Peroxisome proliferator-activated receptor γ 1 and γ 2 isoforms alter lipogenic gene networks in goat mammary epithelial cells to different extents

H. B. Shi,* W. S. Zhao,*† J. Luo,*¹ D. W. Yao,* Y. T. Sun,* J. Li,* H. P. Shi,* and J. J. Looft¹

*Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, China

†Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT

In nonruminants, the alternative splicing of peroxisome proliferator-activated receptor γ (PPARG) generates *PPARG1* and *PPARG2* isoforms. Although transcriptional control differences between isoforms have been reported in human adipose tissue, their roles in ruminant mammary cells are not well known. To assess which of these isoforms is more closely associated with the regulation of mammary lipogenic pathways, their tissue distribution was analyzed and the expression of key genes regulating lipogenic gene networks was measured after overexpression of the 2 isoforms in goat mammary epithelial cells (GMEC). The expression of *PPARG2* was markedly greater in adipose tissue, whereas *PPARG1* is the main isoform in goat mammary tissue (ratio of *PPARG1*:*PPARG2* was close to 37:1). As was reported in previous work, *PPARG1* upregulated the transcription regulators *SREBF1* and *PPARG* and the lipogenic genes *FASN*, *ACACA*, and *SCD*. Along with a tendency for greater expression of *AGPAT6*, *DGAT1*, and *PLIN2*, these data suggest that *PPARG1* is the isoform controlling lipogenesis in mammary cells. Addition of the PPARG ligand rosiglitazone (ROSI) to GMEC overexpressing both isoforms upregulated the expression of *LPL* and *CD36*, which help control uptake of long-chain fatty acids into mammary cells. Other responses to ROSI addition to GMEC overexpressing *PPARG1* and *PPARG2* included upregulation of *AGPAT6*, *DGAT1*, *INSIG1*, *SREBF1*, and *NR1H3*. Although the data suggest that both *PPARG1* and *PPARG2* could affect mammary lipogenesis via control of gene expression when stimulated (e.g., by ROSI), the fact that *PPARG1* is more abundant in mammary tissue and that its overexpression alone upregulated key lipogenic gene networks suggest that it is the more important isoform in goat mammary cells.

Key words: fatty acid metabolism, mammary gland, lactation, gene network, nuclear receptor

INTRODUCTION

Peroxisome proliferator-activated receptor gamma (PPARG) is a ligand-dependent member of the nuclear hormone receptor superfamily with a key role in fat cell differentiation and lipid filling (Rosen and Spiegelman, 2001). Various long-chain fatty acids (LCFA; Kadegowda et al., 2009) and chemical compounds (e.g., thiazolidinediones; Khandoudi et al., 2002) can bind to and activate PPARG, leading to changes in mRNA expression of target genes (Lee et al., 2002). To date, 2 isoforms of PPARG have been identified, *PPARG1* and *PPARG2*, which are generated by alternative use of 2 different promoters (Elbrecht et al., 1996). Compared with *PPARG1*, *PPARG2* has an additional 30 AA in the N terminus sequence in goat (Shi et al., 2013a). In nonruminants, the expression of *PPARG2* is specific to adipose tissue, whereas *PPARG1* is expressed to different extents in several tissues (Fajas et al., 1997).

The specific roles of PPARG isoforms have been extensively studied in humans, leading to the conclusion that *PPARG2* activates adipogenesis in adipose tissue (Ren et al., 2002). The A/B-domain in the N terminus of *PPARG2* plays a gene-specific role for transactivation and co-factor recruitment (Bugge et al., 2009). However, both *PPARG1* and *PPARG2* have the intrinsic ability to stimulate robust adipogenesis in cultured U2OS cells (a human osteosarcoma cell line). In response to low concentrations of rosiglitazone (ROSI), *PPARG2* has a greater ability to induce adipogenesis compared with *PPARG1* (Mueller et al., 2002).

Compared with humans and rodents, the role of PPARG isoforms in coordinating gene network expression in ruminant mammary gland is not well described. Kadegowda et al. (2009), working with bovine mammary cells, were the first to demonstrate that ROSI or saturated LCFA could upregulate lipogenic gene network expression (Kadegowda et al., 2009). Previous work with goat mammary epithelial cells (GMEC) demonstrated that after incubation with ROSI, several

Received December 20, 2013.

Accepted June 3, 2014.

¹Corresponding authors: luojun@nwsuaf.edu.cn and jlloor@illinois.edu

key enzymes related to triacylglycerol (**TAG**) synthesis and secretion were upregulated (Shi et al., 2013b). Conversely, after the knockdown of *PPARG*, key enzymes related to TAG synthesis and secretion were downregulated (Shi et al., 2013b). An important limitation of these earlier studies with ruminant mammary cells is that potential differences of each *PPARG* isoform could not be distinguished.

Although a recent study revealed that both *PPARG* isoforms could affect expression of genes related to desaturation and de novo fatty acid synthesis (Shi et al., 2013a), the role of each *PPARG* isoform in coordinating lipogenic gene networks in mammary cells has not been addressed. Thus, the main objective of this study was to overexpress *PPARG1* and *PPARG2* isoforms in GMEC and measure the mRNA expression of several potential downstream targets of *PPARG*, representing different aspects of lipid metabolism, to evaluate which of these isoforms is more closely associated with the regulation of the lipogenic pathways.

MATERIALS AND METHODS

Tissue Collection

The Animal Care and Use Committee of the Northwest A&F University approved all procedures involving the use of live goats. Three-year-old Xinong Saanen dairy goats at peak lactation and nonlactating ($n = 3$ for each physiological state) from the experimental farm at the Northwest A&F University, Shaanxi, China, were used in the study. Nonlactating goats ($n = 3$) were killed by captive bolt stunning followed by exsanguination. Within 20 min of exsanguination, samples of subcutaneous adipose, skeletal muscle, rumen, mammary gland, liver, spleen, lung, heart, and kidney tissues were collected. Mammary gland tissue was harvested via biopsy from lactating goats ($n = 3$). Percutaneous biopsies from each goat were obtained from the right or left udder as previously described (Farr et al., 1996). Briefly, after making the skin incision, blunt dissection of the mammary capsule was performed to ensure that the tissue obtained was mammary parenchyma during the biopsy. All tissue samples were obtained under sterile conditions and immediately frozen in liquid nitrogen until RNA extraction.

Adenovirus Generation

The adenovirus vectors were generated as described previously (Shi et al., 2013a). Briefly, the cDNA sequences of dairy goat *PPARG1* [National Center for Biotechnology Information (NCBI) no. HQ589347.1] and *PPARG2* (NCBI no. JN854246) were subcloned into

the pAdTrack-CMV plasmid vector between the *XhoI* and *ScaI* (New England BioLabs Inc., Ipswich, MA) restriction sites to generate pAdTrack-CMV-*PPARG1* and pAdTrack-CMV-*PPARG2* vectors. These 2 vectors were inserted into an adenoviral vector (pAdEasy-1) to generate adenoviral plasmids in BJ5183 cells. The adenoviral plasmids linearized by *PacI* (New England BioLabs Inc.) were transfected into 293A cells to generate the adenovirus pAd-*PPARG1* and pAd-*PPARG2*. The adenovirus (**Ad-GFP**) used as a positive control was a gift from Zhijie Chang (Tsinghua University, Beijing, China).

Cell Culture and Treatments

Mammary epithelial cells were isolated from peak-lactation Xinong Saanen goats as described previously (Wang et al., 2010). Details of cell culture were described recently (Lin et al., 2013; Shi et al., 2013b). Cultures of GMEC at approximately 80% confluence were transfected with 1 of the 3 adenovirus supernatants (Ad-*PPARG1*, Ad-*PPARG2*, or Ad-GFP). Transfected GMEC were cultured with the *PPARG*-specific ligand ROSI (Biovision, Milpitas, CA) or control (dimethyl sulfoxide, DMSO, Sigma, St. Louis, MO) at 50 μ M after 24 h of initial culture and then harvested at 48 h (24 h later) for RNA extraction. The 293A cells for adenovirus generation and amplification were cultured in the basal Dulbecco's modified Eagle's medium (Gibco Products International Inc., Langley, OK) containing 10% fetal bovine serum (Gibco Products International Inc.).

Total RNA Extraction, Purification, and Quantitative Real-Time PCR

The procedures for total RNA extraction, purification and quantitative real-time PCR (**qPCR**) were recently described (Lin et al., 2013; Shi et al., 2013b). Total RNA from goat tissues was extracted with Trizol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's directions. Total RNA from GMEC was extracted using the RNA Prep Pure cell kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's protocol. The RNA used in the qPCR was treated with DNAase (Tiangen Biotech Co. Ltd., Beijing, China) to remove genomic DNA contamination. Synthesis of cDNA was conducted using the PrimeScript RT kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions. The qPCR was performed according to manufacturer's instructions using SYBR Green (SYBR Premix Ex Taq II, Perfect Real Time, Takara Bio Inc.).

Genes studied include those related to FA uptake [lipoprotein lipase (**LPL**) or CD36 molecule (thrombo-

spondin receptor, *CD36*], FA de novo synthesis and desaturation [fatty acid synthase (*FASN*), acetyl-CoA carboxylase α (*ACACA*), and stearoyl-CoA desaturase (*SCD*)], triacylglycerol synthesis and hydrolysis [1-acylglycerol-3-phosphate *o*-acyltransferase 6 (*AGPAT6*), diacylglycerolacyltransferase 1 (*DGAT1*), and patatin-like phospholipase domain containing 2 (*PNPLA2*)], lipid droplet formation [perilipin 2 (*PLIN2*) and perilipin 3 (*PLIN3*)], transcription factors [sterol regulatory element-binding transcription factor (*SREBF*) cleavage activating protein (*SCAP*), sterol regulatory element-binding transcription factor 1 (*SREBF1*), liver X receptor α (*NR1H3*), and insulin induced gene 1 (*INSIG1*)]. The sequences for all primers except insulin induced gene 1 (*INSIG1*) used in qPCR were reported previously (Shi et al., 2013b). Primer sequences for *INSIG1* were reported by Bionaz and Loo (2008a). Primer sequences for *PPARG2* were as follows: sense 5'-CCCTGGGAGATGCTCTTATTG-3' and antisense 5'-GTCAAAGGCATGGGAGTGGT-3', which had been tested according to methods described previously (Kadegowda et al., 2009; Shi et al., 2013b) to ensure isoform specificity. Based on current knowledge of the *PPARG1* and *PPARG2* goat sequences, the coding DNA sequence (CDS) of *PPARG2* completely overlaps that of *PPARG1*, precluding us from differentiating *PPARG1* from *PPARG2* using qPCR. The qPCR primers for *PPARG* (i.e., *PPARG1* + *PPARG2*) were as recently described (Shi et al., 2013b).

All the qPCR reactions were performed in a Bio-Rad CFX96 sequence detector (Bio-Rad Laboratories Inc., Hercules, CA) and data were normalized to *GAPDH* because it has been used previously in studies of goat mammary tissue (Ramunno et al., 2005; Wang et al., 2012). However, the limitation of using a single internal control gene is recognized, as reliability of data interpretation increases with the use of at least 3 suitable internal controls (Bionaz and Loo, 2007). Every sample was analyzed in triplicate.

Statistical Analysis

Each treatment was replicated 3 times in culture wells and results are expressed as mean $\pm$ standard errors of the mean. Data of qPCR were analyzed using the $2^{-\Delta\Delta C_t}$ method relative to the Ad-GFP+DMSO control (Lin et al., 2013; Oppi-Williams et al., 2013). The statistical model included the fixed effect of treatment and stage of lactation. Data of qPCR for tissue type were analyzed using the $2^{-\Delta C_t}$ method. Significance of RNA expression between stages of lactation was assessed via *t*-test. Significance of RNA expression across different treatments and among tissues was determined by one-way ANOVA. Significance was declared at $P < 0.05$.

Gene Network Analysis

Networks among the genes studied were generated using Ingenuity Pathway Analysis (IPA; Ingenuity Systems, Redwood City, CA; www.ingenuity.com). The gene network was developed based on results from this study as well as the known relationships available in the IPA knowledge base. That database relies on manually curated data from the published literature (Kadegowda et al., 2009; Moisé et al., 2013).

RESULTS

Distribution of PPARG Isoforms in Various Tissues

As shown in Figure 1A, *PPARG* had the highest ($P < 0.05$) expression in adipose and rumen tissues, followed by spleen, liver, and mammary gland. The lowest ($P < 0.05$) expression was detected in skeletal muscle and kidney. The expression of *PPARG* was also detected in heart and lung, albeit at a very low level. The expression of *PPARG2* appeared to have more restricted distribution, with the greatest expression observed in adipose tissue ($P < 0.05$) followed by lung. The other tissues examined had much lower expression of *PPARG2* (Figure 1B). The analysis also revealed that in mammary, expression of *PPARG* and *PPARG2* mRNA was greater (~ 2 -fold and ~ 1.5 -fold, respectively) at peak lactation compared with the nonlactating period (Figure 1C).

Overexpression of PPARG1 Affects Expression of Key Genes Related to LCFA Metabolism

Overexpression of *PPARG1* without (Ad-PPARG1+DMSO) or with (Ad-PPARG1+ROSI) ROSI markedly upregulated the expression of *PPARG* compared with the control (Ad-GFP+DMSO) and ROSI (Ad-GFP+ROSI) treatments (Figure 2A). However, *PPARG1* overexpression did not ($P > 0.05$) increase mRNA expression of *LPL* or *CD36* compared with control (Ad-GFP+DMSO) or ROSI (Ad-GFP+ROSI) (Figure 2, panels A and B). The addition of ROSI to cultures overexpressing *PPARG1* (Ad-PPARG1+ROSI) upregulated ($P < 0.05$) the expression of these 2 genes relative to other treatments (~ 8.4 -fold and ~ 6.7 -fold, respectively; Figure 2B).

Compared with the control (Ad-GFP+DMSO), genes encoding proteins associated with de novo LCFA synthesis and desaturation (*FASN*, *ACACA*, and *SCD*) had greater ($P < 0.05$) expression when incubated with ROSI and when *PPARG1* was overexpressed (Figure 2C). The highest expression of these genes was observed with the *PPARG1* plus ROSI treatment (Figure 2C). Relative to the control (Ad-GFP+DMSO), the mRNA of *FASN*, *ACACA*, and *SCD* increased by ~ 2.7 -, ~ 1.8 - and ~ 59 -fold, respectively.

Figure 1. Messenger RNA expression of peroxisome proliferator-activated receptor gamma (*PPARG*) and *PPARG2* in goat tissues. (A) Relative mRNA expression distribution of *PPARG* in different goat tissues. The relative expression values are shown on top of each bar. (B) Relative mRNA expression distribution of *PPARG2* in different goat tissues. The relative expression values are shown on top of each bar. (C) Relative to the dry period, mRNA expression of *PPARG* and *PPARG2* at peak lactation increased significantly ($*P < 0.05$). Values are means $\pm$ SEM for 3 individuals. Quantitative PCR data were normalized to *GAPDH*. The different letters denote significant ($P < 0.05$) differences in expression.

Compared with the control (Ad-GFP+DMSO), genes encoding proteins associated with TAG synthesis and lipid droplet formation (*AGPAT6*, *DGAT1*, and *PLIN2*) were upregulated ($P < 0.05$) in cultures incubated with ROSI (Figure 2D). Overexpressing *PPARG1* alone, however, did not increase expression of these genes compared with the control. Addition of ROSI to cells overexpressing *PPARG1* upregulated ($P < 0.05$) expression of *AGPAT6* and *DGAT1* to levels achieved with ROSI alone but enhanced *PLIN2* expression (Figure 2, panels D and E). Overexpression of *PPARG1* had no significant effect on the expression of *PLIN3*. Overexpression of *PPARG1* had no significant effect on the expression of *PNPLA2*, but ROSI alone or overexpression of *PPARG1* plus addition of ROSI upregulated ($P < 0.05$) its mRNA expression above the control (Ad-GFP+DMSO; Figure 2D).

Incubation with ROSI alone upregulated ($P < 0.05$) expression of *SCAP* but did not affect ($P > 0.05$) genes encoding transcription regulators such as *SREBF1*, *INSIG1*, and *NR1H3*. Overexpression of *PPARG1* alone had no significant effect on these genes (Figure 2F). Overexpression of *PPARG1* without ROSI compared with the control (Ad-GFP+DMSO) upregulated ($P < 0.05$) the expression of *SREBF1* by ~ 1.9 -fold. However, addition of ROSI to cultures overexpressing *PPARG1* enhanced ($P < 0.05$) the expression of *SREBF1*, *INSIG1*, and *NR1H3* by ~ 1.8 -, ~ 2.9 -, and ~ 3.5 -fold, respectively.

Overexpression of *PPARG2* Affects the Expression of Key Genes Related to LCFA Metabolism

Relative to the control (Ad-GFP+DMSO), overexpression of *PPARG2* (Ad-*PPARG2*+DMSO) upregulated ($P < 0.05$) the expression of *PPARG* and *CD36* (Figure 3, panels A and B). Overexpression of *PPARG2* had no significant effect on *LPL* under either the treatment with Ad-*PPARG2* or ROSI. Unlike *PPARG1* overexpression, the overexpression of *PPARG2* compared with the control (Ad-GFP+DMSO) did not affect ($P > 0.05$) expression of *FASN* and downregulated ($P < 0.05$) the expression of *ACACA*. Similar to the response observed with the *PPARG1* overexpression, *SCD* was upregulated ($P < 0.05$) in cultures overexpressing *PPARG2* alone (Figure 3C). Addition of ROSI to cultures overexpressing *PPARG2* upregulated ($P < 0.05$) the expression of *PPARG*, *CD36*, *FASN*, and *SCD* relative to the control (Ad-GFP+DMSO; Figure 3, panels A, B, and C).

Relative to the control (Ad-GFP+DMSO), the overexpression of *PPARG2* (Ad-*PPARG2*+DMSO) did not alter ($P > 0.05$) the expression of *AGPAT6*, *DGAT1*, *PNPLA2*, and *PLIN2*, but downregulated ($P < 0.05$)

Figure 2. Changes in gene expression in goat mammary epithelial cells in response to overexpression of peroxisome proliferator-activated receptor gamma (PPARG1; Ad-GFP-PPARG1+DMSO), incubation with rosiglitazone (ROSI) alone (Ad-GFP+ ROSI), or overexpression of PPARG1 with ROSI (Ad-PPARG1+ROSI). The expression of PPARG encompasses both PPARG1 and PPARG2 (panel A). Target genes included those related to FA uptake (*CD36*, *LPL*; panel B); de novo FA synthesis (*FASN* and *ACACA*) and desaturation (*SCD*) (panel C); triacylglycerol synthesis (*AGPAT6* and *DGAT1*) and (*PNPLA2*) (panel D); lipid droplet formation (*PLIN2* and *PLIN3*; panel E); and transcriptional regulation (*SREBF1*, *NR1H3*, *INSIG1*, *SCAP*; panel F). Values are means $\pm$ SEM for 3 individuals. The different letters denote significant ($P < 0.05$) differences in expression among treatments. DMSO = dimethyl sulfoxide.

Figure 3. Changes in gene expression in goat mammary epithelial cells in response to overexpression of peroxisome proliferator-activated receptor gamma (PPARG2; Ad-GFP-PPARG2+DMSO), incubation with rosiglitazone (ROSI) alone (Ad-GFP+ROSI), or overexpression of PPARG2 with ROSI (Ad-PPARG2+ROSI). The expression of PPARG encompasses both PPARG1 and PPARG2 (panel A). Target genes included those related to FA uptake (*CD36*, *LPL*; panel B); de novo FA synthesis (*FASN* and *ACACA*) and desaturation (*SCD*) (panel C); triacylglycerol synthesis (*AGPAT6* and *DGAT1*) and (*PNPLA2*) (panel D); lipid droplet formation (*PLIN2* and *PLIN3*; panel E); and transcriptional regulation (*SREBF1*, *NR1H3*, *INSIG1*, *SCAP*; panel F). Values are means $\pm$ SEM for 3 individuals. The different letters denote significant ($P < 0.05$) differences in expression among treatments. DMSO = dimethyl sulfoxide.

the expression of *PLIN3* (Figure 3, panels D and E). Addition of ROSI to cultures overexpressing *PPARG2* upregulated ($P < 0.05$) the expression of *AGPAT6*, *DGAT1*, *PNPLA2*, and *PLIN2*.

Unlike results observed with the overexpression of *PPARG1*, the overexpression of *PPARG2* alone compared with the control (Ad-GFP+DMSO) resulted in downregulation of *SREBF1* and *SCAP* (Figure 3F). However, the expression of *NR1H3* ($P < 0.05$) and *INSIG1* ($P > 0.05$) was upregulated in response to the overexpression of *PPARG2* (Figure 3, panels A and E). The addition of ROSI to cells overexpressing *PPARG2* led to further upregulation ($P < 0.05$) of *NR1H3* and *INSIG1*.

DISCUSSION

The 2 isoforms of *PPARG*—*PPARG1* and *PPARG2*—were recently cloned in GMEC, and they affected the expression of *FASN*, *ACACA*, *SREBF1*, and *SCD*, which are believed to be key genes encoding lipogenic and desaturation proteins as well as a key lipogenic transcription factor (Shi et al., 2013a). A key question in the present study was whether both *PPARG* isoforms play roles in mammary gland during lactation and whether they differ in the regulation of a broader network of lipogenic genes.

At least in nonruminants, the abundance of *PPARG* isoforms in different tissues is directly related to the specific function they perform (Bionaz et al., 2013). The *PPARG* gene is highly expressed in adipose tissue of mice (Kliwer et al., 1994), human (Fajas et al., 1997), and bovine (Bionaz et al., 2013), all of which agree with the overall higher expression of *PPARG* we observed in goat adipose tissue. The high expression of *PPARG* detected in the rumen, spleen, and lung agrees with previous studies in the bovine (Sundvold et al., 1997; Bionaz et al., 2013). Similar to mouse (Kliwer et al., 1994), human (Fajas et al., 1997), and bovine (Bionaz et al., 2013), the expression of *PPARG* in goat heart, liver, skeletal muscle, and kidney was very low.

The *PPARG2* isoform was reported to be an adipose-specific gene (Tontonoz et al., 1994), but we detected its expression in both adipose and lung tissue. Expression of *PPARG* mRNA was detected in bovine mammary gland tissue and increased markedly in response to lactation (Bionaz and Looor, 2008a). However, in the present study, *PPARG* isoforms were not specifically studied. Despite the inability to directly detect *PPARG1* in the present study, assuming only 2 isoforms are expressed, the difference between *PPARG* and *PPARG2* expression levels can be attributed to *PPARG1*. The present data are therefore highly suggestive that *PPARG1* is the main isoform in goat mam-

mary tissue. Additionally, expression of both *PPARG* and *PPARG2* increased significantly at peak lactation compared with the dry period. Thus, it is possible that both isoforms of *PPARG* play roles in lipid metabolism during lactation. Whether they play different roles remains to be determined.

The genes encoding *LPL* and *CD36* proteins are targets of *PPARG* in nonruminants (Berger and Moller, 2002) and are markedly upregulated in bovine mammary gland during lactation (Bionaz and Looor, 2008a). The similar degree of upregulation of *LPL* and *CD36* in response to ROSI addition to cultures overexpressing *PPARG1* suggests that both genes are targets of *PPARG* in GMEC (Figures 2 and 3). The lack of effect on the expression of *LPL* with the overexpression of *PPARG2* suggests that regulation of lipogenic gene expression through *PPARG* might differ between adipose tissue and mammary epithelial cells (Kadegowda et al., 2009).

In bovine mammary and mammary epithelial cells, *AGPAT6* (Bionaz and Looor, 2008b) and *DGAT1* (Bionaz and Looor, 2008a) are highly expressed and, thus, contribute to the production of milk TAG (Thaller et al., 2003; Schennink et al., 2007; Demeter et al., 2009). The data for *AGPAT6* and *DGAT1* in cultures overexpressing both *PPARG1* and *PPARG2* agree with results from bovine (Kadegowda et al., 2009), demonstrating that ROSI increases their expression in mammary cells. Together with previous data demonstrating that knockdown of *PPARG* downregulated the expression of *AGPAT6* and *DGAT1* (Shi et al., 2013b), it appears that *AGPAT6* and *DGAT1* are likely target genes of *PPARG* in mammary cells (Supplementary Figures S1 and S2; <http://dx.doi.org/10.3168/jds.2013-7863>).

Lipid droplets are secreted into milk via a process that requires lipid droplet-associated proteins such as *PLIN2* (Chong et al., 2011) and *PLIN3* (Wolins et al., 2001; Bulankina et al., 2009). Previous work demonstrated that in GMEC, activation of *PPARG* by ROSI significantly increased the expression of *PLIN2*, but knockdown of *PPARG* still increased the expression of *PLIN2* (Shi et al., 2013b). Although other compensatory factors might still be part of the control of *PLIN2*, together with the present data indicating that both *PPARG1* and *PPARG2* overexpression enhanced *PLIN2*, it appears that *PPARG* regulates the transcription of *PLIN2* in GMEC. Furthermore, the fact that ROSI addition to cultures overexpressing *PPARG1* and *PPARG2* upregulated *PLIN2* beyond the response observed with ROSI alone is suggestive that the regulation of *PLIN2* by *PPARG* is ligand-dependent. The lack of effect on the expression of *PLIN3* with overexpression of *PPARG1* and *PPARG2* suggests that it is not a target gene of *PPARG* in GMEC.

The protein PNPLA2 is involved in the intracellular degradation of TAG in adipose tissue, and its lack of response to *PPARG1* and *PPARG2* overexpression is in accordance with the function of PPARG; that is, a transcription regulator promoting adipogenesis and lipid storage (Rosen and Spiegelman, 2001). However, the increase in *PNPLA2* expression when ROSI was added to cultures overexpressing both PPARG isoforms was similar to results in human adipose cells (Kershaw et al., 2007). This type of response seems to suggest that PPARG might elicit 2 levels of control on gene expression such that the basal expression of a set of lipogenic targets is closely regulated by PPARG, whereas a different set of targets require a ligand. This idea is also supported by the response observed for *PLIN3* in cultures overexpressing *PPARG1* (Figure 2E). In the physiological context of lipogenesis and milk fat synthesis, this type of regulation is not far-fetched given that the lactating mammary cell must synthesize TAG and export it to ensure that intracellular TAG do not accumulate. Several knockout studies in mouse have demonstrated a negative effect of intracellular TAG accumulation on lactation (Ogg et al., 2004; Beigneux et al., 2006).

In murine liver, the transcription factor SREBF1 was demonstrated to regulate several genes related to de novo LCFA synthesis (Le Lay et al., 2002). The maturation (and nuclear activity) of SREBF1 can be controlled via SCAP-INSIG1, which would dictate movement out of the outer endoplasmic reticulum membrane to the Golgi (Shimano, 2001; Eberlé et al., 2004; Kast-Woelbern et al., 2004). The upregulation of *INSIG1* observed with ROSI addition to cultures overexpressing *PPARG1* and *PPARG2* agrees with observations in other species (Kast-Woelbern et al., 2004; Kadegowda et al., 2009; König et al., 2009). As observed in a recent study (Shi et al., 2013a), the link between *SREBF1* and *PPARG* is unique to *PPARG1*, which agrees with the fact that *PPARG1* is the primary isoform expressed in mammary tissue.

If the mechanism of INSIG1 regulation of the mature SREBF1 in rodent white adipose tissue is the same as that in ruminant mammary gland, SREBF1 would be rendered inactive or its function curtailed when *INSIG1* is upregulated (Horton et al., 2003; Kast-Woelbern et al., 2004). As a result, it would be expected that the expression of *ACACA*, *FASN*, and *SCD* would decrease because SREBF1 directly activates their expression in lipogenic tissues including mammary gland (Ntambi, 1995; Roder et al., 2007; Ma and Corl, 2012).

The responses observed for *FASN*, *ACACA*, and *SCD* expression to the specificity of PPARG isoform were reported in a recent study (Shi et al., 2013a). It is noteworthy that after overexpression of *PPARG1* and ad-

dition of ROSI, mRNA expression of *SREBF1*, *FASN*, *ACACA*, and *SCD* reached peak expression (Shi et al., 2013a), despite the fact that *INSIG1* was also markedly upregulated. The responses observed in cells overexpressing *PPARG2* were not alike, and although marked upregulation of *INSIG1* was observed when ROSI was added, the expression of *SREBF1*, *FASN*, and *ACACA* was little affected (Shi et al., 2013a). Thus, alternate mechanisms of control for SREBF1 and target genes seem to exist, which are dependent on PPARG isoform. For instance, it could be possible that the PPARG2 isoform does not target SREBF1, explaining the lack of upregulation even when ROSI was added. In addition, in spite of cleavage inhibition of SREBF1 by INSIG1 protein (Horton et al., 2003; Kast-Woelbern et al., 2004), the present data could suggest that other counter-regulatory mechanisms exist in GMEC such that INSIG1 does not inhibit *SREBF1*. Finally, the inducible upregulation of *SREBF1*, *ACACA*, *FASN*, and *SCD* may have been caused by direct control via PPARG as was recently discussed (Shi et al., 2013a).

The NR1H3 protein is a ligand-activated transcription factor that in nonruminants regulates the synthesis of lipid and controls cholesterol homeostasis in liver (Schultz et al., 2000; Ulven et al., 2005). In rats (Repa et al., 2000; Fiévet and Staels, 2009) and bovine mammary cells (McFadden and Corl, 2010), NR1H3 enhances the expression of *SREBF1*. Other studies reported cross-talk between NR1H3 and PPARG (Villacorta et al., 2007), and NR1H3 can be regulated by PPARG (Chawla et al., 2001). The effect of PPARG on SREBF1 in turn could be mediated by NR1H3 (Kast-Woelbern et al., 2004).

The data revealed that only the overexpression of *PPARG2* upregulated the mRNA expression of *NR1H3*. However, it was surprising that the response was accompanied by a downregulation of *SREBF1* or a lack of change when ROSI was added to the culture medium. Because this isoform is primarily expressed in adipose tissue (Figure 1B), the data suggest that any role of PPARG2 in terms of altering the activity of *SREBF1* and its target genes is more likely to occur in nonmammary tissue. Indeed, PPARG2 seemed to inhibit the SREBF1 signaling pathway (Supplementary Figure S2; <http://dx.doi.org/10.3168/jds.2013-7863>), which agrees with results in human white adipose cells (Kast-Woelbern et al., 2004).

Our group has cloned the promoter of goat *SCD* and, upon performing transcription factor site analysis, we observed that 2 potential PPAR response element sites exist on the *SCD* promoter (data not shown). Jointly with the upregulation of *ACACA* and *FASN*, those data suggest that PPARG1 along with NR1H3 and SREBF1 control de novo LCFA synthesis and desatu-

Table 1. Genes induced in goat mammary epithelial cells after overexpression of peroxisome proliferator-activated receptor gamma (*PPARG*)1 or *PPARG*2¹

Gene	Description	G1/G2	(G1+ROSI)/(G2+ROSI)
Transcription regulators			
<i>INSIG1</i>	Insulin induced gene 1	1.10	0.97
<i>NR1H3</i>	Liver X receptor alpha	0.87	1.15
<i>SCAP</i>	SREBP cleavage activating protein	1.05	1.21
<i>SREBF1</i>	Sterol regulatory element-binding transcription factor 1	2.77	1.37
FA uptake			
<i>CD36</i>	CD36 molecule (thrombospondin receptor)	1.07	1.35
<i>LPL</i>	Lipoprotein lipase	0.61	1.20
Lipid droplet formation			
<i>PLIN2</i>	Perilipin 2	0.73	0.85
<i>PLIN3</i>	Perilipin 3	1.26	1.07
Triacylglycerol synthesis and hydrolysis			
<i>AGPAT6</i>	1-Acylglycerol-3-phosphate O-acyltransferase 6	1.27	0.92
<i>DGAT1</i>	Diacylglycerolacyltransferase 1	1.00	0.99
<i>PNPLA2</i>	Patatin-like phospholipase domain containing 2	0.91	0.93
FA de novo synthesis and desaturation			
<i>ACACA</i>	Acetyl-CoA carboxylase α	2.19	1.06
<i>FASN</i>	Fatty acid synthase	2.36	1.82
<i>SCD</i>	Stearoyl-CoA desaturase	6.55	2.48

¹Shown is a list of genes involved in de novo long-chain fatty acid synthesis, lipid metabolism, and secretion that were induced independently by *PPARG*1 and *PPARG*2. For each gene, the ratio between the expression values obtained with overexpression of *PPARG*1 (G1) versus overexpression of *PPARG*2 (G2) is reported as G1/G2 and (G1+ROSI)/(G2+ROSI), where ROSI is rosiglitazone.

ration in lactating ruminant mammary gland (Table 1, Supplementary Figure S1; <http://dx.doi.org/10.3168/jds.2013-7863>).

The findings pertaining to *PPARG*1 overexpression plus ROSI are in agreement with work in bovine mammary epithelial cells (Kadegowda et al., 2009). In contrast, the responses observed with *PPARG*2 overexpression plus ROSI are more similar to the negative regulation that PPARG elicits on LCFA biosynthesis in white adipose cells (Kast-Woelbern et al., 2004). These data are remarkably consistent with the fact that *PPARG*1 appears to be the primary isoform in the goat mammary gland. Additionally, according to the data observed in the present study, we deduced that PPARG isoforms have different signaling pathways in terms of regulating LCFA biosynthesis and TAG formation. It is likely that *PPARG*2 competes with *PPARG*1 for binding to PPAR response element sites, hence, indirectly affecting the function of *PPARG*1 in ruminant mammary cells.

Milk fat composition in ruminants could be controlled at different levels, including genetic variation. For instance, it has been demonstrated that polymorphisms of *SCD* (Mele et al., 2007; Macciotta et al., 2008), *AGPAT6* (Littlejohn et al., 2014), and *DGAT1* (Argov-Argaman et al., 2013) can determine milk fat composition in ruminant milk. In humans, the common PPARG Pro12Ala polymorphism is associated with lipid metabolism and decreased risk of human type 2 diabetes (Ghoussaini et al., 2005; Ereqat et al., 2009); less information about its polymorphism is known in ruminant milk fat.

The fact that PPARG knockout mice produce a toxic, proinflammatory milk (Wan et al., 2007) and PPARG knockdown in GMEC downregulated the genes related to fatty acid synthesis and TAG synthesis (Shi et al., 2013b) suggest that PPARG is also related to an inflammatory response during lactation.

CONCLUSIONS

In summary, our data indicate that, when activated, both *PPARG*1 and *PPARG*2 could participate in regulating the hydrolysis of circulating TAG and uptake of FA. In spite of the inability to directly differentiate *PPARG*1 from *PPARG*2, lipid droplet formation and de novo lipogenesis in mammary cells appear more closely related to PPARG1 activation, with this nuclear receptor acting through its control of SREBF1 and to some extent NR1H3. If, as the present data suggest, the PPARG2 isoform has a unique role in adipose compared with mammary tissue, it might help explain the marked effect on *CD36* and *PLIN2* because these proteins would be essential in terms of allowing adipose tissue to store fatty acids in the form of TAG. Although mammary cells rely on exogenous fatty acids for synthesis of TAG, the process of de novo synthesis is an inherent feature of this tissue. In conclusion, future work using high-throughput technology such as gene microarrays or RNA sequencing (RNA-seq) could help address questions about the unique transcriptional difference between PPARG1 and PPARG2 in ruminant mammary gland.

ACKNOWLEDGMENTS

This research was jointly supported by the Special Fund for Agro-scientific Research in the Public Interest (Beijing, China; 201103038) and the National Natural Science Foundation of China (Beijing, China; 31072013).

REFERENCES

- Argov-Argaman, N., K. Mida, B.-C. Cohen, M. Visker, and K. Hettinga. 2013. Milk fat content and DGAT1 genotype determine lipid composition of the milk fat globule membrane. *PLoS ONE* 8:e68707.
- Beigneux, A. P., L. Vergnes, X. Qiao, S. Quatela, R. Davis, S. M. Watkins, R. A. Coleman, R. L. Walzem, M. Philips, K. Reue, and S. G. Young. 2006. Agpat6—A novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. *J. Lipid Res.* 47:734–744.
- Berger, J., and D. E. Moller. 2002. The mechanisms of action of PPARs. *Annu. Rev. Med.* 53:409–435.
- Bionaz, M., S. Chen, M. J. Khan, and J. J. Loor. 2013. Functional role of PPARs in ruminants: Potential targets for fine-tuning metabolism during growth and lactation. *PPAR Res.* 2013:684159.
- Bionaz, M., and J. Loor. 2008a. Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* 9:366.
- Bionaz, M., and J. J. Loor. 2007. Identification of reference genes for quantitative real-time PCR in the bovine mammary gland during the lactation cycle. *Physiol. Genomics* 29:312–319.
- Bionaz, M., and J. J. Loor. 2008b. ACSL1, AGPAT6, FABP3, LPIN1, and SLC27A6 are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation. *J. Nutr.* 138:1019–1024.
- Bugge, A., L. Grøntved, M. M. Aagaard, R. Borup, and S. Mandrup. 2009. The PPAR γ 2 A/B-domain plays a gene-specific role in transactivation and cofactor recruitment. *Mol. Endocrinol.* 23:794–808.
- Bulankina, A. V., A. Deggerich, D. Wenzel, K. Mutenda, J. G. Witmann, M. G. Rudolph, K. N. J. Burger, and S. Höning. 2009. TIP47 functions in the biogenesis of lipid droplets. *J. Cell Biol.* 185:641–655.
- Chawla, A., W. A. Boisvert, C. H. Lee, B. A. Laffitte, Y. Barak, S. B. Joseph, D. Liao, L. Nagy, P. A. Edwards, L. K. Curtiss, R. M. Evans, and P. Tontonoz. 2001. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol. Cell* 7:161–171.
- Chong, B. M., P. Reigan, K. D. Mayle-Combs, D. J. Orlicky, and J. L. McManaman. 2011. Determinants of adipophilin function in milk lipid formation and secretion. *Trends Endocrinol. Metab.* 22:211–217.
- Demeter, R. M., G. C. B. Schopen, A. G. J. M. Oude Lansink, M. P. M. Meuwissen, and J. A. M. van Arendonk. 2009. Effects of milk fat composition, *DGAT1*, and *SCD1* on fertility traits in Dutch Holstein cattle. *J. Dairy Sci.* 92:5720–5729.
- Eberlé, D., B. Hegarty, P. Bossard, P. Ferré, and F. Foufelle. 2004. SREBP transcription factors: Master regulators of lipid homeostasis. *Biochimie* 86:839–848.
- Elbrecht, A., Y. L. Chen, C. A. Cullinan, N. Hayes, M. D. Leibowitz, D. E. Moller, and J. Berger. 1996. Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. *Biochem. Biophys. Res. Commun.* 224:431–437.
- Erekat, S., A. Nasereddin, K. Azmi, Z. Abdeen, and R. Amin. 2009. Impact of the Pro12Ala polymorphism of the PPAR-gamma 2 gene on metabolic and clinical characteristics in the Palestinian type 2 diabetic patients. *PPAR Res.* 2009:874126.
- Fajas, L., D. Auboeuf, E. Raspe, K. Schoonjans, A. M. Lefebvre, R. Saladin, J. Najib, M. Laville, J. C. Fruchart, S. Deeb, A. Vidal-Puig, J. Flier, M. R. Briggs, B. Staels, H. Vidal, and J. Auwerx. 1997. The organization, promoter analysis, and expression of the human PPAR gamma gene. *J. Biol. Chem.* 272:18779–18789.
- Farr, V. C., K. Stelwagen, L. R. Cate, A. J. Molenaar, T. B. McFadden, and S. R. Davis. 1996. An improved method for the routine biopsy of bovine mammary tissue. *J. Dairy Sci.* 79:543–549.
- Fiévet, C., and B. Staels. 2009. Liver X receptor modulators: Effects on lipid metabolism and potential use in the treatment of atherosclerosis. *Biochem. Pharmacol.* 77:1316–1327.
- Ghoussaini, M., D. Meyre, S. Lobbens, G. Charpentier, K. Clement, M.-A. Charles, M. Tauber, J. Weill, and P. Froguel. 2005. Implication of the Pro12Ala polymorphism of the PPAR-gamma 2 gene in type 2 diabetes and obesity in the French population. *BMC Med. Genet.* 6:11.
- Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown, and J. L. Goldstein. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA* 100:12027–12032.
- Kadegowda, A. K. G., M. Bionaz, L. S. Piperova, R. A. Erdman, and J. J. Loor. 2009. Peroxisome proliferator-activated receptor- γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. *J. Dairy Sci.* 92:4276–4289.
- Kast-Woelbern, H. R., S. L. Dana, R. M. Cesario, L. Sun, L. Y. de Grandpre, M. E. Brooks, D. L. Osburn, A. Reifel-Miller, K. Klausning, and M. D. Leibowitz. 2004. Rosiglitazone induction of Insig-1 in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor γ and sterol regulatory element-binding protein in the regulation of adipogenesis. *J. Biol. Chem.* 279:23908–23915.
- Kershaw, E. E., M. Schupp, H.-P. Guan, N. P. Gardner, M. A. Lazar, and J. S. Flier. 2007. PPAR γ regulates adipose triglyceride lipase in adipocytes in vitro and in vivo. *Am. J. Physiol. Endocrinol. Metab.* 293:E1736–E1745.
- Khandoudi, N., P. Delerive, I. Berrebi-Bertrand, R. E. Buckingham, B. Staels, and A. Bril. 2002. Rosiglitazone, a peroxisome proliferator-activated receptor γ , inhibits the Jun NH2-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury. *Diabetes* 51:1507–1514.
- Kliwer, S. A., B. M. Forman, B. Blumberg, E. S. Ong, U. Borgmeyer, D. J. Mangelsdorf, K. Umesono, and R. M. Evans. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* 91:7355–7359.
- König, B., A. Koch, J. Spielmann, C. Hilgenfeld, F. Hirche, G. I. Stangl, and K. Eder. 2009. Activation of PPAR α and PPAR γ reduces triacylglycerol synthesis in rat hepatoma cells by reduction of nuclear SREBP-1. *Eur. J. Pharmacol.* 605:23–30.
- Le Lay, S., I. Lefrère, C. Trautwein, I. Dugail, and S. Krief. 2002. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. *J. Biol. Chem.* 277:35625–35634.
- Lee, G., F. Elwood, J. McNally, J. Weiszmann, M. Lindstrom, K. Amaral, M. Nakamura, S. Miao, P. Cao, R. M. Learned, J.-L. Chen, and Y. Li. 2002. T0070907, a selective ligand for peroxisome proliferator-activated receptor γ , functions as an antagonist of biochemical and cellular activities. *J. Biol. Chem.* 277:19649–19657.
- Lin, X., J. Luo, L. Zhang, W. Wang, H. Shi, and J. Zhu. 2013. MiR-27a suppresses triglyceride accumulation and affects gene mRNA expression associated with fat metabolism in dairy goat mammary gland epithelial cells. *Gene* 521:15–23.
- Littlejohn, M. D., K. Tiplady, T. Lopdell, T. A. Law, A. Scott, C. Harland, R. Sherlock, K. Henty, V. Obolonkin, K. Lehnert, A. MacGibbon, R. J. Spelman, S. R. Davis, and R. G. Snell. 2014. Expression variants of the lipogenic AGPAT6 gene affect diverse milk composition phenotypes in *Bos taurus*. *PLoS ONE* 9:e85757.
- Ma, L., and B. A. Corl. 2012. Transcriptional regulation of lipid synthesis in bovine mammary epithelial cells by sterol regulatory element binding protein-1. *J. Dairy Sci.* 95:3743–3755.
- Macciotta, N. P. P., M. Mele, G. Conte, A. Serra, M. Cassandro, R. Dal Zotto, A. C. Borlino, G. Pagnacco, and P. Secchiari. 2008. As-

- sociation between a polymorphism at the stearoyl CoA desaturase locus and milk production traits in Italian Holsteins. *J. Dairy Sci.* 91:3184–3189.
- McFadden, J. W., and B. A. Corl. 2010. Activation of liver X receptor (LXR) enhances de novo fatty acid synthesis in bovine mammary epithelial cells. *J. Dairy Sci.* 93:4651–4658.
- Mele, M., G. Conte, B. Castiglioni, S. Chessa, N. P. P. Macciotta, A. Serra, A. Buccioni, G. Pagnacco, and P. Secchiari. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. *J. Dairy Sci.* 90:4458–4465.
- Moisá, S. J., D. W. Shike, W. T. Meteer, D. Keisler, D. B. Faulkner, and J. J. Loor. 2013. Yin yang 1 and adipogenic gene network expression in longissimus muscle of beef cattle in response to nutritional management. *Gene Regul. Syst. Bio.* 7:71–83.
- Mueller, E., S. Drori, A. Aiyer, J. Yie, P. Sarraf, H. Chen, S. Hauser, E. D. Rosen, K. Ge, R. G. Roeder, and B. M. Spiegelman. 2002. Genetic analysis of adipogenesis through peroxisome proliferator-activated receptor γ isoforms. *J. Biol. Chem.* 277:41925–41930.
- Ntambi, J. M. 1995. The regulation of stearoyl-CoA desaturase (SCD). *Prog. Lipid Res.* 34:139–150.
- Ogg, S. L., A. K. Weldon, L. Dobbie, A. J. H. Smith, and I. H. Mather. 2004. Expression of butyrophilin (Btln1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. *Proc. Natl. Acad. Sci. USA* 101:10084–10089.
- Oppi-Williams, C., J. K. Suagee, and B. A. Corl. 2013. Regulation of lipid synthesis by liver X receptor alpha and sterol regulatory element-binding protein 1 in mammary epithelial cells. *J. Dairy Sci.* 96:112–121.
- Ramunno, L., G. Cosenza, A. Rando, A. Pauciuolo, R. Illario, D. Gallo, D. Di Berardino, and P. Masina. 2005. Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland. *Gene* 345:289–299.
- Ren, D., T. N. Collingwood, E. J. Rebar, A. P. Wolffe, and H. S. Camp. 2002. PPAR gamma knockdown by engineered transcription factors: Exogenous PPAR γ 2 but not PPAR γ 1 reactivates adipogenesis. *Genes Dev.* 16:27–32.
- Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J.-M. A. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXR α and LXR β . *Genes Dev.* 14:2819–2830.
- Roder, K., L. Zhang, and M. Schweizer. 2007. SREBP-1c mediates the retinoid-dependent increase in fatty acid synthase promoter activity in HepG2. *FEBS Lett.* 581:2715–2720.
- Rosen, E. D., and B. M. Spiegelman. 2001. PPAR γ : A nuclear regulator of metabolism, differentiation, and cell growth. *J. Biol. Chem.* 276:37731–37734.
- Schennink, A., W. M. Stoop, M. H. P. W. Visker, J. M. L. Heck, H. Bovenhuis, J. J. van der Poel, H. J. F. van Valenberg, and J. A. M. van Arendonk. 2007. DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. *Anim. Genet.* 38:467–473.
- Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, and B. Shan. 2000. Role of LXRs in control of lipogenesis. *Genes Dev.* 14:2831–2838.
- Shi, H. B., J. Luo, D. W. Yao, J. J. Zhu, H. F. Xu, H. P. Shi, and J. J. Loor. 2013a. Peroxisome proliferator-activated receptor-Y stimulates the synthesis of monounsaturated fatty acids in dairy goat mammary epithelial cells via the control of stearoyl-coenzyme A desaturase. *J. Dairy Sci.* 96:7844–7853.
- Shi, H. B., J. Luo, J. J. Zhu, J. Li, Y. T. Sun, X. Z. Lin, L. P. Zhang, D. W. Yao, and H. P. Shi. 2013b. PPAR γ regulates genes involved in triacylglycerol synthesis and secretion in mammary gland epithelial cells of dairy goats. *PPAR Res.* 2013:310948.
- Shimano, H. 2001. Sterol regulatory element-binding proteins (SREBPs): Transcriptional regulators of lipid synthetic genes. *Prog. Lipid Res.* 40:439–452.
- Sundvold, H., A. Brzozowska, and S. Lien. 1997. Characterisation of bovine peroxisome proliferator-activated receptors γ 1 and γ 2: Genetic mapping and differential expression of the two isoforms. *Biochem. Biophys. Res. Commun.* 239:857–861.
- Thaller, G., W. Kramer, A. Winter, B. Kaupe, G. Erhardt, and R. Fries. 2003. Effects of *DGAT1* variants on milk production traits in German cattle breeds. *J. Anim. Sci.* 81:1911.
- Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPAR gamma 2: Tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* 8:1224–1234.
- Ulvén, S. M., K. T. Dalen, J.-Å. Gustafsson, and H. I. Nebb. 2005. LXR is crucial in lipid metabolism. *Prostaglandins Leukot. Essent. Fatty Acids* 73:59–63.
- Villacorta, L., M. Garcia-Barrio, and Y. Chen. 2007. Transcriptional regulation of peroxisome proliferator-activated receptors and liver X receptors. *Curr. Atheroscler. Rep.* 9:230–237.
- Wan, Y., A. Saghatelian, L.-W. Chong, C.-L. Zhang, B. F. Cravatt, and R. M. Evans. 2007. Maternal PPAR γ protects nursing neonates by suppressing the production of inflammatory milk. *Genes Dev.* 21:1895–1908.
- Wang, W., J. Luo, Y. Zhong, X. Lin, H. Shi, J. Zhu, J. Li, Y. Sun, and W. Zhao. 2012. Goat liver X receptor α , molecular cloning, functional characterization and regulating fatty acid synthesis in epithelial cells of goat mammary glands. *Gene* 505:114–120.
- Wang, Z., J. Luo, W. Wang, W. Zhao, and X. Lin. 2010. Characterization and culture of isolated primary dairy goat mammary gland epithelial cells. *Sheng Wu Gong Cheng Xue Bao* 26:1123–1127.
- Wolins, N. E., B. Rubin, and D. L. Brasaemle. 2001. TIP47 associates with lipid droplets. *J. Biol. Chem.* 276:5101–5108.