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Feed restriction alters lipogenic and adipokine gene expression in visceral and subcutaneous fat depots in lamb

Jinli Yang [a,*], Xinxing Lu [a], Xianzhi Hou [a], Hairong Wang [a], Caixia Shi [a], Guichao Wang [a], Shuqing Wu [b], Aiwu Gao [c,*]

[a] College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, People's Republic of China
[b] College of Veterinary Medicine, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, People's Republic of China
[c] College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, People's Republic of China

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ABSTRACT

Currently, lipogenic and adipokine gene expression patterns in different fat depots during long-term feed restriction in growing lambs are poorly understood. This study aimed to determine the effects of long-term nutritional deficiency on lipogenic and adipokine gene expression in subcutaneous and visceral fat depots in lambs. Twenty male lambs (4 months old; 21.81 ± 0.75 kg body weight) were randomly assigned to either control or feed-restricted groups. After 60 days of maintenance-level feed restriction, femoral, omental, mesenteric, epicardial, and perirenal fat were collected. Gene expressions were estimated by quantitative real-time PCR, and plasma parameters were also determined. The results showed that feed restriction increased the plasma concentration of NEFA ($P < 0.05$) at 30 days, and decreased the plasma concentration of triglycerides, glucose, and insulin ($P < 0.05$) at 30 days and 60 days. Feed restriction decreased lipogenic gene expression (acetyl coenzyme A carboxylase alpha and fatty acid synthase) in all five fat depots. The related key regulating gene PPARγ mRNA decreased ($P < 0.05$) in femoral, omental, and mesenteric fat depots, while sterol regulatory element binding transcription factor 1 decreased ($P < 0.05$) in femoral fat depots. Perilipin, as a barrier to lipolysis, decreased ($P < 0.05$) in femoral, omental and perirenal fat depots of the feed restriction group. Adipose tissue-derived hormone leptin mRNA decreased ($P < 0.05$) in all five fat depots, while adiponectin decreased ($P < 0.05$) only in omental, mesenteric, and epicardial depots. Macrophage marker CD68 mRNA increased ($P < 0.05$) in femoral and mesenteric fat depots. In addition, tumor necrosis factor-α mRNA increased in femoral, omental, and mesenteric depots, whereas IL-1β mRNA increased in femoral and perirenal depots. IL-6 expression decreased in femoral, omental, and epicardial fat depot at the end of feed restriction. Nutritional deficiency induced different changes in lipogenic and adipokine gene expression between visceral and subcutaneous adipose tissue. The decreased leptin, adiponectin, and increased inflammatory factors may inhibit lipogenesis and stimulate lipolysis to protect the body against the threat of starvation.

1. Introduction

White adipose tissue (WAT) is a complex, essential, and highly active metabolic and endocrine organ that has received increased attention in the past decade (Hotamisligil, 2006). In addition to secreting several endocrine hormones (such as leptin and adiponectin), fat cells also synthesize a number of cytokines including tumor necrosis factor-α (TNFα) and interleukin (IL)-6 that participate in the pathogenesis of metabolic syndrome (Galic et al., 2010). Adipocytes are also thought to be immunologically active and share striking commonalities with immune cells by producing a number of antimicrobial peptides (Zhang et al., 2015).

Immune and metabolic processes are coordinately regulated by adipose tissue (Hotamisligil, 2006). Excess energy intake induces adipocyte expansion, triggering macrophage infiltration and chronic inflammation in WAT (Weisberg et al., 2003). This is now recognized as an important cause of obesity-related insulin resistance (Gregor and Hotamisligil, 2011). Interestingly, short-term feed restriction (FR) in mice, which mobilizes triglyceride stores and increases extracellular free fatty acid levels, also induces recruitment of macrophages and changes the cytokine expression patterns in adipose tissue (Kosteli et al., 2010). In dairy cows during dry periods and early lactation, insufficient feed intake

* Corresponding authors.
E-mail addresses: yangjinli207@aliyun.com (J. Yang), 171544952@qq.com (X. Lu), houxz05@sina.com (X. Hou), wanghairong97@163.com (H. Wang), shicx98@163.com (C. Shi), 1186726537@qq.com (G. Wang), wushuqing2009@126.com (S. Wu), gao_69nm@163.com (A. Gao).

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induces mobilization of body fat and increases plasma concentrations of non-esterified fatty acids (NEFA), and TNFα expression in subcutaneous adipose. This suggests down regulation of maternal insulin sensitivity helps ensure an adequate nutrient supply to the fetus and mammary tissue (Sano et al., 1993; Sadri et al., 2010; Sordillo and Raphael, 2013). However, different WAT depots possess different anatomical, cellular, and molecular compositions (Ibrahim, 2010). The varying energy signaling, endocrine, and cytokine secretion pattern between subcutaneous and visceral WAT in response to changes in energy availability have been studied in both human and rodents (Del Mar Romero et al., 2009; Sitticharoon et al., 2014).

Young animals, such as calves and lambs, after a period of growth restriction, may show a greater growth rate than unrestricted animals after restoring free access to good-quality feed (Berge, 1991 Yang et al., 2014). During this process, both the weight and composition of the animal adipose tissues changes significantly (Yambayamba et al., 1996). To date, lipogenic and adipokine gene expression patterns in different fat depots during long-term FR in growing lamb remain poorly defined (Meale et al., 2014). Therefore, this study was designed to delve further into the effects of long-term nutritional deficiency on lipogenic and adipokine gene expression in femoral, omental, mesenteric, epicardial, and perirenal fat depots in lambs.

2. Materials and methods

The animal study was approved by Animal Experimentation Ethics Committees of Inner Mongolia Agriculture University.

2.1. Animals

Twenty male lambs (4 months old; 21.81 ± 0.75 kg body weight) from a local breeder were used in this study, and randomly assigned to either control (CON) or FR groups. The feed restriction experiment lasted for 60 d, ten lambs in the FR groups were fed limited amounts of chopped grass hay to meet minimal maintenance requirements and ten lambs in CON group were fed a mixed ration, which supplied maintenance requirements and a daily gain of 150 g (Zhang and Zhang, 1998). At the end of 60 days feed restriction, five lambs in each group whose weight was similar to the average weight of their group were slaughtered to collect samples. To assess the compensatory growth capacity of lambs in the FR group, the remaining lambs were returned to a fattening diet and *ad libitum* feeding for 60 d after shearing the wool (realimentation period). The composition and nutrient content of the diets are presented in Table 1. Lambs were fed at 0800 h and 1800 h each day. Feed and oats were weighed and recorded daily in the morning before feeding, and the amount of feed offered adjusted at 10-d intervals after weighing the lambs. Fresh water and mineral mixture blocks (Yuantongweiye Co., Ltd., Inner Mongolia, China) were freely available.

2.2. Blood sampling and biochemical analyses

Blood samples were collected at 30 and 60 d before morning feeding by venipuncture using vacuette tubes (HuaBo Medical Instrument Co., Ltd, Shandong, China) containing sodium heparin. Samples were immediately centrifuged at 3000 g for 15 min and the plasma stored at −70 °C. Total cholesterol, triglycerides, glucose, and NEFA content were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). An ovine insulin ELISA kit (Mercodia AB, Uppsala, Sweden) was used to measure insulin concentrations according to the method described by Mahjoubi et al. (2014). Ninety-six-well microplates were read at 450 nm using a multi-detection microplate reader (Bio-Tek Synergy HT, Winooski, VT, USA).

Table 1
Ingredients and chemical composition of diets.

Item	Restriction period		Realimentation period
	CON	FR	
Ingredients, % of DM			
Grass hay	69.5	100	50
Corn	10		32
Soybean meal	18		15.5
Limestone	0.5		0.5
NaCl	1		1
Premix[a]	1		1
Total	100	100	100
Chemical composition			
ME[b], MJ/kg	8.95	7.84	9.85
Crude protein, %	10.68	8.28	12.22
Fat, %	2.49	2.13	3.01
NDF, %	47.19	62.11	37.23
ADF, %	29.27	38.92	22.87
Ash, %	7.31	7.35	6.98

CON, control group; FR, feed restricted group.

[a] Premix provided per kilogram of basal diet: Ca 1.52 g, P 0.41 g, Zn 31 mg, Fe 24 mg, Cu 8 mg, I 0.8 mg, Co 0.1 mg, Mn 19.1 mg, Se 0.23 mg, nicotinic acid 80 mg, VA 3500 IU, VE 20 IU, VD3 1500 IU.

[b] Metabolizable energy was calculated according to the data of Zhang and Zhang (1998).

2.3. Adipose tissue sampling, RNA extraction, and cDNA synthesis

Water and feed were removed from the animals 12 h before slaughter. Femoral subcutaneous fat, omental fat, and mesenteric, epicardial, and perirenal fat were rapidly isolated, submerged in cold (4 °C) sample protector for RNA/DNA (Takara Bio Company, Dalian, China), and stored at −80 °C until processing.

Frozen samples (200 mg) were homogenized in lysing matrix D tubes (MP Biomedicals, Illkrich, France) containing RNAiso plus (Takara Bio Company) with a FastPrep-24 instrument (MP Biomedicals). Homogenates processed for RNA extraction following the manufacturer's instruction. RNA (2 μg) was reverse transcribed in a final volume of 40 μL using PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio Company). Potential contaminating genomic DNA was eliminated by treatment for 2 min at 42 °C with gDNA eraser, and reverse-transcription performed for 15 min at 37 °C and inactivated at 85 °C for 5 s according to the manufacturer's instructions.

2.4. Analysis of gene expression

Expression of WAT acetyl coenzyme A carboxylase alpha (ACACA), fatty acid synthase (FASN), perilipin, peroxisome proliferator activated receptor-γ (PPARγ), sterol regulatory element binding transcription factor 1 (SREBP1), adiponectin, leptin, as well as CD68, IL-1β, IL-6, and TNFα were estimated by quantitative real-time PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serves as a suitable endogenous reference gene in adipose and used as a control in all samples (Murthi et al., 2008; Zang et al., 2011). Specific primer pairs for SREBP1, IL-1β, TNFα, and GAPDH were obtained from previous studies (French et al., 2006; Viturro et al., 2009; Yan et al., 2011a, 2011b). For all other genes, primers were designed using Primer 3 software (Rozen and Skaletsky, 2000). All primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Co. Ltd. (Shanghai,