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Research paper

Regulation of lipid metabolism in adipose depots of fat-tailed and thin-tailed lambs during negative and positive energy balances

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ABSTRACT

This study aimed to evaluate the effects of negative and positive energy balances on gene expression of regulators and enzymes controlling lipogenesis and lipolysis in muscle and adipose depots of fat-tailed and thin-tailed lambs. Lambs were slaughtered during neutral, negative and positive energy balances for sample collection. Real time q-PCR was conducted to measure the gene expression. Expression of *PPARγ* was increased in response to positive energy balance regardless of genotype and type of tissue ($P < 0.04$). Expression of *SREBF1* was reduced in response to negative and positive energy balances in fat-tailed lambs, whereas in thin-tailed lambs, downregulated *SREBF1* was restored during positive energy balance ($P < 0.01$). Enhancement in *FABP4* expression in response to negative and positive energy balances was respectively higher in thin-tailed and fat-tailed lambs affected by interaction of genotype and energy balance ($P < 0.11$). In thin-tailed lambs, the enhanced *FABP4* expression in response to negative energy balance was considerably higher in mesenteric adipose depot, whereas in fat-tailed lambs, positive energy balance induced enhancement in *FABP4* expression was considerably higher in fat-tail adipose depot. The results demonstrate that transcription regulation of lipogenesis and lipolysis during negative and positive energy balances occurs differently in fat-tailed and thin-tailed lambs. Thin-tailed and fat-tailed lambs are respectively more responsive to negative and positive energy balances and mesenteric and fat-tail adipose depots respectively in thin-tailed and fat-tailed lambs are the main adipose depots responsible for higher responsiveness of thin-tailed and fat-tailed lambs to negative and positive energy balances.

1. Introduction

Acquisition of fat-tail is a unique characteristic of all sheep breeds in semi-arid and arid regions of the world. Despite their higher body fat content comparing to thin-tailed breeds, fat-tailed breeds can easily tolerate periods of seasonal feed shortage without suffering from detrimental effect of harsh non-esterified fatty acids (NEFA) mobilization during negative energy balance (Atti et al., 2004; Almeida, 2011). These breeds have high proportion of body fat accumulated in fat-tail region, whereas thin-tailed breeds accumulate considerable proportion of body fat in visceral adipose depots. Fat-tail as a body reserve has been reported to be less responsive to negative energy balance and lipolytic stimulus (Khachadurian et al., 1966) and due to its great mass, low and constant release of NEFA from this site can guarantee the viability of animal through periods of feed shortage (Atti et al., 2004). On the other hand, visceral adipose depots have been reported to be more responsive to lipolytic stimulus and variation in energy balance (Van Harmelen et al., 1997; Giorgino et al., 2005). Moreover, visceral adipose depots have more capacity for preformed fatty acid uptake, whereas subcutaneous adipose depot is more dependent on de novo lipogenesis (Ji et al., 2014a). Despite above mentioned differences in metabolism of adipose depots, it is not clear how the transcription factors are regulated in various adipose depots in response to negative energy balance which lead to different level of fat mobilization in fat-tailed and thin-tailed lambs (Atti et al., 2004). Furthermore, the lipolytic sensitivity and relative contribution of de novo synthesis and preformed fatty acid uptake in lipogenesis is not defined in various adipose depots of fat-tailed and thin-tailed lambs. As peroxisome

Abbreviations: NEFA, non-esterified fatty acids; TMR, total mixed ration; CNCPS, Cornell net carbohydrate and protein system; cDNA, complementary DNA; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PPIA, peptidylprolyl isomerase A; YWHAZ, Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide; PPARγ, peroxisome proliferator activated receptor gamma; SREBF1, sterol regulatory element-binding transcription factor 1; FABP4, fatty acid binding protein 4; LPL, lipoprotein lipase; ACAC, acetyl-CoA carboxylase; SCD, stearoyl-CoA desaturase; ACLY, ATP citrate lyase; HSL, hormone sensitive lipase; Ct, threshold cycle; NE_L, energy for lactation

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Table 1
Sequence and some characteristics of primers designed for reference and target genes.

Genes[1]	Accession number	Forward and reverse sequence	Fragment length (bp)	Annealing temperature (°C)
GAPDH	NM_001190390.1	ACGCTCCCATGTTTGTGATG	146	58.83
		CATAAGTCCCTCCACGATGC		58.13
PPIA	NM_001308578.1	TTGCAGACAAAGTCCCGAAG	121	58.41
		CCACCCTGGCACATAAATCC		58.60
YWHAZ	NM_001267887.1	GTTCTTGATCCCAAACGCTTC	119	57.80
		CCACAATCCCTTTCTTGTCATC		57.29
PPARγ	NM_001100921.1	GCATTTCTGCTCCGCACTAC	120	59.62
		ATACAGGCTCCACTTTGATTGC		58.98
SREBF1	XM_015098336.1	CTGCCCATCAACCGACTTG	100	58.83
		AGCGGTAGCGTTTCTCGATG		60.53
FABP4	NM_001114667.1	AAACTGGGATGGGAAATCAACC	109	58.83
		TGCTCTCTCGTAAACTCTGGTAGC		61.93
LPL	NM_001009394.1	CCAGCAGCATTATCCAGTGTC	120	59.05
		CCCAAGAGATGCACATTACCC		58.70
ACAC	NM_001009256.1	CGACTGCAACCACATCTTCC	101	59.20
		GACTTCCATAGCGCATCACC		58.78
SCD	NM_001009254.1	AGTACCGCTGGCACATCAAC	100	60.67
		AAGACGGCAGCCTTGGATAC		60.11
HSL	NM_001128154.1	ACAGCAGCGACACAACAGAC	108	61.15
		CAGATTCATCCTCAGACCCAAG		58.19
ACLY	XM_012185121.2	ATGTCCTGTTCCACCACGAG	109	59.68
		TTCTTGATGTCCTCGGGATTC		57.46

GAPDH; glyceraldehyde 3-phosphate dehydrogenase, PPIA; peptidylprolyl isomerase A, YWHAZ; Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide, PPARγ; peroxisome proliferator activated receptor gamma, SREBF1; sterol regulatory element-binding transcription factor 1, FABP4; fatty acid binding protein 4, LPL; lipoprotein lipase, ACAC; acetyl-CoA carboxylase, SCD; stearoyl-CoA desaturase, ACLY; ATP citrate lyase, HSL; hormone sensitive lipase.

proliferator activator receptor γ (*PPARγ*) and sterol regulatory element-binding transcription factor 1 (*SREBF1*) are two main transcription factors controlling lipogenesis in adipose tissue (Lapsys et al., 2000; Ferre and Foufelle, 2010) and lipoprotein lipase (*LPL*), fatty acid binding protein 4 (*FABP4*), acetyl-CoA carboxylase (*ACAC*), stearoyl-CoA desaturase (*SCD*) and ATP citrate lyase (*ALCY*) are their main target genes responsible for preformed fatty acid uptake and de novo lipogenesis respectively and hormone sensitive lipase (*HSL*) is responsible for stimulated lipolysis, this study was conducted to evaluate the expression of these genes in muscle and adipose depots of Lori-Bakhtiari pure fat-tailed breed and Lori-Bakhtiari × Romanov cross-breed thin-tailed male lambs during negative and positive energy balances.

2. Material and methods

2.1. Experimental design, animals, diet, and sampling

The experiment was conducted at Natural Resources & Agricultural Research Farm of Tehran University, Karaj, Iran. Thirty-six male lambs with average body weight of 41.08 ± 4.59 and age of 5–6 months were divided into 3 groups (6 Lori-Bakhtiari pure breed and 6 Lori-Bakhtiari and Romanov F1 cross breed lambs in each group) according to their body weight and placed in individual pens. The experiment began after two weeks of adaptation to pen and lasted for 42 days. Lambs were fed balanced total mixed ration (TMR) formulated by Cornell net carbohydrate and protein system (CNCPS) software program 1.5 fold of their maintenance requirement during the adaptation period. The amount of feed was adjusted weekly according to the lamb's body weight change during the whole experiment. At the end of adaptation period, the first group (6 fat-tailed and 6 thin-tailed lambs) was randomly selected and slaughtered by weighting after 16 h of feed deprivation. The two remained groups were fed 90, 80 and 70% of their maintenance requirement respectively during weeks 1, 2 and 3 of experiment. At the end of week 3, the second group was randomly selected and slaughtered and the remained group was fed ad-libitum until to the end of experiment (days 42) and then slaughtered for sample collection. Samples of longissimus dorsi muscle from the 12–13 ribs site and adipose tissue from fat-tail, subcutaneous, perirenal and mesenteric depots were collected immediately after slaughtering and kept in liquid nitrogen until the subsequent analysis.

2.2. Total RNA extraction and complementary DNA (cDNA) synthesis

Total RNA was extracted from muscle and adipose tissues according to the method of Chomczynski and Sacchi (2006) using YTzol kit (Yekta Tajhiz Azma Co., Cat No; YT9063). Extracted RNA was treated with RNase-free DNase I in order to remove the remnant genomic DNA from samples (TaKaRa, Shuzo, Kyoto, Japan). The RNA abundance was estimated by nanodrop spectrophotometry at 260 nm, and purities were checked by determining the absorption ratios at 260/280 nm. Quality of the extracted RNA was assessed by electrophoresis at 1% agarose-gel containing ethidium bromide. First-strand cDNA was synthesized from 100 ng of total RNA by oligo (dT) primer, random hexamers and cDNA synthesis kit (M-MuLV Reverse Transcriptase, Cinaclon Co, Cat No; PR911658) according to manufacturer's instructions. Process of cDNA synthesis was initiated by connection of primers at 37 °C for 1 min followed by cDNA synthesis at 42 °C for 60 min and terminated by inactivation of reverse transcriptase enzyme at 85 °C for 5 min. Synthesized cDNA was kept at − 20 °C to be used.

2.3. Primer designing

Nucleotide sequences of reference genes including glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), peptidylprolyl isomerase A (*PPIA*), Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (*YWHAZ*), and target genes including *PPARγ*, *SREBF1*, *FABP4*, *LPL*, *ACAC*, *SCD*, *ACLY* and *HSL* belonging to sheep (*Ovis aries*) were obtained from public databases (GenBank, National Center for Biotechnology Information). Primers were designed according to these sequences (optimal T_m at 61 °C and GC between 45 and 50%) using primer3Plus (Untergasser et al., 2007) online software program and primer suitability was evaluated using OligoAnalyzer 3.1 (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/) and OligoCalc (Kibbe, 2007). Specificity of designed primers was examined by PrimerBLAST software of NCBI database (Ye et al., 2012). Sequence and some characteristics of designed primers are presented in Table 1.