Meat Science 96 (2014) 704–711

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Gene expression profiling of SIRT1, FoxO1, and PPARγ in backfat tissues and subcutaneous adipocytes of Lilu bulls

Xiaomu Liu, [1], Guifen Liu [1], Xiuwen Tan, Hongbo Zhao, Haijian Cheng, Fachun Wan, Naike Wu, Enliang Song *

Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Sangyuan Road Number 8, Ji'nan City, Shandong Province, 250100, China
Shandong Key Lab of Animal Disease Control and Breeding, Sangyuan Road Number 8, Ji'nan City, Shandong Province, 250100, China

ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 12 March 2013
Accepted 20 September 2013

Keywords:
Backfat tissue
Subcutaneous adipocytes
Sirtuin 1
Forkhead box O1
Peroxisome proliferator-activated receptor-γ

ABSTRACT

The temporal pattern of gene expression of sirtuin 1 (*SIRT1*), forkhead box O1 (*FoxO1*), and peroxisome proliferator-activated receptor-γ (*PPARγ*) in differentiating bovine preadipocytes and in backfat tissue from Lilu bulls 12, 18, 24, and 30 months old was investigated using real-time quantitative PCR; Carcass characteristics and adipocyte diameters were also measured. The upregulation of *PPARγ* and the downregulation of *SIRT1* and *FoxO1* were observed in the backfat tissue of Lilu cattle with increasing age. Moreover, the results showed that fat accumulation in Lilu cattle may primarily be related to an increase in mature fat cell numbers after 18 months of age. The present study indicates *SIRT1* may play an important role in the development of bovine adipose tissue *in vivo*. Although *SIRT1*, *FoxO1*, and *PPARγ* expression appeared to be nonlinear during the stages of preadipocyte differentiation, these genes play an important role during bovine adipocyte development in Lilu cattle.

1. Introduction

In many countries, fat is an unpopular constituent of meat for consumers and is considered to be unhealthy. However, the quantity and distribution of fat and fatty acids in the adipose tissue and muscles are important contributors to various aspects of meat quality and are central to the nutritional quality of the meat (Cho & Baik, 2010; Wood et al., 2008). An understanding of the mechanisms regulating fat deposition and metabolism in cattle is important, because this knowledge may lead to the production of meat that will be more healthful and appealing to consumers.

The adipocyte plays a central role in overall metabolic regulation, serving both as a storage depot for fatty acids (Morrison & Farmer, 2000) and as an endocrine cell to regulate energy utilization and feeding behavior. Adipose tissue mass is controlled by a balance of cell proliferation and increases in fat cell size, known as hyperplasia and hypertrophy, respectively (Roh, Hishikawa, Hong, & Sasaki, 2006). Further, dysregulation of adipocyte proliferation and differentiation can lead to a reduction in animal meat quality (Hausman et al., 2009).

The sirtuin family of proteins possesses NAD-dependent deacetylase and ADP-ribosyltransferase activities and regulates numerous biological functions, such as longevity and metabolism (Wang & Tong, 2009). Sirtuin type 1 (*SIRT1*), the best characterized of the 7 sirtuin family members, plays a key modulatory role in animal fat deposition (Picard et al., 2004). Moreover, this enzyme is involved in adipogenesis and can promote fat mobilization in white adipocytes (Chalkiadaki & Guarente, 2012; Picard et al., 2004; Wolf, 2006). Ghinis-Hozumi et al. (2011) first reported the expression of bovine sirtuins in the liver, muscle, and adipose tissue and established a tissue-specific, developmentally regulated expression profile for *SIRT1* and *SIRT3*. Nevertheless, the mechanism of their action at the cellular level in beef cattle has not yet been determined. *SIRT1* regulates several transcription factors that govern fat metabolism, including peroxisome proliferator-activated receptor-γ (*PPARγ*) (Picard et al., 2004), forkhead-box transcription factors (Kobayashi et al., 2005; Yang, Hou, Haller, Nicosia, & Bai, 2005), and adiponectin (Qiang, Wang, & Farmer, 2007; Qiao & Shao, 2006). The activation of *SIRT1* promotes fat mobilization by repressing *PPARγ*, which is involved in fat storage (Picard & Auwerx, 2002; Picard et al., 2004). The expression of forkhead box O1 (*FoxO1*) is induced in the early stages of adipocyte differentiation; however, its activation is delayed until the end of the clonal-expansion phase. *SIRT1* regulates the activity of *FoxO1* (Brunet et al., 2004; Nemoto, Fergusson, & Finkel, 2004), while *SIRT2* suppresses adipogenesis by deacetylating *FoxO1* to promote *FoxO1* binding to *PPARγ* and to thereby repress *PPARγ* transcriptional activity (Wang & Tong, 2009). As *SIRT1* is also known to deacetylate *FoxO1* (Kobayashi et al., 2005), the *SIRT1* inhibition of adipocyte differentiation may also be mediated through the regulation of the FoxO1–PPARγ interaction.

The genetic heritage of Lilu beef cattle is 62.5% Limousin and 37.5% local Luxi cattle (Liu et al., 2012). We previously investigated the growth performance, carcass characteristics, and meat quality of Lilu beef cattle at 4 different ages during their growth and

* Corresponding author at: Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Sangyuan Road Number 8, Ji'nan City, Shandong Province 250100, China. Tel.: +86 0531 88676857; fax: +86 0531 88967665.
E-mail address: enliangs@126.com (E. Song).
[1] These authors contributed equally to this work.

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.09.019

Table 1
Ingredients and chemical composition of the basal diet (%DM).

Ingredients	Content
Chinese wild rye	61.61
Corn	27.00
Wheat bran	2.89
Cottonseed meal	6.75
Calcium hydrogen phosphate	0.35
Calcium carbonate	0.58
Sodium chloride	0.39
Sodium bicarbonate	0.39
Mineral premix	0.04
Vitamin premix	0.19
Total	100.00
Analyzed composition	
CP%	9.55
ADF%	27.68
NDF%	52.27
Ca%	0.86
Calculated composition	
NEm/(MJ/kg)	6.48

Note: The mineral premix provides the following components per kg: 40 g of Mn, 60 g of Zn, 20 g of Fe, 30 g of Cu, 1.5 g of I, 1 g of Se, and 400 mg of Co. The vitamins premix provides the following components per kg: 30,000,000 IU of vitamin A; 100,000,000 IU of vitamin D; and 6000 mg of vitamin E.

Table 2
Primer sequences of genes selected for analysis by real-time qRT-PCR.

Gene	Accession number	Primer	bp	Temp (°C)
SIRT1	NM_001192980.1	F: AGTGGCGGCTGAGAGGGAGG R: GTACCCAATAGCGGCCGCCG	331	57
FoXO1	XM_002691748.1	F: GCAACGCGTGGGGCAACCTGT R: GGGCACGCTCTTCACCATCCACTC	115	58
PPARγ	NM_181024.2	F: AATCAAAGTGGAGCCTGTATC R: CCTGATGGCATTATGAGACA	313	56
β-actin	NM_173979.3	F: TGACCCAGATCATGTTTGAGA R: CAAGGTCCAGACGCAGGAT	256	56

investigated 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMGR*) expression levels in muscle and adipose during these 4 different ages (Liu et al., 2012). In addition, *in vivo* and *in vitro* studies have recently been performed on the physiological functions of genes and factors related to adipogenesis in beef (Cho & Baik, 2010; Gallardo, Varela-Echavarria, Miyasaka, & Izaguirre, 2008; Ghinis-Hozumi et al., 2011; Liu et al., 2012; Wang et al., 2010).

Based on the previous studies, we hypothesize that *SIRT1*, *FoxO1*, and *PPARγ* may play an important role during bovine adipocyte development in Lilu cattle, and that fat accumulation may primarily be related to mature fat cell numbers. For testing the accuracy of our hypothesis, the goal of the current study was to investigate the molecular mechanisms underlying the accumulation of backfat tissue in cattle at 4 different ages and the differentiation of subcutaneous (s.c.) adipocytes at various developmental stages by analyzing the expression patterns of *SIRT1*, *FoxO1*, and *PPARγ*. Understanding the genetic interaction profile in these tissues and cells will provide knowledge about the biochemical pathways involved in the adipogenesis of bovine adipocytes in Lilu beef cattle, which will enable a greater understanding of the molecular and cellular factors pertaining to meat quality and fat deposition in Lilu beef cattle.

2. Materials and methods

2.1. Animals and sample collection

Forty Lilu bulls were maintained under identical conditions at 12 months of age. The housing facility conformed with Chinese national standards (Laboratory Animal Requirements of Environment and Housing Facilities, GB 14925-2010). The animals were nurtured and ultimately slaughtered according to Chinese national law (China Administration Rule of Laboratory Animal; Operating Procedure of Cattle Slaughtering GB/T 19477-2004). The basal diet (Table 1) consisted of a total mixed ration (TMR) formulated to meet the nationally mandated nutritional requirements for cattle (Feeding Standard of Cattle of the People's Republic of China, NY/T815-2004). The TMR was offered to the cattle in equal amounts on a twice-daily basis, and all cattle had free access to fresh water.

All the animals began the trial at the same time (12 months), and serial slaughters were conducted every 6 months over the following 18 months, with 10 bulls slaughtered at each age. Details in terms of animals and tissue collection have been previously outlined by our group (Liu et al., 2012). At slaughter, the hot carcasses were weighed, and the dressing percentage was calculated. After a 24-h post-slaughter chill, the ribeye area and the thickness of the 12th rib backfat were determined. A sample of the longissimus dorsi muscle was removed as a standard 2.5-cm thick steak, and s.c. fat near the last 12th and 13th rib was frozen in liquid nitrogen and stored at −80 °C until further use. A digital tenderness meter (Model C-LM3B, Northeast Agricultural University, China) was used to measure the meat tenderness once the center of the muscle samples had reached 70 °C. A drill with a round sampler removed 10 meat columns (1.27 cm in diameter) in a direction parallel to the muscle fiber. Each meat column was sheared in the tenderness meter, and the data were recorded. An average of 10 values was used to determine the shear force of this muscle.

2.2. Tissue lipid content and adipocyte diameter analyses

The total intramuscular lipid content was determined in the muscle after extraction with chloroform-methanol precipitation (Gardan, Gondret, & Louveau, 2006). Adipocyte diameters were determined for 5 serial cross-sections (10 μm thick, at 40-μm intervals) from frozen s.c. fat cut with a cryostat (603269; YONGFENG, Zhejiang, China), as described previously by Gardan et al. (2006). Cross sections were fixed for 10 min in 100 mM phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde and were stained for 4 min in isopropanol containing 0.5% Oil Red O. Image acquisition of the adipocyte areas was achieved with the Olympus DP controller software (DP controller 3.3.1.292; Olympus Imaging Corp, Tokyo, Japan), and individual adipocyte areas were investigated by using an image analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD, USA). The rare visible cells that displayed a diameter smaller than 10 μm were not considered (Gondret & Lebret, 2002). One hundred fat cells per cross section were measured. The results corresponded to the mean values obtained for analyses performed on 5 sections for each sample and were expressed in terms of the diameter (μm) of visible adipocytes. The coefficient of variation for cell diameter between the 5 successive sections was 9.9%.

2.3. Cell culture and induction of preadipocyte differentiation

To investigate the induction of preadipocyte differentiation, cells from s.c. adipose tissues of three 12-month-old Lilu bulls were isolated and cultured using a method previously described by Aso et al. (1995) as well as our group (Liu et al., 2009). In brief, the cells were propagated and maintained in DMEM/F12 (Hyclone, Logan, UT, USA) containing 10% FBS (Gibco, Grand Island, NY, USA) with an antibiotic–antimycotic agent (containing 100 IU/mL penicillin and 100 μg/mL streptomycin; Gibco). The cells were incubated at a density of 2×10^4 cells/cm^2 at 37 °C under a humidified 5% CO_2 atmosphere. To induce adipogenesis of bovine preadipocytes, 2-day post-confluent preadipocytes (designated Day 0) were incubated in differentiation-induction medium with DMEM/F12 containing 10 μg/mL insulin (Sigma, St. Louis, MO, USA), 0.25 μM DEX (Sigma), and 0.5 mM IBMX (Sigma) for 2 days. The cells were then transferred into differentiation medium with DMEM/F12