Visfatin regulates genes related to lipid metabolism in porcine adipocytes


*Department of Animal Science and Technology, National Taiwan University, Taipei 106, Taiwan, ROC;
†Livestock Research Institute, Council of Agriculture, Executive Yuan, Tainan 712, Taiwan, ROC;
and ‡Department of Animal Science and Biotechnology, Tunghai University, Taichung 407, Taiwan, ROC

ABSTRACT: Visfatin is a visceral adipose tissue-specific adipocytokine that plays a positive role in attenuating insulin resistance by binding to the insulin receptor. Visfatin has been suggested to play a role in the regulation of lipid metabolism and inflammation; however, the mechanism remains unclear. We investigated the effects of visfatin on the regulation of gene expression in cultured porcine preadipocytes and differentiated adipocytes. In preadipocytes, the mRNA abundance of lipoprotein lipase and PPARγ were significantly increased by visfatin or insulin treatment after 8 d (all \( P < 0.05 \)). In the presence of insulin, the mRNA abundance of adipocyte fatty acid-binding protein was 24.7-fold greater than in the untreated group (\( P < 0.05 \)), whereas visfatin alone had no effect on adipocyte fatty acid-binding protein mRNA abundance. Adipocyte differentiation was induced by insulin treatment for 8 d. In differentiated porcine adipocytes, exposure to insulin or visfatin for 24 h increased \( P < 0.05 \) fatty acid synthase mRNA abundance but had no effect on the expression of sterol regulatory element binding-protein 1c mRNA. We also found a 5.8-fold upregulation of IL-6 expression in porcine adipocytes after 24 h of treatment with visfatin (\( P < 0.05 \)). These results demonstrated that visfatin upregulated lipid protein lipase expression in preadipocytes, potentially facilitating lipid uptake, and increased the gene expression of fatty acid synthase in differentiated adipocytes to potentially enhance lipogenic activity. Furthermore, visfatin can upregulate IL-6 expression in differentiated porcine adipocytes. The information presented in this study provides insights into the roles of visfatin in lipid metabolism in pigs.

Key words: adipocyte, lipid metabolism, visfatin

INTRODUCTION

Adipose tissue is an important endocrine organ that expresses and secretes more than 50 different bioactive peptides (Trayhurn and Wood, 2004), referred to as adipokines. These adipokines appear to be involved in a broad range of physiological or physiopathological processes, including lipid metabolism, insulin sensitivity, blood pressure regulation, energy balance, and angiogenesis (Lago et al., 2009).

Visfatin was originally identified as the pre-B-cell colony-enhancing factor 1, and its expression was correlated with obesity. Visfatin participates in the differentiation of pre-B cells (Samal et al., 1994). In mammalian cells, pre-B-cell colony-enhancing factor 1 functions as a nicotinamide phosphoribosyltransferase, a rate-limiting enzyme for the biosynthesis of NAD, and therefore influences cellular differentiation and metabolic responses (Wang et al., 2009).

Pre-B-cell colony-enhancing factor 1 was renamed visfatin to distinguish its exclusive expression in visceral adipose tissue (VAT). It plays a positive role in attenuating insulin resistance and facilitates adiogenesis (Sethi and Vidal-Puig, 2005; Chen et al., 2006). Visfatin exerts insulin mimetic properties; however, it does not bind to the same region of the receptor as insulin (Chen et al., 2006). As an autocrine/paracrine signaling factor, visfatin facilitates the differentiation of adipose tissue through its pro-adiogenic and lipogenic actions (Sethi and Vidal-Puig, 2005).

The underlying cellular mechanism by which visfatin regulates adipogenic differentiation and lipid accumula-
tion in adipose tissue is not well understood. Accordingly, the aim of the present study was to explore the relationship between visfatin and lipid metabolism-associated genes by the analysis of mRNA profiles after treatment with recombinant porcine visfatin.

MATERIALS AND METHODS

The procedures for the animal portions of this study were approved by the Institutional Animal Care and Use Committee of National Taiwan University.

Construction of Recombinant Visfatin Plasmid

Forward (5′-TCCGGGATCCCGATGAATGCT-3′) and reverse (5′-ACGCAGCTTTACACACACC-3′) primers for the visfatin gene were designed according to the published porcine sequence (GenBank accession No. DQ020218). The primers included recognition sites for the restriction enzymes, BamHI and HindIII (restriction sites underlined in primer sequences). We used the AccuPrime Pfu DNA Polymerase Kit (Invitrogen, Carlsbad, CA) for PCR. Conditions for the thermal cycling were as follows: 95°C for 3 min, 30 cycles of 95°C for 30 s, 55°C for 30 s, 68°C for 90 s, and a final extension at 68°C for 10 min. The amplified product was purified and then inserted into the pCR-Blunt II-TOPO (Invitrogen) vector. To create an in-frame fusion protein with a N-terminal 6×His-tag, the visfatin DNA fragment was digested with BamHI and HindIII restriction enzymes (Takara, Shiga, Japan) and ligated into the pQE31 (Qiagen, Hilden, Germany) vector. The recombinant plasmid pQE31-visfatin was transformed into BL21(DE3) competent cells (Novagen, Madison, WI) for expression. Its authenticity was confirmed by sequencing.

Expression and Purification of Recombinant Visfatin

Protein expression was induced by 1 mM isopropyl-thiogalactopyranoside. Recombinant His-tagged visfatin was purified using the Pro bond Purification System (Invitrogen) according to the manufacturer’s protocol under native conditions. In brief, isopropyl-thiogalactopyranoside-induced cells were harvested, resuspended in a native binding buffer (50 mM NaH_2PO_4, 500 mM NaCl, pH 7.8), and sonicated for 10 s and repeated 15 times. The sonicated-cell extracts were subjected to centrifugation at 800 × g for 5 min at 37°C, and the supernatant was loaded on a nickelcharged affinity column (Invitrogen). This column was washed twice with the wash buffers (20 mM imidazole, 50 mM NaH_2PO_4, 500 mM NaCl at pH 7.8, 6.0, and 5.3, respectively). The visfatin-His fusion protein was eluted with an elution buffer (250 mM imidazole, 50 mM NaH_2PO_4, 500 mM NaCl, pH 4.0). Purity was verified by Coomassie staining of SDS-PAGE gels. The eluate was concentrated using a centrifugal filter device with a 30 kDa cut-off (Millipore, Bedford, MA) and then filtered through an Ultrafree-MC 0.22-µm filter (Millipore) at 4,000 × g for 5 min to eliminate dust, microcrystals, and precipitated protein.

Generation of Monoclonal Antibody

Mice (BALB/c) were purchased from the Animal Center of the College of Medicine at the National Taiwan University. Immunization of the mice and cell fusion were carried out by a standard procedure which was described in Chen et al. (2002) and modified in the following manner. Briefly, six 4-wk-old BALB/c female mice were immunized intraperitoneally with an emulsion of Freund’s complete adjuvant (Sigma-Aldrich, St. Louis, MO) containing 100 µg of purified recombinant visfatin. Four or five booster shots of 50 µg of purified recombinant visfatin in Freund’s incomplete adjuvant (Sigma-Aldrich) were given every 2 wk. A final dose was administered 3 d before fusion. Cell fusion was induced by mixing 1.0 × 10^8 spleen cells from the immunized mice with the myeloma cell line SP2/0-Ag14 (2.0 × 10^7 cells). The selection of positive hybridomas was carried out using an ELISA procedure, and they were subcloned by the limiting dilution method. For the large-scale production of visfatin monoclonal antibody, the ascites fluid was prepared by injecting 1.0 × 10^7 hybridoma cells into BALB/c female mice primed with prestatin (Sigma-Aldrich). To determine the titer and specificity of the antibody, a Western blot analysis was performed. Purified immunoglobulins were stored at −80°C.

Western Blot Analysis of Porcine Visfatin

Electrophoresis was performed using protocols according to the method described previously (Laemmli, 1970) with 12% SDS-PAGE gels at 80 V for 90 min. Subsequent immunoblottings were performed using the visfatin monoclonal antibody (1:2,000) prepared from the culture supernate of hybridoma cells. Protein was electro-transferred from the gel onto a Polyscreen PVDF membrane (PerkinElmer, Boston, MA). The membrane was blocked with 5% BSA in TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20) before incubation with the primary antibody at an appropriate dilution at 4°C overnight. After washing with 0.05% TBST, the membrane was incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000; Invitrogen) antibody for 2 h at room temperature. Bound peroxidase-conjugate was detected using the enhanced chemiluminescence system (Millipore) and Hyperfilm ECL photographic film (GE Healthcare, Munich, Germany) following the manufacturer’s instructions.
Isolation of Porcine Stromal Vascular Cells and Culture System

Two-week-old Landrace piglets were obtained from a commercial producer and killed by electrocution combined with exsanguination. Stromal vascular (S/V) cells were isolated and cultured followed the procedure described previously (Wang et al., 2006) and modified by Chen et al. (2008). In the preadipocyte experiment, the medium on confluent S/V cells was replaced with 100 ng/mL of visfatin- or 100 ng/mL of insulin-containing preadipocyte growth medium. For the adipocyte experiment, approximately 50% of the adipocytes were well differentiated; the medium was changed to the 100 ng/mL of visfatin- or 100 ng/mL of insulin-containing Dulbecco’s modified Eagle/F12 medium. Cells were used for transcript analysis or stained with Oil Red-O (Sigma-Aldrich) according to a previously described protocol (Ding et al., 2002) to evaluate the degree of adipocyte differentiation.

Reverse Transcription Real-Time PCR Analyses

Total RNA was extracted from washed preadipocytes and adipocytes using the TRI reagent (Ambion, Austin, TX), according to the manufacturer’s protocol. The quantity of extracted RNA was measured at OD260 and OD280. The quality of RNA was determined by examination of the ethidium bromide-stained 18S and 28S ribosomal RNA bands after electrophoresis under denaturing condition. Reverse transcription was performed using 2 µg of total RNA with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA), according to the manufacturer’s protocol. Amplification reactions were performed using the Fast-Start SYBR Green Master Mix kit (Roche, Mannheim, Germany) and a DNA Engine Opticon 2 Real-Time PCR Detection System (Bio-Rad Laboratories, Richmond, CA) according to the instruction manual, but the reactions were scaled down to 10 µL. Each reaction, containing 10 ng of template, was performed in duplicate, and the abundance of β-actin mRNA was used as the internal control. The primer sequences used are described in Table 1. The following thermal cycling conditions for amplification were used: 94°C for 10 min, 40 cycles of 94°C for 30 s, then 60°C for 30 s, and then 72°C for 30 s with a final extension at 72°C for 7 min. Cycle threshold (CT) values were determined with Opticon Monitor 3 software (Bio-Rad Laboratories). Relative differences for a gene under treatment with the recombinant visfatin, insulin, or both were determined using the comparative cycle threshold (ΔΔCT) method (Livak and Schmittgen, 2001). The resulting values were converted to fold-changes compared with the control by 2−ΔΔCT.

Statistical Analyses

Data were presented as the mean ± SEM. Data represent 3 replicates, each using cells isolated from a different pig. For each replicate, the control value for a variable was set to 1.0. A factorial ANOVA (SAS Inst. Inc., Cary, NC) was performed to determine the effect of recombinant visfatin or insulin stimulation. Duncan’s multiple range test was used to evaluate the different response values between treatment groups. Differences were considered statistically significant when the P-value was ≤0.05.

Table 1. The primer sequences used for real-time PCR

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Accession No.</th>
<th>Primer sequence</th>
<th>Location</th>
<th>Expected length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>AY550069</td>
<td>5′-CAGGTCATCACCATCAGGCAC 823–842 93</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-TTCTGGGATGCGCGAGGA 898–915</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visfatin</td>
<td>DQ02018</td>
<td>5′-TATTGGCTTGTGTTGCTGTTGTC 1131–1150 154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>AY307771</td>
<td>5′-CCTCTGCTCTCTCAGGCAAC 1011–1030 229</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-GACCCGCTCTCCATAGACAA 1220–1239</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td>AB097930</td>
<td>5′-CATGTGCTGATGGGCTGTAAC 29–48 188</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-TCATTAGGATGGCGAGCTTC 197–216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>AY559454</td>
<td>5′-TGGACCGTGACAGGATTGTA 378–397 237</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-AAGGCTGTATCCACGATGGGTGA 555–572</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aP2</td>
<td>AJ416020</td>
<td>5′-GGTGTCTGCAGCTACCGAAT 364–383 156</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-CAAAATCACTGTCGGGGAAG 501–519</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAS</td>
<td>EF589048</td>
<td>5′-GGACCTGTTGATGAAGCTCT 4510–4529 225</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-CGGAATTTGGAGGAGGTGTGA 4715–4734</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>AF309651</td>
<td>5′-ATGGCCAGAAAAAGACGGATT 328–347 215</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-GTGGTGGCTTTGGTGTGATT 523–542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>AF052691</td>
<td>5′-TGCTCTCTGCTCAGTCCAAG 1440–1459 239</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-AGATGGAAACCTGGTGGATTG 1659–1678</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>AY135647</td>
<td>5′-TGAAAGGATGGTGAAGTACG 589–608 221</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5′-AGGAGGCTGCTGAAAGTTGGA 780–799</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1SREBP-1c, sterol regulatory binding transcription factor 1c; LPL, lipoprotein lipase; aP2, adipocyte fatty acid-binding protein; FAS, fatty acid synthase.
RESULTS

Expression and Purification of Recombinant Visfatin

Protein extracts from bacteria expressing recombinant visfatin were separated by SDS-PAGE gel, and the purity of the recombinant protein carrying an N-terminal His tag was confirmed as a single band of 52 kDa after Coomassie brilliant blue staining (Figure 1). The purified recombinant visfatin protein was used to immunize mice and to investigate function in adipocytes.

Characterization of Porcine Visfatin in Gene Expression Patterns

The endogenous visfatin protein was harvested from porcine blood plasma and detected by western blot analyses using the visfatin monoclonal antibody. Three visfatin protein variants of approximately 52, 27, and 14 kDa were detected (Figure 2A). Tissue distribution of visfatin gene expression was examined (Figure 2B). The mRNA for visfatin was expressed in all tissues examined. In these tissues, the relative mRNA expression of visfatin was greatest ($P < 0.05$) in the liver and muscle.

Effects of Visfatin on Porcine Preadipocytes

Preadipocytes were treated with visfatin or insulin for 4 or 8 d, and the mRNA abundance of sterol regulatory element binding protein 1c (SREBP-1c), PPARγ, lipoprotein lipase (LPL), and adipocyte fatty acid-binding protein (aP2) were determined by real-time RT-PCR analyses. Visfatin did not increase any of the mRNA abundance at 4 d (Figure 3A). Visfatin increased the mRNA abundance of LPL and PPARγ after 8 d of differentiation by approximately 3.5- and 2.1-fold ($P < 0.05$), respectively. Insulin had a similar effect on LPL and PPARγ mRNA abundance, but also increased the mRNA at 4 d. The increase at 8 d was approximately 5.0- and 10.7-fold ($P < 0.05$), respectively. In contrast, neither visfatin nor insulin had an effect on the gene expression of SREBP-1c ($P > 0.05$). Treatment of preadipocytes with insulin induced a 24.7-fold increase ($P < 0.05$) in the mRNA abundance of aP2; visfatin alone had no effect. After 8 d, cells were fixed and stained with Oil Red-O. Compared with visfatin-treated preadipocytes, insulin-treated preadipocytes accumulated lipid at a more rapid rate during differentiation/adipogenesis; fully differentiated cells contained greater amounts of lipid with larger cytoplasmic lipid droplets (Figure 3B).

DISCUSSION

Multiple Alternative Splicing Events of Porcine Visfatin

The porcine visfatin gene, localized on chromosome SSC9, is composed of at least 11 exons and has exactly the same exon-intron structure as the human ortholog (Karnuah et al., 2001; Chen et al., 2007). Accumulating evidence indicates that there are at least 8 alternatively spliced transcripts of porcine visfatin that are expressed in a tissue-specific manner (Chen et al., 2007; Palin et al., 2008). Six visfatin transcript variants are the products of alternative splicing or polyadenylation (Palin et al., 2008). Among them, 4 protein isoforms, 46, 54, 60, and 120 kDa, have been identified with Western blot analyses using antibodies against human visfatin. Variant 1 is the predominant form observed in every species and contains an open reading frame of 1,473 bp encoding a 491-AA protein with a molecular mass of 52 kDa (Chen et al., 2007). In this study, we produced a specific monoclonal antibody against
porcine visfatin and identified 3 isoforms of visfatin. The 52-kDa protein had the greatest expression. The 14-kDa protein could be detected, but 100 µg of total protein was needed. In support of our findings in the current study, these isoforms corresponded to the primary calculated molecular weight for the open reading frames published on GenBank (National Center for Biotechnology Information, Bethesda, MD) under the accession numbers DQ001974 (2.2 kb, approximately 52 kDa), DQ231167 (1.9 kb, approximately 27 kDa), and DQ231168 (1.2 kb, approximately 14 kDa; Chen et al., 2007). It is suggested that the isoforms result from multiple alternative splicing events and tissue-specific expression. The results also confirmed that the monoclonal antibody against porcine visfatin was successfully prepared with the desired specificity and immunological reactivity. Our data from the current study are in agreement with the report of Chen et al. (2007), but we could not confirm the results of Palin et al. (2008) that revealed the presence of 4 distinct porcine visfatin bands (46, 54, 60, and 120 kDa). This discrepancy may also due to the different specificity of the monoclonal antibody developed by the current study to the human antibody used by the Palin et al. (2008). Therefore, related data should be interpreted with caution before more detailed characterization is confirmed. Pagano et al. (2006) suggested that the amplification of different visfatin transcripts in adipose tissue may be an explanation for the contradictory results obtained in various studies. In humans, genetic variation in visfatin was found to be associated with lipid metabolism (Jian et al., 2006). It is not clear whether these porcine visfatin mRNA variants are all translated into protein with physiological properties. The functional significance of the splice variants in pigs remains unknown.

**Tissue Distribution of Porcine Visfatin mRNA Expression**

In Sprague-Dawley rats, visfatin is expressed to a greater extent in VAT than in muscle; the greater VAT expression may result from the extensive adipogenesis occurring in adipocytes in this physiological model (Lv et al., 2009). However, in our studies with 2-wk-old pig tissues, we failed to confirm that there was a difference in visfatin mRNA abundance between VAT and subcutaneous adipose tissue (SAT). Our results in the current study showed that the visfatin gene was transcribed in many porcine tissues; its quantities were greater in the liver and muscle than in the adipose tissue. These observations were in agreement with previous studies using human (Samal et al., 1994), dog (McGlothlin et al., 2005), chicken (Krzysik-Walker et al., 2008), and pig (Palin et al., 2008) tissues. The exact physiological functions of visfatin, however, remain controversial. Such conflicting observations regarding the source of visfatin and the proposed role of visfatin in each insulin-sensitive tissue still need clarification.

**Regulation of Visfatin in S/V Cell Differentiation**

Visfatin exhibits insulin-like activity that are dose-dependent and has been shown to activate the insulin receptor in various insulin-sensitive cell lines in vitro, resulting in the enhancement of glucose uptake, suppression of glucose release, accumulation of triglycerides, and induction of gene markers for adipocyte differentiation (Chan et al., 2006; Chen et al., 2006). Glucose treatments cause a time- and dose-dependent visfatin release from subcutaneous adipocytes (Haider et al., 2006). The binding of visfatin for the insulin receptor shows similar affinities to that of insulin, but visfatin
inserts its function through a distinct binding site (different from insulin binding site) on the insulin receptor (Kim et al., 2006). At a concentration of 2 nM, visfatin and insulin stimulate nearly identical increase in glucose uptake in partially differentiated 3T3-L1 adipocytes (Morgan et al., 2008). At a concentration of 100 nM, insulin and visfatin also significantly upregulate glucose uptake in adipocytes (Moschen et al., 2007). Increasing visfatin concentrations causes increasing uptake of glucose, and the maximal effect was reached at 2 µM visfatin (or 100 ng/mL) in cultured mesangial cells and osteoblast cells (Xie et al., 2007; Song et al., 2008). To further examine the insulin-mimetic effect of visfatin on lipid metabolism and to determine whether visfatin and insulin share a common signaling pathway, we studied the mRNA abundance of several key genes involved in the insulin-signaling cascade in the presence of exogenous visfatin.

Insulin is implicated in the regulation of LPL, a key enzyme in lipid metabolism, in adipose tissue via the activation of PPARγ (Hanyu et al., 2004). Porcine PPARγ is the master regulator that governs the differentiation of adipocytes (Yu et al., 2006, 2008). The active form of PPARγ binds to the consensus peroxisome proliferator response element (PPRE) sequence in the LPL promoter to trigger its expression (Hanyu et al., 2004). Therefore, an increase in the expression of PPARγ may lead to an increase in the expression of LPL. We observed similar adipogenesis-inducing effects for visfatin, an increase in PPARγ and LPL mRNA expressions, thus suggesting visfatin may play a role in adipogenesis.

Expression of aP2 in preadipocytes is well-correlated with cell differentiation and regarded as a molecular marker for terminal differentiation. Neither the aP2 protein nor mRNA transcripts are detected in undiff-

Figure 3. Effect of visfatin and insulin on the expression of adipogenesis-related genes in porcine preadipocytes. Postconfluent undifferentiated stromal/vascular (S/V) cells were treated with serum-free growth medium containing 100 ng/mL of visfatin or insulin, respectively, for 4 and 8 d. (A) Total RNA was extracted and used for cDNA synthesis and real-time PCR. The expression of each gene was normalized against β-actin and presented as fold changes compared with untreated control cells. The control value was set as 1.0. Error bars represent the mean ± SEM from 3 independent experiments, each with preadipocytes isolated from a different pig. Analyses were performed in duplicate. White bar: control (no treatment), black bar: visfatin treatment, hatched bar: insulin treatment. Different letters (a, b) indicate column means were significantly different, $P \leq 0.05$ using Duncan’s new multiple range test. SREBP-1c = sterol regulatory element binding protein 1c; LPL = lipoprotein lipase; aP2 = adipocyte fatty acid-binding protein. (B) Oil Red-O staining of S/V cells incubated with visfatin (left panel) or insulin (right panel) for 8 d. The lipid droplets turned red (light spheres as pointed at by arrows) with Oil Red-O staining, whereas the cytoplasms showed no color. The images were photographed at 400× magnification. Color version available in the online PDF.
differentiated adipocytes (Ding et al., 1999). Activation of PPARγ by ligands is sufficient to stimulate aP2 expression and adipocyte, as well as macrophage, differentiation (Takahashi et al., 2003; Thompson et al., 2004). In contrast, we observed no significant changes in aP2 in S/V cells (preadipocytes) treated with visfatin. Visfatin-induced cells had less lipid accumulation than insulin-induced cells after 8 d of differentiation. This observation is in agreement with the fact that aP2 expression is closely associated with lipid droplet accumulation in many types of cell lines (Ding et al., 1999; El-Jack et al., 1999; Sun et al., 2003; Liu and Nambi, 2004; Kazemi et al., 2005; Makowski et al., 2005). Adipocyte fatty acid-binding protein acts as a cytosolic fatty acid chaperone and facilitates the utilization of lipids in metabolic pathways (Maeda et al., 2005), but mechanisms for the regulation of aP2 expression in different cell types are not well-characterized (Sun et al., 2003; Shum et al., 2006). Collectively, data in the current study indicated that visfatin possesses the potential to induce mRNA of adipose differentiation markers during the differentiation process, but did not result in accumulation of large amounts of lipid or formation of large lipid droplets. The function of visfatin may be attributed to another insulin-like effect.

**Regulation of Visfatin in Differentiated Adipocytes**

The crucial transcription factor, SREBP-1c, has been implicated in the insulin-mediated induction of genes associated with glucose and fatty acid metabolism in hepatocytes, adipocytes, and myocytes (Chakravarty et al., 2001; Ducluzeau et al., 2001). Fatty acid synthase is a marker for the lipogenic pathway, and some studies suggest that there is a positive correlation between mRNA abundance of FAS and SREBP-1c for the synthesis of fatty acids stimulated by insulin (Gondret et al., 2001; Li and Yang, 2008). The current data, however, showed contradictory results in which the expression of SREBP-1c mRNA only slightly increased, but mRNA abundance of FAS increased during the visfatin- or insulin-stimulated differentiation of porcine S/V cells. These results imply that there is a potential limit to the regulation of lipogenesis in adipocytes through the induction of SREBP-1c to activate the transcription of FAS. The result is further supported by recent reports claiming that the mRNA abundance of SREBP-1c do not coincide with the changes in adipose lipogenic gene expression (Bertile and Raclot, 2004; Sekiya et al., 2007). In the present study we demonstrated that in adipocytes, visfatin and insulin activated the expression of FAS mRNA, but SREBP-1c mRNA did not change, implying the involvement of other transcription factors.

**Effect of Visfatin on the Induction of Inflammatory Responses**

In the 3T3-L1 adipocyte cell line, IL-6 is a strong inhibitor of adipogenesis (Ohsumi et al., 1994) and directly influences the metabolism of human adipocytes by decreasing the activity of LPL (Greenberg et al., 1992). Treatment of adipocytes with visfatin resulted in upregulation of IL-6 in the present study; thus, visfatin

---

**Figure 4.** Effect of visfatin and insulin on the expression of adipogenesis-related genes in porcine adipocytes. Preadipocytes were differentiated from 8 d and then treated with Dulbecco’s modified Eagle/F12 medium containing 100 ng/mL of visfatin or insulin, respectively, for 24 h. Total RNA was extracted from the cells and used for cDNA synthesis and real-time PCR. (A) Sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS), and (B) IL-6, adiponectin (ADN), and leptin. The expression of each gene was normalized using β-actin expression in the same sample. Normalized data are presented as fold changes compared with untreated control cells. The control value was set as 1.0. Data presented are means ± SEM from 3 independent experiments, each using cells from a different pig. White bar: control (no treatment), black bar: visfatin treatment, hatched bar: insulin treatment. Different letters (a,b) indicate column means were significantly different, \( P \leq 0.05 \) using Duncan’s new multiple range test.
may play a role in triggering and coordinating immune responses (Ognjanovic and Bryant-Greenwood, 2002). Lagathu et al. (2003) demonstrated that IL-6 produced by adipocytes is a pro- and anti-inflammatory cytokine and is capable of inducing insulin resistance in differentiating and differentiated adipocytes. Acutely, IL-6 mimics the action of insulin on ERK1/2 and Akt activation and chronically induces insulin resistance via the induction of cytokine signaling-3 proteins (Lagathu et al., 2003; Senn et al., 2003; Gabler and Spurlock, 2008). Berndt et al. (2005) indicated that IL-6 expression, induced by visfatin, might be involved in the pathogenesis of insulin resistance associated with visceral obesity.

We found that the abundance of 2 major adipocyte mRNA, leptin and adiponectin, were not affected by treatments with visfatin. Although adiponectin and leptin are adipocyte cytokines, the biological functions and regulation of their secretion are obviously different and even, to a certain extent, opposite of IL-6 (Zhang et al., 2006). The increase of IL-6 by visfatin treatment may inhibit the expression of adiponectin (Fasshauer et al., 2003); however, we did not observe such an effect. The lack of an adiponectin response may result from the balance of an inhibition effect of abundantly expressed IL-6 and other inducing regulators increased by visfatin.

In conclusion, we have presented results of the effects of visfatin on the regulation of genes related to lipid metabolism in porcine adipocytes. Transcriptional changes in visfatin- and lipid metabolism-related genes revealed insulin-mimetic properties of visfatin; however, there are differences in the effects of insulin and visfatin on preadipocyte differentiation and lipid accumulation. Taken together, these results provide further evidence for the molecular mechanisms underlying the effects of visfatin on lipid metabolism and also suggest there are important differences between insulin and visfatin effects.

**LITERATURE CITED**


Porcine visfatin regulates lipid metabolism


