were plated in 2 mL of growth medium without antibiotics to yield 90% confluent cells at the time of transfection. Negative control siRNA (Neg.-siRNA) was supplied by Ambionn and the control group was transfected with the empty plasmid. For RES and Sirt1-siRNA synergistic regulation of FABP3, adipocytes were treated with 50 μM RES in conjunction with transfection with Sirt1-siRNA (RES + Sirt1-siRNA group) or 50 μM RES (RES group) on d 2. In addition, to verify the roles of PPARγ in the regulation process of FABP3 by Sirt1, the PPARγ specific-siRNA (PPARγ-siRNA) was also constructed and the RNAi conditions were optimized as the above description. The control group was transfected with the empty plasmid. For NAM or RES treatments, adipocytes were treated with 100 μM NAM or 50 μM RES. For NAM/RES and PPARγ-siRNA synergistic regulation, cells were treated with 100 μM NAM or 50 μM RES in conjunction with transfection with PPARγ-siRNA (NAM/RES + PPARγ-siRNA group) on d 2. Gene expression was determined 48 h (on 4 d) after transfection.

TOTAL RNA EXTRACTION AND REVERSE TRANSCRIPTION

Cells were collected and total RNA was isolated from the collected cells using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. All RNA was treated with RNase-free DNase I (Takara Biotechnology Co. Ltd.) to remove contaminating genomic DNA. The purity and concentration of total RNA were measured with a spectrophotometer at 260 nm and 280 nm. Ratios of absorption (260/280 nm) of all samples were between 1.8 and 2.0. For each sample, cDNA was synthesized from 2 μg of total RNA using a MMLV-RT kit with random hexamer primers and an RNase inhibitor (Takara Biotechnology Co. Ltd.) according to previous studies [Shan et al., 2008].

QUANTITATIVE REAL-TIME PCR

Quantitative real-time PCR was carried out in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, Calif., USA) with a SYBR Premix Ex Taq Kit (Takara Biotechnology Co. Ltd.) and gene-specific primers 18S rRNA was used as the reference gene. Primer sequences are available upon request. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative changes in each gene expression [Livak and Schmittgen, 2001]. Briefly, 1 μL of cDNA template was added to each well in a 96-well reaction plate, and the transcripts of each gene were amplified in triplicate. Average CT values were calculated, and the ΔCT values relative to the 18S rRNA control were computed for each gene. Subsequently, ΔΔCT was computed for each gene by subtracting the average ΔCT for the control group. The final fold differences were computed as $2^{-\Delta\Delta CT}$ for each gene. These measurements were repeated three times.

DATA ANALYSIS

All experimental data are presented as means ± S.E.M. Comparisons were made by unpaired two-tailed Student's *t*-tests or one-way

ANOVA, as appropriate. Effects were considered significant at $P < 0.05$.

RESULTS

Sirt1 INHIBITOR (NAM) UPREGULATION OF FABP3 GENE EXPRESSION

To address the question whether Sirt1 could modulate the gene expression of FABP3, we first treated with the Sirt1 inhibitor (NAM). Treatment with different doses of NAM significantly decreased ($P < 0.05$) the levels of Sirt1 mRNA (Fig. 1A). Compared with the control, 50 or 100 μM NAM administration decreased the Sirt1 mRNA levels by 10.76% ($P < 0.05$) and 36.05% ($P < 0.01$) (Fig. 1A), while it increased the mRNA levels of FABP3 by 48.59% ($P < 0.01$) and 146.82% ($P < 0.01$) (Fig. 1B), respectively. Furthermore, treatment with 100 μM NAM for 24 or 48 h significantly decreased ($P < 0.01$) the mRNA expression of Sirt1 (Fig. 2A), while it significantly increased ($P < 0.01$) the mRNA levels of FABP3 (Fig. 2B). This finding suggested that Sirt1 may regulate the expression of FABP3 in cultured porcine adipocytes.

siRNA-MEDIATED KNOCKDOWN OF Sirt1 INCREASED FABP3 GENE EXPRESSION

To confirm the regulation of Sirt1 on the FABP3 gene expression in adipocytes, we treated porcine adipocytes with Sirt1-specific siRNA (Sirt1-siRNA). Compared with the control, transfection of the porcine adipocytes with Sirt1-siRNA for 48 h resulted in a 49.70% ($P < 0.01$) knockdown of porcine Sirt1 mRNA (Fig. 3A). Conversely, the mRNA level of porcine FABP3 was increased by 37.01% ($P < 0.01$) (Fig. 3B). These results demonstrated that Sirt1 downregulates FABP3 gene expression in cultured adipocytes.

Sirt1 ACTIVATOR (RES) DOWNREGULATION OF FABP3 GENE EXPRESSION

To further confirm the role of Sirt1 on the regulation of FABP3 transcription in adipocytes, the effects of the Sirt1 activator RES on the expression of the Sirt1 and FABP3 genes were studied. The results showed that exposure of cultured adipocytes to 50 μM RES significantly increased ($P < 0.01$) the mRNA levels of Sirt1 (Fig. 4A) while significantly decreasing decreased ($P < 0.01$) the gene expression of FABP3 (Fig. 4B). These results suggested that RES stimulates the gene expression of the Sirt1, but downregulates the FABP3 mRNA expression in porcine adipocytes.

RES AND Sirt1-siRNA SYNERGISTIC REGULATION OF FABP3 GENE EXPRESSION

The Sirt1 activator RES and Sirt1-siRNA synergistic regulation results showed that RES treatment upregulated Sirt1 gene expression (48.34%, $P < 0.01$) (Fig. 5A), but decreased ($P < 0.01$) FABP3 gene expression (Fig. 5B). Compared with RES treatment, RES + Sirt1-siRNA treatment reduced Sirt1 gene expression (28.48%, $P < 0.01$) (Fig. 5A), while increased FABP3 gene expression (Fig. 5B). These findings provided further evidence for the negative modulation of FABP3 in porcine adipocytes by Sirt1. However, the dramatic increase of FABP3 expression by RES and Sirt1-siRNA synergistic regulation will be interesting in the future.

PPARγ PLAYS AN IMPORTANT ROLE IN THE REGULATION OF FABP3

As Sirt1 was demonstrated to modulate FABP3 in adipocytes, we next sought to determine whether the PPARγ plays any role in this process. Treatment with 50 or 100 μM NAM significantly increased the mRNA levels of PPARγ by 134.65% ($P < 0.01$) and 187.96% ($P < 0.01$), respectively (Fig. 6A). Treatment with 100 μM NAM for 24 or 48 h significantly increased ($P < 0.01$) the mRNA levels of PPARγ (Fig. 6B). Furthermore, knockdown of Sirt1 with Sirt1-siRNA, significantly increased ($P < 0.01$) the mRNA levels of PPARγ (Fig. 6C). Conversely, treating cells with the Sirt1 agonist RES significantly suppressed the PPARγ mRNA expression (Fig. 6D). In addition, synergistic regulation by RES and Sirt1-siRNA caused a significant increase in PPARγ gene expression in the RES + Sirt1-siRNA group as compared with the control or the RES group (Fig. 6E). Furthermore, PPARγ exhibited a parallel pattern of regulation similar to that of seen for FABP3 in the current study.

To verify the role of PPARγ in this process, we treated cells with RES or NAM after knockdown of PPARγ with its specific-siRNA (PPARγ-siRNA). The results showed that NAM treatment increased ($P < 0.01$) PPARγ (Fig. 7A) and FABP3 (Fig. 7B) gene expression, while RES treatment, PPARγ-siRNA treatment, and NAM/

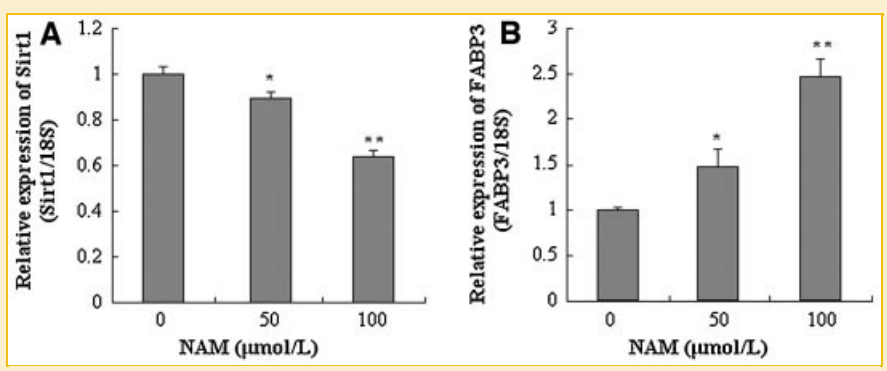

Fig. 1. Effect of various concentrations of the Sirt1 inhibitor NAM on the gene expression of Sirt1 (A) and FABP3 (B) in porcine adipocytes. Gene expression was determined by real-time quantitative PCR and was normalized to 18S ribosomal RNA and expressed relative to gene expression in the control group (0 μmol). Each column represents the means ± S.E.M. from three replicates. $^{*}P < 0.05$, $^{**}P < 0.01$.

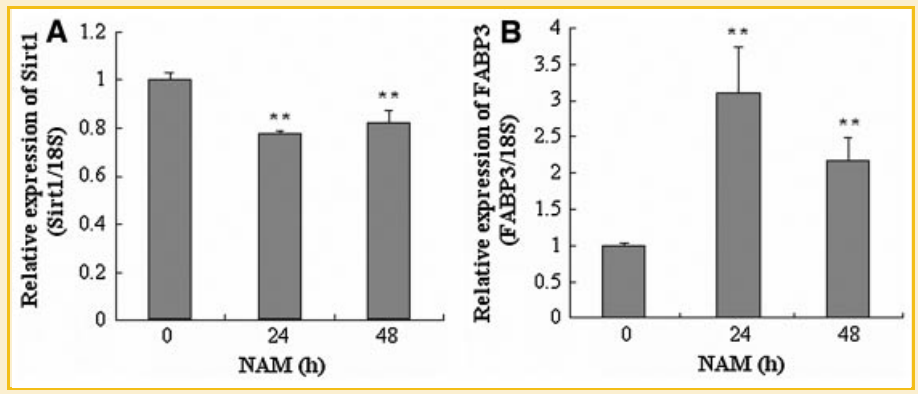

Fig. 2. Time course for the expression of Sirt1 (A) and FABP3 (B) genes were generated by incubating porcine adipocytes with 100 μmol NAM for the time indicated. Gene expression is presented as in Fig. 1.

RES + PPARγ-siRNA treatment significantly lowered ($P < 0.01$) PPARγ and FABP3 gene expression (Fig. 7) compared with the control group. NAM + PPARγ-siRNA treatment reduced the gene expression of PPARγ (Fig. 7A) and FABP3 (Fig. 7B) compared with the NAM treatment. Likewise, RES + PPARγ-siRNA treatment reduced the gene expression of PPARγ (Fig. 7C) and FABP3 (Fig. 7D) compared with the RES treatment. There were no significant difference ($P > 0.05$) between the NAM + PPARγ-siRNA treatment and PPARγ-siRNA treatment. However, RES + PPARγ-siRNA treatment decreased PPARγ (Fig. 7C) and FABP3 (Fig. 7D) gene expression ($P < 0.01$) compared with PPARγ-siRNA treatment.

DISCUSSION

This study investigated the regulatory role of Sirt1 on gene expression of FABP3 in cultured porcine adipocytes. Sirt1 was inhibited by the inhibitor NAM, knocked down by Sirt1-siRNA or activated by the agonist RES. Conversely, the mRNA levels of FABP3 significantly increased or decreased after incubation of porcine adipocytes with Sirt1 inhibitor NAM and decreased after incubation with the activator RES, and was significantly increased in Sirt1-siRNA transfected knockdown cells. Together, these results demonstrated that Sirt1 could downregulate FABP3 gene expression in porcine adipocytes, suggesting that FABP3 and Sirt1 are directly involved in adipocyte function.

FABPs are abundant intracellular proteins that play important roles in intracellular fatty acid transport, regulation of gene expression and cell growth [Haunerland and Spener, 2004; Chmurzynska, 2006; Szczerbal et al., 2007]. The FABPs have unique tissue-specific distributions and many FABPs are prominently expressed in a single tissue or cell type. However, FABP3 displays a broad tissue distribution [Storch and Corsico, 2008]; it is highly expressed in heart and skeletal muscle, with lower expression seen in brain, renal cortex, lung, testis, aorta, adrenal gland, mammary gland, placenta and ovary [Chmurzynska, 2006]. Recent studies have demonstrated that FABP3 also expressed in adipose tissue [Chen et al., 2006] and in adipocytes [Gardan et al., 2007; Qiang et al., 2007], suggesting that FABP3 influences adipose tissue and adipocyte function. However, few data are available concerning the expression and regulation of FABP3 in adipocytes.

In this context, the findings of the current study are particularly relevant. Nicotinamide, a strong noncompetitive inhibitor of Sirt1 in vivo and in vitro [Bitterman et al., 2002; Jackson et al., 2003], inhibited Sirt1 activity [Kim et al., 2007] or decreased the expression

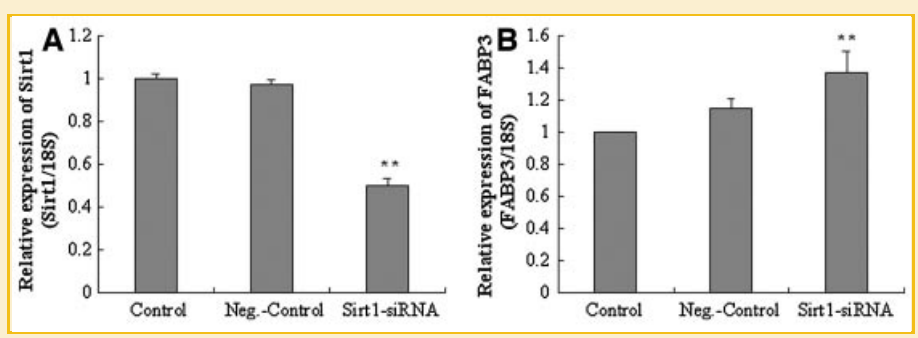

Fig. 3. Knockdown of Sirt1 in porcine adipocytes. siRNA-mediated knockdown of Sirt1 was achieved by transfecting porcine adipocytes with Negative control siRNA (Neg.-siRNA group), empty plasmid (Control) or Sirt1-specific siRNA. The mRNA expression of Sirt1 (A) and FABP3 (B) were determined 48 h after transfection. Gene expression is presented as in Fig. 1.

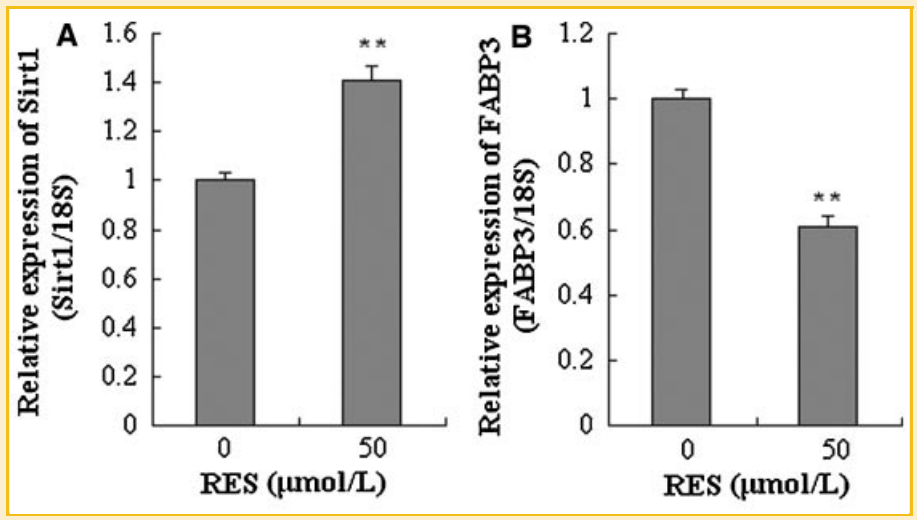

Fig. 4. Effect of the Sirt1 agonist RES on the expression of Sirt1 (A) and FABP3 (B) genes in porcine adipocytes. Gene expression is presented as in Fig. 1.

of Sirt1 mRNA and greatly stimulated the proliferation and differentiation [Bai et al., 2008]. In the current study, we found that NAM inhibited Sirt1 gene expression in cultured adipocytes confirming the well-established actions of NAM on Sirt1 [Bitterman et al., 2002; Jackson et al., 2003]. In addition, however, FABP3 mRNA expression was increased by nicotinamide, while glycerol release was decreased (data not shown) suggesting that Sirt1 may regulate the expression of FABP3, and therefore fat metabolism, in porcine adipocytes.

The RNA interference (RNAi) experiments performed in the present study also provide useful information. RNAi is an evolutionary conserved posttranscriptional mechanism of gene silencing induced by sequence-specific double-stranded RNA [Fire et al., 1998] and has found extensive use in functional genomics. It also offers innovative approaches for the development of novel therapeutics [Xu et al., 2006]. Gene knockdown of Sirt1 by siRNA resulted in a significant inhibition of Sirt1 mRNA and decreased the mRNA abundance of FABP3 in cultured adipocytes. It is well known that interference with gene expression by small interfering RNA (siRNA) or short hairpin RNA (shRNA) is an important biological strategy for gene silencing in multiple organisms [Xia et al., 2002], as siRNA, triggers RNAi processing resulting in specific endonucleolytic cleavage of mRNA [Bernstein et al., 2001; Elbashir et al., 2001]. These results further demonstrated that Sirt1 could negatively regulate the expression of FABP3.

Treatment of porcine adipocytes with the general Sirt1 activator RES also significantly increased the mRNA levels of Sirt1 while significantly decreasing the mRNA levels of FABP3 in cultured cells. RES, an activator of Sirt1 [Picard et al., 2004; Backesjo et al., 2006], has various pharmacological effects including protection of cells from lipid accumulation, chemoprevention, immunomodulation, antiproliferation and promotion of differentiation [Pervaiz, 2003]. Previous studies have shown that RES reduces blood TG content and stimulates free fatty acid (FFA) release, lowers blood fat, and inhibits adipocyte differentiation and fat accumulation by activation of Sirt1 [Picard et al., 2004; Kim et al., 2008]. Based on the previous reports and the results of this study, Sirt1 appears to downregulate porcine FABP3 gene expression in porcine adipocytes, a supposition further supported by the results of the synergistic siRNA/RES findings reported here. Taken together, these findings suggested that Sirt1 promotes fat mobilization partly through negative regulation of FABP3 gene expression in adipocytes.

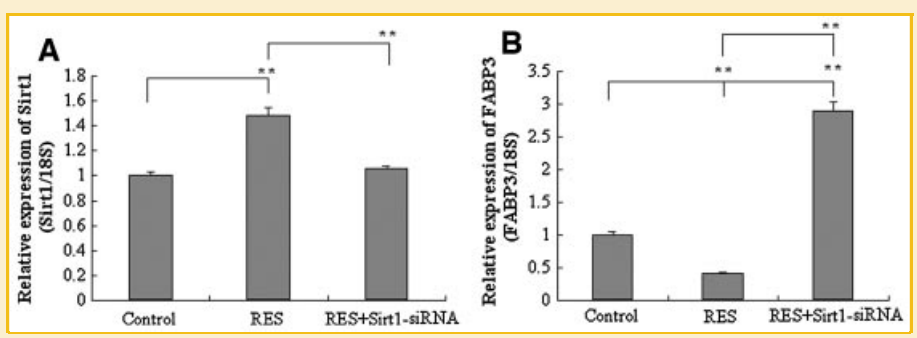

Fig. 5. RES and Sirt1-siRNA synergistic regulation of the gene expression of Sirt1 (A) and FABP3 (B). Gene expression data are presented as in Fig. 1.

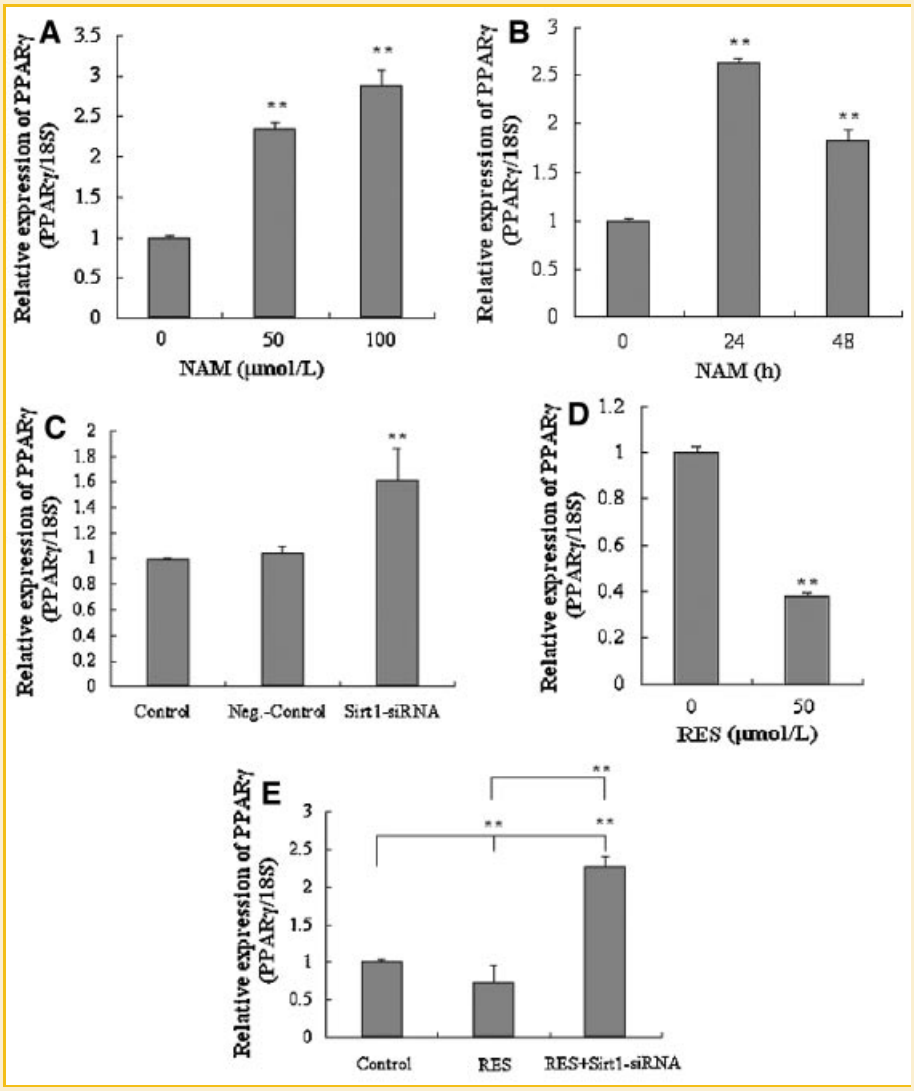

Fig. 6. Effect of NAM, RES and Sirt1-specific siRNA on the expression of PPARγ gene in porcine adipocytes. The effects of NAM (A and B), RES (C), Sirt1-specific siRNA (D) and RES + Sirt1-siRNA (E) on the PPARγ gene expression were determined by real-time PCR. Each column represents the means ± S.E.M. from three replicates. $^{*}P < 0.05$, $^{**}P < 0.01$.

Sirt1, an ortholog of Sir2 in mammals, controls the metabolism of white adipose tissue [Wolf, 2006] and plays an important role in mediating the response of adipocytes to perturbations in overall metabolism [Qiang et al., 2007]. Sirt1 has been shown to inhibit insulin signaling in fat, thereby reducing adipocyte differentiation and increasing FFA release, potentially by suppressing expression of the fatty acid-binding protein aP2 [Picard et al., 2004]. Furthermore, the activation of Sirt1 by its activator RES or overexpression of Sirt1 both reduced the expression of aP2 [Picard et al., 2004; Wolf, 2006; Bai et al., 2008]. In addition, knockdown or inhibition of the Sirt1 increased the expression of the fatty acid-binding protein aP2, suggesting that Sirt1 could regulate the expression of ap2 in adipocytes [Qiang et al., 2007].

Recent studies have shown that FABP3 is also expressed in adipose tissues and adipocytes at a moderate level (more than 10% of that of aP2), and exhibits a parallel pattern of upregulation similar to that of aP2 during adipose tissue development [Qiang et al., 2007]. This suggests that FABP3 may play an important role in adipose tissue fat metabolism, and could be regulate by Sirt1 in a similar fashion to that of aP2. Our current study proved that Sirt1 could negatively regulate the gene expression of FABP3 in cultured adipocytes. However, the regulation mechanism is as yet unclear.

The peroxisome proliferator-activated receptor gamma (PPARγ), a key element for the adipocyte differentiation, is a nuclear receptor that is regarded as a master regulator of adipogenesis and metabolic homeostasis [Knouff and Auwerx, 2004]. Recent studies have shown that FABP3, aP2 and LPL are response genes of PPARγ and are indicators for in vivo PPARγ activation in adipose tissue [Yang et al., 2004]. PPARγ levels have already been demonstrated to correlate with FABP3 in human muscle [Schmitt et al., 2003]. In human mesenchymal cells transfected with PPARγ-siRNA, FABP3 expression was inhibited [Xu et al., 2006]. Lycopene intake also significantly decreased the expression of PPARγ and its target gene, FABP3, in the adrenal glands and kidney [Zaripheh et al.,

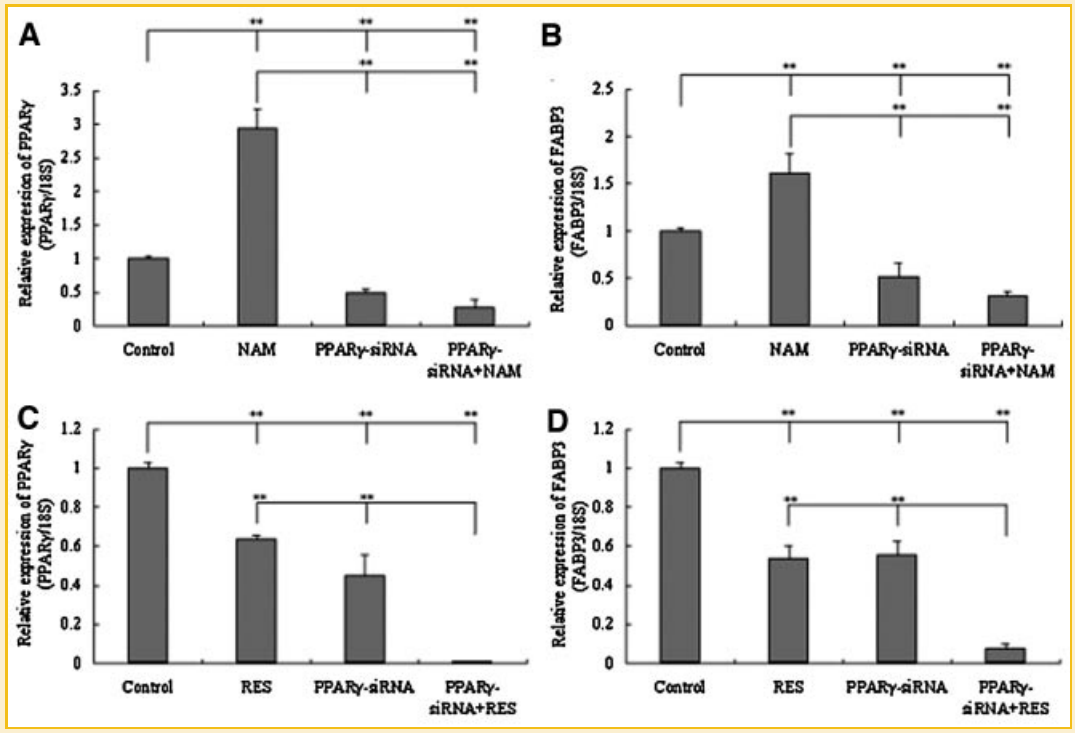

Fig. 7. NAM/RES and Sirt1–siRNA synergistic regulation of the gene expression of PPARγ (A and C) and FABP3 (B and D). Gene expression data are presented as in Fig. 1.

2006]. FABP3 showed highly correlated expression patterns with porcine adipogenesis as well as expression patterns of PPARγ [Samulin et al., 2008]. Therefore, FABP3 expression, a marker of adipogenesis, was also apparently regulated by PPARγ [Xu et al., 2006]. A previous study has shown that Sirt1 promotes fat mobilization by repressing PPARγ, and potentially by suppressing expression of aP2 in adipocytes [Picard et al., 2004]. FABP3, a protein highly homologous to FABP4, exhibited a parallel pattern of upregulation similar to that of aP2 during adipose tissue development [Li et al., 2007].

Based on previous reports and on our current study, we hypothesized that the regulation of FABP3 by Sirt1 may be raised through the PPARγ signals in adipocytes. In the current study, we found that treatment of porcine adipocytes with the Sirt1 inhibitor NAM, with the activator RES or by knockdown Sirt1 with the Sirt1-specific siRNA had similar regulatory effects on the expression of PPARγ as were seen for FABP3 in adipocytes. In addition, NAM/RES + PPARγ-siRNA treatment reduced the gene expression of PPARγ and FABP3 compared with the NAM or RES treatment. Furthermore, there were not significant difference ($P > 0.05$) between the NAM + PPARγ-siRNA treatment and PPARγ-siRNA treatment. This suggests that PPARγ regulates FABP3 expression in adipocytes and, combined with previous reports, indicates that the action of Sirt1 on FABP3 regulation may occur partly through repression of the action of PPARγ in adipocytes. However, RES + PPARγ-siRNA treatment decreased PPARγ and FABP3 gene expression compared with PPARγ-siRNA treatment. Therefore, the synergistic regulation RES and PPARγ-siRNA will be interesting and need further studies in the future.

Previous studies have shown that PPARs regulate gene transcription by heterodimerising with the 9-cis-RXR and binding to a peroxisome proliferator responsive element (PPRE). PPREs in turn have been found in the promoter region of the FABP3 gene [Schachtrup et al., 2004; Kawabe et al., 2005]. FAPBs and PPARs can also interact through direct protein–protein interactions, either in ligand-dependent or in a ligand-independent manner to activate the transcription of PPAR target genes [Adida and Spener, 2006]. In the current study, we showed that PPARγ plays an important role in regulation process of FABP3 by Sirt1. However, recent study has suggested that PPARα expression has also been correlated to FABP3 expression (Samulin et al., 2008). Therefore, the exact mechanisms for the regulation of FABP3 by Sirt1 in the adipocytes are as yet unclear and require more in-depth investigation.

In conclusion, Sirt1 was able to downregulate the gene expression of FABP3 in adipocytes and the PPARγ signal pathway appeared to play an important role in this process. These results reveal new insights for the manipulation of Sirt1 and FABP3 expression in terms of regulating lipid metabolism. However, additional studies are required to further characterize the role of Sirt1 and FABP3 in adipose tissues and to assess their potential contribution to disorders such as obesity and diabetes.

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