Livestock Science 168 (2014) 168–176

Contents lists available at ScienceDirect

Livestock Science

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

Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle

Myunggi Baik [a,b,*,1], Jin Young Jeong [c,1], Thi- Thu Thao Vu [d], Min Yu Piao [a,b], Hyeok Joong Kang [a]

[a] Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea
[b] Institute of Green Bio Science Technology, San 967-2, Sin-ri, Daehwa-myeon, Pyeungchang-gun, Gangwond-do, Republic of Korea
[c] National Institute of Animal Science, RDA, Suwon 441-706, Republic of Korea
[d] Department of Molecular Biotechnology, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 10 June 2014
Received in revised form
11 August 2014
Accepted 18 August 2014

Keywords:
Fat depots
Adipogenesis
Castration
Intramuscular fat
Korean cattle

ABSTRACT

Castration increases intramuscular (IM) fat deposition in the *musculus longissimus dorsi*, a highly desirable trait in Korean beef cattle. However, castration may also affect accumulation in other fat depots. We examined whether castration affects adipose cellularity and lipid metabolism gene expression in various fat depots, including subcutaneous (SC), abdominal (AB), perirenal (PR), and IM fat. First, frozen sections taken from fat depots were stained with hematoxylin and eosin and the mean fat cell size was determined. Steers showed larger cell sizes in AB ($P=0.01$), SC ($P=0.05$), and PR ($P=0.01$) fat compared to bulls. Next, lipid metabolism gene expression in AB fat was compared between bulls and steers. In AB fat, steers showed increased mRNA expression of CCAAT/enhancer binding protein alpha (C/EBPα), a gene related to adipogenesis, compared to bulls ($P<0.01$). In contrast, steers showed decreased protein expression of medium-chain acyl-CoA dehydrogenase (MCAD), an enzyme involved in fatty acid β-oxidation, compared to bulls ($P<0.05$). Our results demonstrate that castration induces hypertrophy in body fat cells, and that the up-regulation of adipogenesis and down-regulation of fatty acid β-oxidation may in part contribute to this effect. We compared fat cell sizes among the various fat depots in Korean cattle steers. The order of fat cell size was AB > PR > SC > IM. The expression levels of lipid metabolism genes were compared among various fat depots. The mRNA levels of C/EBPα and peroxisome proliferator-activated receptor-γ were highest ($P<0.05$) in AB fat and lowest in IM fat. The mRNA levels of fatty acid binding protein-4 and lipogenic acetyl-coenzyme A carboxylase and fatty acid synthase genes were highest ($P<0.05$) in AB fat, and lowest in IM fat. Expression levels of lipolytic hormone-sensitive lipase and fatty oxidation MCAD genes were also highest ($P<0.05$) in AB fat and lowest in IM fat. Our results suggest that combined effects of higher adipogenesis, lipogenesis, and cellular fatty acid transport are responsible for the largest AB and smallest IM fat cells among the fat depots examined.

* Correspondence to: Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea. Tel.: +82 2 880 4809; fax: +82 2 873 2271.
E-mail address: mgbaik@snu.ac.kr (M. Baik).
[1] Authors made equal contribution.

http://dx.doi.org/10.1016/j.livsci.2014.08.013
1871-1413/

1. Introduction

Castration of bulls markedly increases intramuscular (IM) fat deposition in the *musculus longissimus dorsi* (LM), resulting in improved beef quality in Korean cattle (Bong et al., 2012; Jeong et al., 2012). In Korea, steers are slaughtered at a later age and heavier weight than are bulls, which is intended to maximize the degree of marbling. However, delayed slaughter may also negatively impact profits due to excessive fat deposition and decreased tenderness. In a previous study, we showed that steers had increased subcutaneous (SC) fat deposition compared to bulls, as determined by backfat thickness (Bong et al., 2012). Fat depots in other parts of the body may also be affected, including abdominal (AB) and perirenal (PR) fats. Excessive deposition of SC, AB, and PR fat does not contribute to meat quality; instead, this fat is trimmed from the carcass before sale and thus represents an economic loss. Thus, the site and quantity of fat deposited in beef cattle are of major importance for optimizing meat quality against efficient production. The ability to produce highly marbled beef, without excessive deposition elsewhere, requires an understanding of the distinct mechanisms regulating fat deposition in IM fat *vs*. other depots.

Fat deposition is regulated through both, increases in adipocyte number (hyperplasia) and adipocyte size (hypertrophy). Hyperplasia is the first step in fat accumulation during the early life of animals, and generally does not occur in the late fattening period in cattle (Cianzio et al., 1985). Instead, increases in whole-body fat content are strongly correlated with the size of individual adipocytes (Robelin, 1986). Little information exists on adipose cellularity in Korean cattle breed.

Body fat deposition is affected by several lipid metabolism pathways, including adipogenesis, lipogenesis, and fatty acid β-oxidation. Previously, we reported that castration modulates gene expression associated with lipid metabolism in LM muscle and SC fat depots (Bong et al., 2012; Jeong et al., 2012, 2013). However, little is known regarding the effects of castration on adipose cellularity and the expression of genes related to lipid metabolism in other fat depots, including AB fat.

Therefore, this study examined whether castration affects adipose cellularity in several fat depots. First, we hypothesized that steers would show differences in cell size in SC, AB, PR, and IM fat compared to bulls. Next, because differences in adipocyte size might be associated with changes in the expression of genes controlling lipid metabolism, we examined the expression of adipogenesis, lipogenesis, and fatty acid β-oxidation genes in AB fat collected from steers and bulls. We also compared fat cell sizes and lipid metabolic gene expression levels among the various fat depots in Korean cattle steers.

2. Material and methods

All experimental procedures involving animals were approved by the Chonnam National University Institutional Animal Use and Care Committee (CNUIAUCC), Republic of Korea. The experiments were conducted in accordance with the Animal Experimental Guidelines provided by CNUIAUCC.

2.1. Animals

In this study, we used samples from 10 bulls and 10 steers collected during a previous study, and the feeding method was described previously (Bong et al., 2012). Briefly, animals were fed concentrate diets that consisted of 15% crude protein (CP) and 71% total digestible nutrient (TDN) until 14 months of age, 13% CP/72% TDN until 20 months of age, and 11% CP/73% TDN after 21 months of age. Roughage was offered *ad libitum*, and the animals had free access to freshwater. Slaughter age was 20 months for bulls and 28 months for steers, with mean carcass weights of 347 kg and 398 kg, respectively. Carcass characteristics are described in more detail in our previous paper (Bong et al., 2012). Briefly, steer LM had a 3.7-fold greater IM fat content (11%) than bull LM (3.0%, $P < 0.001$).

2.2. Fat samples

SC fat samples were taken from the dorsal area of the 13th rib, approximately 13 cm lateral to the midline. PR fat samples were collected from fat attached to the kidney. AB fat samples were dissected from the abdomen. In steers, IM fat was excised from the LM; however, it was not possible to isolate IM fat from bulls, as LM fat deposition was negligible and barely visible to the naked eye. Fat samples were stored at −80 °C or used for the preparation of frozen sections.

2.3. Frozen sections and hematoxylin and eosin staining

Six bulls and six steers were randomly chosen for analysis of adipose cellularity. Fat samples were fixed in 10% buffered formalin, immersed in 10–15% sucrose with 1 × phosphate-buffered saline (PBS), embedded in optimal cutting temperature (OCT) compound, flash-frozen in liquid nitrogen-cooled isopentane, and stored at −80 °C. The OCT-embedded frozen tissues were cut into 12-μm sections using a cryostat at −20 °C and stained using a standard protocol. Briefly, frozen sections were washed in distilled water, stained in hematoxylin solution for 5 min, and washed in running tap water for 5 min. After soaking the sections in 1% acid alcohol for 10–30 s, they were washed again in running tap water for 30 s, counterstained in eosin solution for 10–30 s, and rinsed in running tap water for 30 s. The sections were then passed through an ascending series of ethanol solutions (70%, 80%, 95%, and 100% ethanol) for 1 min each, and cleared in two changes of xylene for 10 min each. Slides were mounted using a xylene-based mounting medium.

2.4. Determination of adipocyte number and size

The size and number of adipocytes were examined in a cross-sectional area of 1.44 mm^2. Cell number was determined under a light microscope at 40 × magnification and analyzed using image analysis software (Image Pro Analyzer, Media Cybernetics, USA). Due to the relatively small area of