

Molecular and functional characterization of the adiponectin (*AdipoQ*) gene in goat skeletal muscle satellite cells

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Objective: It is commonly accepted that adiponectin binds to its two receptors to regulate fatty acid metabolism in adipocytes. To better understand their functions in the regulation of intramuscular adipogenesis in goats, we cloned the three genes (adiponectin [*AdipoQ*], adiponectin receptor 1 [*AdipoR1*], and *AdipoR2*) encoding these proteins and detected their mRNA distribution in different tissues. We also determined the role of *AdipoQ* in the adipogenic differentiation of goat skeletal muscle satellite cells (SMSCs).

Methods: SMSCs were isolated using 1 mg/mL Pronase E from the *longissimus dorsi* muscles of 3-day-old female Nanjiang brown goats. Adipogenic differentiation was induced in satellite cells by transferring the cells to Dulbecco's modified Eagle's medium supplemented with an isobutylmethylxanthine, dexamethasone and insulin cocktail. The pEGFP-N1-AD plasmid was transfected into SMSCs using Lipofectamine 2000. Expression of adiponectin in tissues and SMSCs was detected by quantitative polymerase chain reaction and immunocytochemical staining.

Results: The three genes were predominantly expressed in adipose and skeletal muscle tissues. According to fluorescence and immunocytochemical analyses, adiponectin protein expression was only observed in the cytoplasm, suggesting that adiponectin is localized to the cytoplasm of goat SMSCs. In SMSCs overexpressing the *AdipoQ* gene, adiponectin promoted SMSC differentiation into adipocytes and significantly ($p < 0.05$) up-regulated expression of *AdipoR2*, acetyl-CoA carboxylase, fatty-acid synthase, and sterol regulatory element-binding protein-1, though expression of CCAAT/enhancer-binding protein- α , peroxisome proliferator-activated receptor γ , and *AdipoR1* did not change significantly.

Conclusion: Adiponectin induced SMSC differentiation into adipocytes, indicating that adiponectin may promote intramuscular adipogenesis in goat SMSC.

Keywords: Adiponectin; Intramuscular Adipogenesis; Skeletal Muscle Satellite Cells; Fatty Acid Synthesis

INTRODUCTION

Adiponectin, an adipokine belonging to the complement factor C1Q protein family [1], is expressed in liver sinusoid endothelial cells, skeletal muscle and bone-forming cells as well as in adipocytes [2-4]. Adiponectin, which is expressed by the *AdipoQ* gene, consists of four parts: an amino-terminal signal peptide, a variable region, a collagenous domain and a carboxyl-terminal globular domain [5]. There are two receptors for adiponectin, AdipoR1 and AdipoR2. Both are seven-transmembrane domain proteins with an intracellular N-terminus and highly expressed in skeletal muscle (AdipoR1) and adipose tissue (AdipoR2) [6]. Adiponectin circulates in the plasma in several forms: globular adiponectin (gAd) interacts with AdipoR1; whereas full-length adiponectin (fAd) binds to both AdipoR1 and AdipoR2

to regulate glucose and fatty acid metabolism [7].

Emerging evidence suggests that adiponectin enhances glucose uptake by increasing GLUT4 translocation to the cell membrane [8] and promotes fatty acid oxidation by activating adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), p38-mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha pathways [9,10]. In addition, adiponectin inhibits gluconeogenesis by phosphorylating AMPK and acetyl-CoA carboxylase (ACC) [11]. For example, intravenous infusion of adiponectin in mice leads to lower expression of PEPCK and G6Pase in the liver, which inhibits endogenous glucose production [12]. In myocytes, fAd increases the number of mitochondria and enhances fatty acid oxidation by activating CaMKK β and PGC1- α [13]. Studies have also reported that overexpression of the *AdipoQ* gene in fat tissue and fibroblasts results in increased lipid accumulation [14]. Therefore, the results of these studies suggest that adiponectin plays important roles in regulating glucose uptake and fatty acid metabolism.

It has been shown that ACC and fatty-acid synthase (FASN) are two key enzymes in *de novo* fatty acid synthesis. ACC couples with FASN to increase fatty acid production in *Escherichia coli* and humans [15,16]. In addition, sterol regulatory element-binding protein-1 (SREBP-1), CCAAT/enhancer-binding protein- α (C/EBP α) and peroxisome proliferator-activated receptor γ (PPAR γ) are master adipogenic transcription factors that have been proven to promote the formation of white adipose tissue [17]. As previously demonstrated, PPAR γ and SREBP-1 cooperatively play key roles in regulating the redifferentiation of bovine adipofibroblasts [18]. Moreover, C/EBP α has important functions in regulating cell proliferation and differentiation, especially adipocyte differentiation [19], and PPAR γ and C/EBP α together induce adipogenesis and adipocyte differentiation [20]. These studies demonstrate that these genes play pivotal roles in fatty acid synthesis and adipocyte differentiation. Although adiponectin has been shown to regulate fatty acid metabolism, it remains unknown whether adiponectin regulates the expression levels of these adipogenic-related genes.

An increasing number of studies on adiponectin are being carried out in humans and mice, but information on the gene encoding adiponectin in domestic animals is still limited. In addition, the role of adiponectin in the adipogenic differentiation of muscle satellite cells is unknown. In this study, we cloned and characterized the goat *AdipoQ*, *AdipoR1*, and *AdipoR2* genes and detected their mRNA expression levels in different tissues. In addition, we quantified the expression levels of these adipogenic-related genes in skeletal muscle satellite cells (SMSCs) overexpressing adiponectin. The results of this study provide information on the expression patterns of the *AdipoQ*, *AdipoR1*, and *AdipoR2* genes in goat as well as the functions of adiponectin in regulating fatty acid synthesis.

MATERIALS AND METHODS

Animals and tissue collection

All procedures were performed according to the guidelines established by the Sichuan Province Committee for the Protection of Laboratory Animals. Eleven tissues, namely, the ovary, spleen, rumen, liver, brain, heart, *gastrocnemius* muscle, *biceps femoris* muscle, *longissimus dorsi* muscle (LD), and mesenteric and subcutaneous adipose tissue, were dissected from three three-day-old female Nanjiang brown goats. All tissues were immediately frozen in liquid nitrogen and then stored at -80°C .

RNA isolation and cDNA synthesis

Total RNA was extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The integrity of total RNA was examined by agarose gel electrophoresis, and the purity and concentration were determined using a NanoDrop (Thermo Fisher, Waltham, MA, USA) instrument. First-strand complementary DNA (cDNA) was synthesized using 2 μg of total RNA and a PrimeScript RT Reagent Kit (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol.

Primer design and gene cloning

Based on the conserved regions of the human and cattle *AdipoQ*, *AdipoR1*, and *AdipoR2* gene sequences, primer pairs for gene cloning and quantitative polymerase chain reaction (qPCR) were designed by Primer Premier 5.0 (Table 1) and synthesized by Shanghai Sangon Biotechnology Company (Shanghai, China). The total reaction volume was 10 μL and included 1 μL cDNA, 5 μL 2 \times Taq PCR Master Mix, 0.8 μL (8 pM) primer pairs (ADF/R for *AdipoQ*, ADR1F/R for *AdipoR1* and ADR2F/R for *AdipoR2*) and 3.2 μL ddH $_2$ O. The PCR procedures were as follows: initial denaturation at 95°C for 3 min; 35 cycles of 95°C for 30 s, optimum annealing temperature (Table 1) for 30 s and 72°C for 1 to 2 min; and a final extension at 72°C for 5 min. All products were separated by agarose gel electrophoresis, purified using a gel extraction reagent kit (Tiangen, Beijing, China), cloned into the pMD19-T vector (TaKaRa, Japan) and sequenced by Shanghai Invitrogen Biotechnology Company (Shanghai, China).

Semi-quantitative reverse transcription-PCR analysis

To detect the mRNA tissue distributions of the *AdipoQ*, *AdipoR1*, and *AdipoR2* genes, we performed semi-quantitative reverse transcription (RT)-PCR. The total reaction volume was 20 μL and included 2 μL cDNA, 10 μL 2 \times Taq PCR Master Mix, 1.2 μL (12 pM) primer pairs (ADQF/R for *AdipoQ*, ADR1QF/R for *AdipoR1* and ADR2QF/R for *AdipoR2*) and 6.8 μL ddH $_2$ O. The PCR procedure was as follows: initial denaturation at 95°C for 3 min; 26 to 32 cycles at 95°C for 30 s,

Table 1. Primer pairs used in this study

Gene name	Primer name	Primer sequence (5'-3')	Size (bp)	Tm (°C)
<i>AdipoQ</i>	ADF	CACCTGAGGGACTCAGGAT	1,163	59.5
	ADR	ACTTAGGCACACAGCTCTC	142	59.5
	ADQF	GTTCCATTGCTTTACCAA	735	59.0
	ADQR	GGCTGACCTTCACATCCTTC		
	Hind III	CCAAGCTTATGCTGCTGCTGGGAGCTCT		
	BamH I	CGGGATCCATTTCGATGTTATGGTAGAGAA		
<i>AdpoR1</i>	ADR1F	TGATGTCGTCTACAAGGGA	1,132	62.0
	ADR1R	GCTCAGAGGAGGGAGTCGT		
	ADR1QF	GCTGGGCTTTGTGCTGTTTC	100	61.0
	ADR1QR	CATCCCGAACACCACCTT		
<i>AdpoR2</i>	ADR2F	CCTCTTTGTGAGAAAGCCT	1,221	59.5
	ADR2R	ACCTCCAGCCAACGTCTCA		
	ADR2QF	GAGCGGTGACAGTGATGC	211	61.0
	ADR2QR	ACCTCCAGACCTTACAGA		
ACC	ACC-F	CCGTCTGTGATGACTTTGA	191	56.4
	ACC-R	CTTTCTGGGTTGGGTGAG		
FASN	FASN-F	GAAGGGTGTGACCTCGTCC	126	58.4
	FASN-R	AGAGGGTGGTGTGGAAAG		
SREBP-1	SREBP1-F	CTGCTGACCGACATAGAAGACAT	81	56.4
	SREBP1-R	GTAGGGCGGGTCAAACAGG		
C/EBP α	CEBP-F	CCGTGGACAAGAACAGCAA	141	58.4
	CEBP-R	GGCGGTCAATTGCTACTGG		
PPAR γ	PPAR1-F	GTGCTACTCTGAACGAAAT	156	56.4
	PPAR1-R	GGAATGCTGGAGAAGTCAA		
β -actin	BA-F	CCTGCGGCATTACGAAACTAC	87	59.7
	BA-R	ACAGCACCGTGTGGCGTAGAG		

AdipoQ, adiponectin; *AdpoR*, adiponectin receptor; *ACC*, acetyl-CoA carboxylase; *FASN*, fatty-acid synthase; *SREBP-1*, sterol regulatory element-binding protein-1; *C/EBP α* , CCAAT/enhancer-binding protein- α ; *PPAR γ* , peroxisome proliferator-activated receptor γ .

optimum annealing temperature (Table 1) for 30 s and 72°C for 10 s; and a final extension at 72°C for 5 min. All products were examined by agarose gel electrophoresis to estimate mRNA abundance in the 11 tissues tested; β -actin was used as an internal control gene.

Quantitative real-time PCR

qPCR was carried out using a SYBR Green-based kit in 10- μ L reaction volumes containing 5 μ L SYBR Green real-time PCR Master Mix (TaKaRa, Japan), 0.8 μ L cDNA and 0.4 μ L each primer; a Bio-Rad CFX96 qPCR instrument (Bio-Rad, Hercules, CA, USA) was used. The qPCR procedure was as follows: initial denaturation at 95°C for 3 min; 40 cycles at 95°C for 30 s, alternative annealing for 20 s, and 72°C for 15 s; and a final extension for 5 min. Melting curve analysis was used to confirm specific PCR products. Gene expression levels were quantified relative to β -actin expression using the comparative Ct ($2^{-\Delta\Delta Ct}$) value method.

Cell culture

SMSCs were isolated using 1 mg/mL Pronase E (Sigma, St. Louis, MO, USA) from the *longissimus dorsi* muscles from

3-day-old female Nanjiang brown goats. After enzymatic digestion, cells were separated from tissue fragments by repeated centrifugation at 500 \times g for 5 min, followed by filtration through a 200- μ m filter and then a 50- μ m Nytex filter. SMSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin under conditions of 5% CO₂ and 37°C. When the cells had grown to 80% to 90% confluence, adipogenic differentiation of satellite cells was induced by transferring the cells into DMEM supplemented with an isobutylmethylxanthine, dexamethasone and insulin (MDI) cocktail (0.5 mM 1-methyl-3-isobutylxanthine, 1 μ M dexamethasone, and 10 μ g/mL insulin), followed by supplementation with 10 μ g/mL insulin for 1 day. The medium was replaced every two days. Cells were visualized by staining with oil red O solution.

Plasmid construction and transient transfection

Using Primer Premier 5.0, a primer pair (Hind III-F, BamH I-R) was designed to amplify the coding region of *AdipoQ* for plasmid construction. PCR products and the pEGFP-N1 (Clontech, San Francisco, CA, USA) vector were digested with

Hind III and *Bam*H I restriction enzymes (TaKaRa, Japan), purified with a gel extraction reagent kit and ligated using T4 DNA ligase (TaKaRa, Japan). The resulting plasmid (pEGFP-N1-AD) was sequenced to confirm the correct orientation and reading frame.

Transient transfection was performed according to the manufacturer's protocol: 4 µg plasmid and 10 µL Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were mixed, cultured for 20 min and then transfected into cells. Twenty-four hours after transfection, the cells were rinsed with phosphate-buffered saline; a portion was fixed with 4% formaldehyde for 30 min at room temperature to detect the cellular distribution of the adiponectin fusion protein using a fluorescence microscope (Nikon, Tokyo, Japan); other cells were suspended in TRIzol reagent for RNA extraction and expression analysis. The results were normalized to the expression of β -actin, and the expression of the control group (pEGFP-N1) was set as 1 for expression analysis of each gene. The relative mRNA expression analysis was performed using SAS 9.0 software with the generalized linear model process. Duncan's new multiple range test was applied to analyze statistical significance.

Immunocytochemistry

Expression of adiponectin in SMSCs was detected by immunocytochemical staining. Briefly, cells were fixed with 4% paraformaldehyde for 30 min and then permeabilized in 0.25% Triton X-100 with shaking at room temperature for 30 min, followed by blocking with 1% bovine serum albumin for 1 h. The cells were then incubated overnight at 4°C with an anti-adiponectin primary antibody (1:100) (Boster Company, Wuhan, China). The secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:200), was incubated with the cells for 30 min. Cells incubated without the primary antibody were used as the negative control.

Bioinformatic analysis

ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) and DNASTAR were used to predict the open reading frames of the three genes. The domains of the three proteins were predicted by ProtParam (<http://cn.expasy.org/tools/protparam.html>) and SMART (<http://smart.embl-heidelberg.de/>), and the functional domain diagram was generated with DOG 2.0 software [21]. Amino acid sequences were aligned using ClustalX (<http://www.ebi.ac.uk/clustalw/>).

RESULTS

Molecular characterization of *AdipoQ*, *AdipoR1*, and *AdipoR2*

The full-length coding sequences of the goat *AdipoQ*, *AdipoR1*, and *AdipoR2* genes were obtained (accession numbers:

JX573539, KC286912 and JX573540). The coding regions of the *AdipoQ*, *AdipoR1*, and *AdipoR2* are 720 bp, 1,128 bp, and 1,176 bp, respectively. The *AdipoQ* gene encodes a 239-aa protein with a molecular weight of 25.95 kDa. The *AdipoR1* gene encodes a 375-aa protein with a molecular weight of 42.41 kDa, and the *AdipoR2* gene encodes a 391-aa protein with a molecular weight of 43.81 kDa. These three genes share high similarity with other mammalian orthologous genes (Supplementary Table S1). Goat *AdipoR1* shows the highest homology with other mammals, with the amino acid sequence sharing more than 96% identity with other mammalian sequences. Goat *AdipoQ* and *AdipoR2* exhibit relatively high homology with other mammalian sequences. The amino acid sequence of *AdipoQ* is 81.17%, 87.45%, 81.59%, and 99.16% identical to those of *Homo sapiens*, *Bos taurus*, *Mus musculus*, and *Ovis aries*, respectively. The amino acid sequence of *AdipoR2* shows similar identity to those of humans (86.27%), *Bos taurus* (92.49%), *Mus musculus* (84.20%), and *Ovis aries* (94.04%). Prediction of the deduced protein structure showed that adiponectin harbors a 137-aa C1Q domain; although the receptors both contain a 220-aa seven-transmembrane domain, these receptors are not identical (Figure 1).

Tissue distribution of goat *AdipoQ*, *AdipoR1*, and *AdipoR2*

The mRNA tissue distribution of these three genes was detected by semi-quantitative RT-PCR, and the agarose gel electrophoresis results are shown in Figure 2. *AdipoQ* expression was predominantly observed in mesenteric and subcutaneous adipose tissues but was weakly expressed in the *gastrocnemius* muscle, LD muscle and *biceps femoris* muscle; expression was not detected in other tissues. *AdipoR1* mRNA was found to be expressed in almost all tissues, with high expression in skeletal muscles and the rumen, followed by adipose tissue; its expression in other tissues was relatively weak. Similarly, *AdipoR2* was expressed in all tissues but was most abundant in adipose and skeletal muscle tissues; it was weakly expressed in other tissues. The expression patterns of these three genes were further quantitated by qPCR (Figure 3). *AdipoQ* mRNA was predominantly expressed in adipose tissue, whereas its expression in skeletal muscles was relatively weak. *AdipoR1* and *AdipoR2* mRNA were expressed in all tissues examined, with the highest expression in adipose and skeletal muscle tissues.

Subcellular localization of goat adiponectin in SMSCs

Fluorescence analysis showed a green fluorescent signal throughout cells of the control group transfected with the pEGFP-N1 plasmid; In contrast, the green fluorescence of the adiponectin fusion protein was only observed in the cytoplasm of cells transfected with the pEGFP-AD plasmid (Figure 4), suggesting that adiponectin is localized to the cytoplasm.

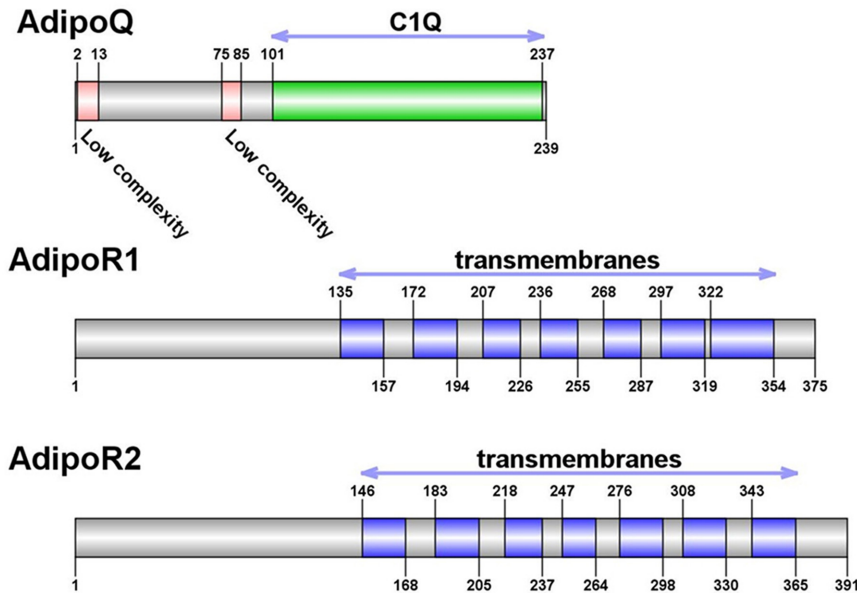


Figure 1. Diagram of the deduced protein structures of AdipoQ, AdipoR1, and AdipoR2. Adiponectin has a C1Q domain, and AdipoR1 and AdipoR2 both possess a seven-transmembrane domain. Domains are color coded: pink indicates low-complexity segments, green indicates the C1Q domain, and blue indicates transmembrane domains. AdipoQ, adiponectin; AdipoR, adiponectin receptor.

Furthermore, immunostaining in SMSCs using an antibody against the human adiponectin protein revealed strong positive staining within the goat SMSC cytoplasm (Figure 5A) compared with the controls (Figure 5B).

Adiponectin promotes fatty acid synthesis in skeletal muscle satellite cells

To determine the role of adiponectin in the adipogenic differentiation of goat MSCs, cells were transfected with the pEGFP-N1-AD plasmid to overexpress the goat *AdipoQ* gene. To evaluate effective overexpression of adiponectin, whole-cell lysates were harvested for analysis of expression levels. In SMSCs, adiponectin was overexpressed approximately 80-fold compared with that in control cells transfected with the

pEGFP-N1 vector (Figure 6). These results demonstrate that cells transfected with the pEGFP-N1-AD plasmid can overexpress adiponectin compared with the expression levels of control goat SMSCs expressing green fluorescent protein (GFP).

Next, satellite cells were induced with an MDI adipogenic cocktail and stained with oil red O seven days later. As shown in Figure 7, more oil red O-positive cells were observed in the pEGFP-N1-AD-transfected group than in the control group, indicating that adiponectin promoted MSC differentiate into adipocytes. In addition, several markers of adipogenic differentiation were detected by qPCR. The results showed that adiponectin overexpression significantly ($p < 0.01$) up-regulated the mRNA expression levels of *AdipoR2*, *ACC*, *FAS*, and *SREBP-1* (Figure 8), which were 1.85, 4.55, 4.88, and 4.25 times

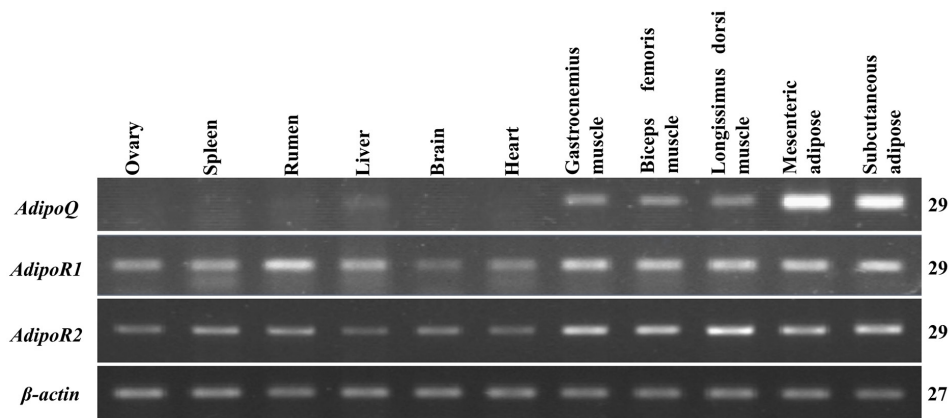


Figure 2. mRNA distribution of *AdipoQ*, *AdipoR1*, and *AdipoR2* genes in 11 tissues of three-day-old female Nanjiang brown goats; β -actin was used as the internal standard control gene. Cycle numbers are indicated in the right margin. *AdipoQ*, adiponectin; *AdipoR*, adiponectin receptor.

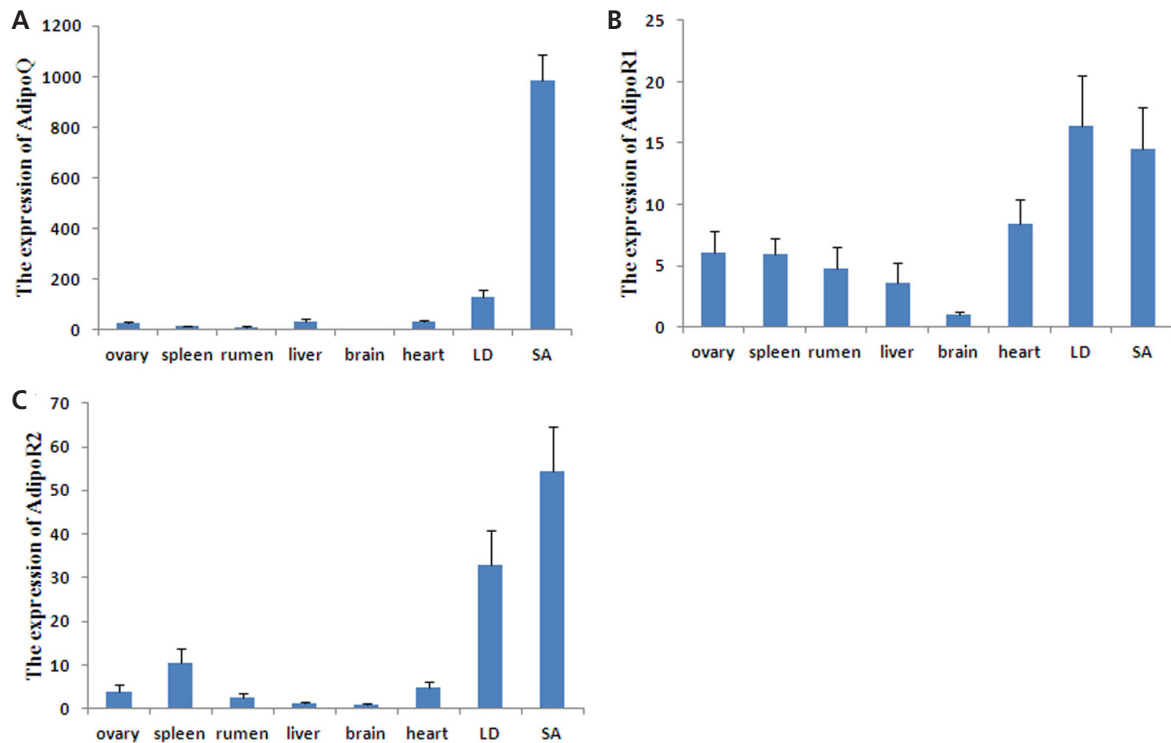


Figure 3. mRNA expression levels of AdipoQ, AdipoR1, and AdipoR2 in different tissues were determined by quantitative polymerase chain reaction. The samples represent the *longissimus dorsi* muscle (LD) and subcutaneous adipose (SA) tissue. The bars represent the mean \pm standard error of the mean. (n = 3); β -actin was used as a control. AdipoQ, adiponectin; AdipoR, adiponectin receptor.

higher, respectively, than those of the control group. Conversely, no significant changes in expression were found for *C/EBP α* , *PPAR γ* , and *AdipoR1* (data not shown).

DISCUSSION

Recently, many studies have focused on the functions of the

AdipoQ gene in regulating glucose and fat metabolism and on its anti-inflammatory and anti-atherosclerosis effects in some species [12,22,23]. In the present study, we cloned the goat *AdipoQ*, *AdipoR1*, and *AdipoR2* genes to investigate their expression and the functions of the encoded proteins. We found these gene sequences to be conserved among species. Adiponectin belongs to the complement factor C1Q family,

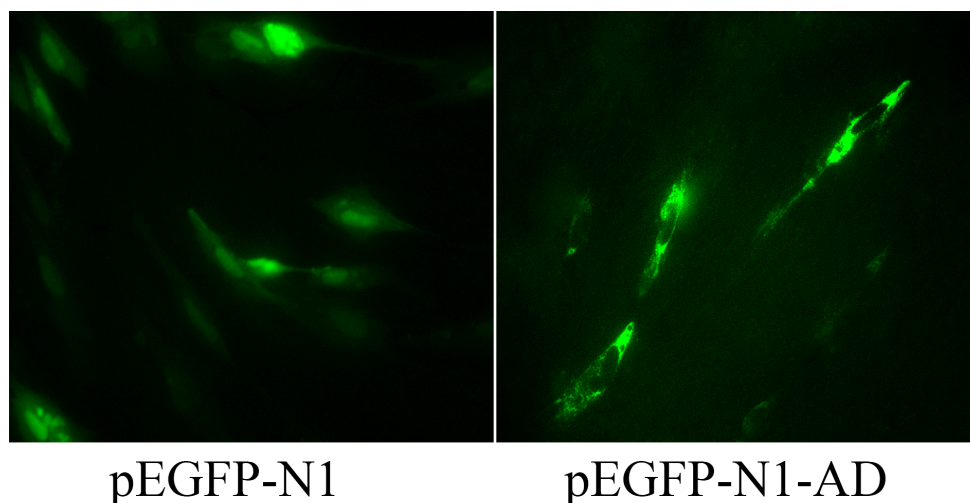


Figure 4. Adiponectin protein subcellular localization in skeletal muscle satellite cells (SMSCs). Green fluorescent protein (GFP) in the control group was expressed throughout the cell, whereas the GFP-adiponectin fusion protein was only found in the cytoplasm.

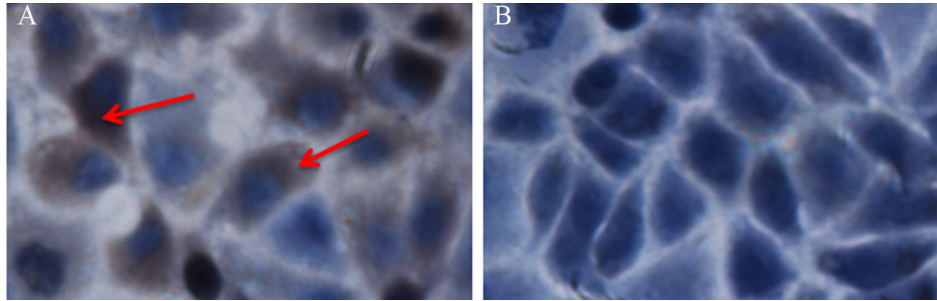


Figure 5. Adiponectin protein expression in goat skeletal muscle satellite cells (SMSCs). Immunocytochemistry analysis of the goat adiponectin protein using a human anti-adiponectin antibody in SMSCs (A) and compared with controls (B). Arrows indicate positive staining.

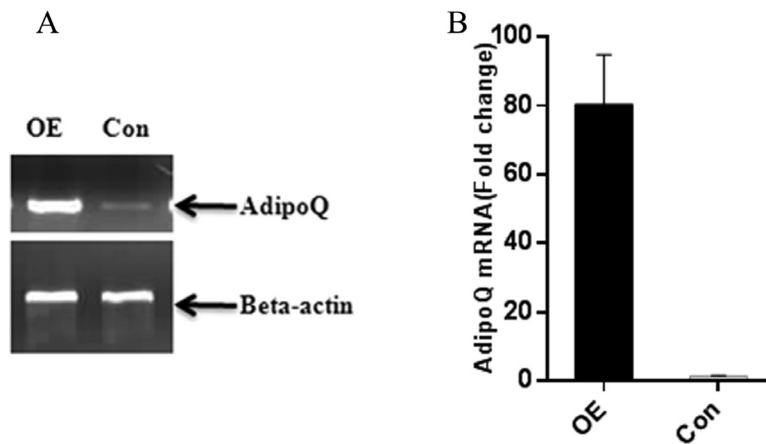


Figure 6. The effect of goat adiponectin overexpression. Cells were transfected with the pEGFP-N1-AD (OE) plasmid and then harvested for mRNA extraction after 48 hours. Effective overexpression of goat adiponectin was identified by semi-quantitative reverse transcription–polymerase chain reaction (RT-PCR) (A) and quantitative PCR (B). Cells transfected with the pEGFP-N1 plasmid were used as the control (Con).

and studies have proven that the C1Q domain has particular functions, including inducing SMC differentiation and fusion [24] and enhancing muscle fat oxidation and glucose transport [12]. The two receptors of adiponectin, AdipoR1 and AdipoR2, are seven-transmembrane domain proteins, but their protein structures lack homology with other mammalian proteins. In general, AdipoR1 and AdipoR2 are activated by different types of adiponectin. However, Narasimhan found

that in yeast, the AdipoR1 and AdipoR2 proteins interact with osmotin, which has no sequence homology but does possess structural similarity to adiponectin [25]. This finding indicates that AdipoR1 and AdipoR2 might serve as receptors for other proteins.

Previous studies have shown that chicken *AdipoQ* is predominantly expressed in adipose tissue, whereas *AdipoR1*, and *AdipoR2* are widely expressed in various tissues. *AdipoR1*

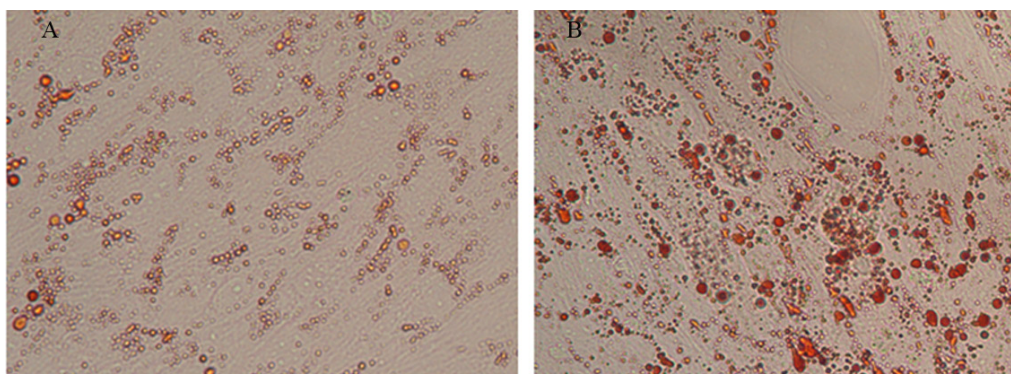


Figure 7. Lipid accumulation in cells of the AdipoQ-overexpression and control groups after 7 d of adipogenic differentiation, as determined by oil red O staining.

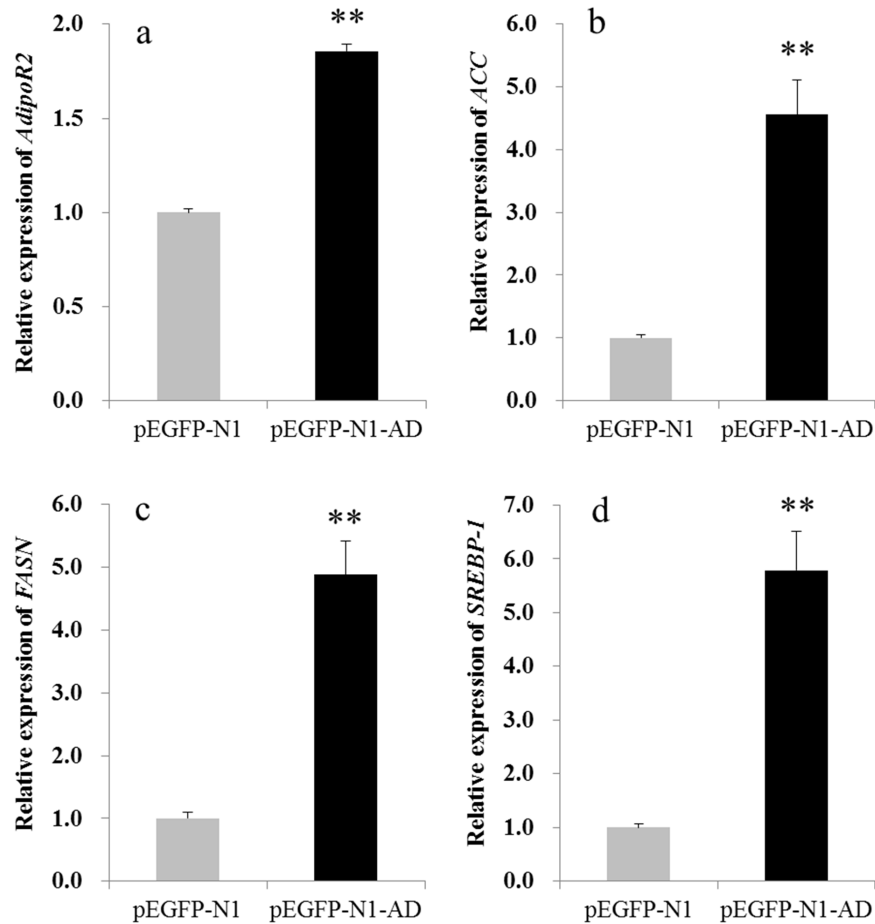


Figure 8. Expression levels of *AdipoR2* (a), *ACC* (b), *FASN* (c) and *SREBP-1* (d) in skeletal muscle satellite cells (SMSCs) overexpressing adiponectin. The expression levels of *AdipoR2*, *ACC*, *FASN* and *SREBP-1* in adiponectin-overexpressing cells were 1.85, 4.55, 4.88, and 4.25 times, respectively, higher than in the control group. The results were normalized to β -actin expression, and expression in the control group was set as 1. Error bars represent SE (n = 3), ** p<0.01. *AdipoR*, adiponectin receptor; *ACC*, acetyl-CoA carboxylase; *FASN*, fatty-acid synthase; *SREBP-1*, sterol regulatory element-binding protein-1.

mRNA is highly expressed in skeletal muscles, adipose tissue and the diencephalon; *AdipoR2* mRNA is most highly expressed in adipose tissue, followed by skeletal muscles and the liver [26]. Research in pigs showed that *AdipoQ* mRNA expression is abundant in adipose tissue, that *AdipoR1* mRNA expression is abundant in skeletal muscle and adipose tissues, and that *AdipoR2* mRNA is predominantly expressed in adipose and skeletal muscle tissues [27]. Overall, these three genes are mainly expressed in adipose and skeletal muscle tissues, where they are responsible for energy metabolism. Hence, the observed tissue distribution profile corresponds with their function in regulating lipid and fatty acid metabolism. In our study, the mRNA tissue distribution of *AdipoQ* and its two receptor genes was similar to that in chickens and pigs, suggesting a similar function among species.

A previous report stated that adiponectin expression and secretion are increased during the human adipocyte differentiation process, which is regarded as a distinctive marker of adipocyte differentiation [28]. Overexpression of adiponectin

in 3T3-L1 fibroblasts increases the number and size of lipid droplets and promotes adipogenic differentiation [14]. Accordingly, these results suggest that adiponectin is able to promote fatty acid synthesis and adipocyte differentiation.

In this study, the adiponectin fusion protein was only found in the cytoplasm of SMSCs overexpressing the *AdipoQ* gene, indicating that adiponectin functions in the cytoplasm. Based on our qPCR results, the expression levels of *AdipoR2*, *ACC*, *FASN*, and *SREBP-1* were significantly up-regulated, suggesting that adiponectin interacts with *AdipoR2* to regulate expression of *ACC*, *FASN*, and *SREBP-1* genes in SMSCs. It has been reported that *ACC* and *FASN* are two important factors for *de novo* fatty acid synthesis; *ACC* catalyzes the production of malonyl-CoA from acetyl-CoA, which is then converted into long-chain fatty acids by *FASN* [29]. Moreover, *SREBP-1* is an important transcription factor that regulates *ACC* and *FASN* transcription for fat metabolism and adipogenic differentiation [30]. Thus, increased expression of these three adipogenic genes may result in fatty acid synthesis and

lipid accumulation, which indicates that adiponectin may promote fatty acid synthesis and adipogenic differentiation.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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