Cloning and Expression of Lactate Dehydrogenase H Chain Gene in Adipose Tissues of Korean Cattle

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ABSTRACT : To understand molecular mechanisms that regulate deposition and release of intramuscular fat, a fasting-induced clone was identified by differential screening from cDNA library of adipose tissues of Korean cattle. The clone had a total length of 1,319 nucleotides coding for 334 amino acids. It was identified as one encoding L-lactate dehydrogenase H chain (LDH-B). Comparison of the deduced amino acid sequences of bovine LDH-B with those of pig, human, rat, and mouse showed 98%, 98%, 97%, and 96% identity, respectively. Food deprivation for 48 h increased mRNA levels of LDH-B gene in adipose tissues of Korean cattle compared to fed- and 6 h refed- tissues. The expression of obese mRNA was examined for individual adipose tissue from several fat depots. Fasting induced expression of LDH-B gene in subcutaneous adipose tissues, but it did not affect expression levels in abdominal, perirenal and intramuscular tissues. Results demonstrate that induction of LDH-B gene during fasting may represent a metabolic shift from anaerobic state to aerobic predominance in fasted adipose tissues and that its responses to fasting are different among several adipose tissues. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1670-1674)*

Key Words : Korean Cattle, Lactate Dehydrogenase B Gene, Adipose Tissues, Fasting

INTRODUCTION

The deposition of intramuscular adipose tissue (marbling) is positively related to beef flavor and palatability (Judge et al., 1989) and it is an important factor for high quality beef, especially in Korean cattle and Japanese Black cattle. Efforts have been made to produce high-marble beef using by nutritional program, castration, and immunological treatment. However, the molecular mechanism that regulates intramuscular fat deposition and its release has not been well studied. To produce high marble beef, a study is required to understand the differential mechanism that regulates the deposition and release of fat between intramuscular tissues and external adipose tissues, especially subcutaneous adipose tissues.

This study was performed to understand molecular events that regulate the deposition of fat and its release in adipose tissues. The primary objective was to identify fasting-induced clones by differential screening of bovine adipocyte cDNA library. One clone was identified as a full-length cDNA encoding bovine lactate dehydrogenase H (LDH-B) chain. Northern analysis was conducted to examine expression levels of LDH-B mRNA at feeding and fasting states in several adipose tissues of Korean cattle.

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MATERIALS AND METHODS

Tissue samples

Nine Korean cattle (Hanwoo) weighing about 550 kg and at 21 months of age were used. Three bulls were fed normally, three were starved for 48 h, and three were refed 6h after 48 h fasting. After their slaughter, the adipose tissues were collected from abdominal, perirenal, subcutaneous, intermuscular and intramuscular fat depots. Pieces of intramuscular fat tissues were dissected from *longissimus dorsi* muscle tissues. Care was taken not to contaminate with muscular tissue and intermuscular fat tissues connected to them.

Construction of cDNA library of bovine adipose tissues

Total RNA was extracted from pooled adipose tissues containing equal amounts of abdominal, perirenal, subcutaneous, intermuscular and intramuscular tissues by the acid/guanidinium thiocyanate/phenol chloroform method (Chomzynski and Sacchi, 1987). A cDNA library was constructed by using poly A RNA extracted from the pooled total RNAs of several adipose tissues (abdominal, perirenal, subcutaneous, intermuscular and intramuscular) from both fed and fasted animals. The λZAP express vector was used to construct the directional cDNA library according to the manufacturer's protocol (Stratagene, USA). Briefly, the first strand cDNA was synthesized using the poly A RNA, Xho 1-$(dT)_{15}$ primer and AMV reverse transcriptase. The second strand cDNA was synthesized using RNase H, DNA polymerase and DNA ligase. The EcoR I-adapted cDNAs were ligated to the λZAP express vector, and the recombinant vectors were subjected to *in*

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                                                                GCGCGCCTGCAGGTCGAC
ACTAGTGGATCCAAAGAATTCGGCACGAGCTGACTTTGTCTTCGTGCAGCCCTTATCACTCTCTGGAGAACTTTCTCTCCCCTGGGCACA  108
ATGGCAACTCTTAAGGAAAAATTGATTGCACCAGTTGCAGAAGAAGAGACAACGATCCCAAACAATAAGATCACTGTAGTGGGTGTTGGA  198
 M  A  T  L  K  E  K  L  I  A  P  V  A  E  E  E  T  T  I  P  N  N  K  I  T  V  V  G  V  G    30
CAAGTTGGTATGGCATGTGCCATCAGCATTCTGGGAAAGTCTCTGACTGACGAGCTTGCTCTTGTGGATGTTTTGGAAGATAAACTCAAA  288
 Q  V  G  M  A  C  A  I  S  I  L  G  K  S  L  T  D  E  L  A  L  V  D  V  L  E  D  K  L  K    60
GGAGAAATGATGGACCTGCAGCACGGGAGCTTATTCCTTCAGACACCAAAAATTGTGGCAGACAAAGATTACTCTGTCACTGCCAATTCC  378
 G  E  M  M  D  L  Q  H  G  S  L  F  L  Q  T  P  K  I  V  A  D  K  D  Y  S  V  T  A  N  S    90
AAGATCGTGGTGGTAACTGCAGGAGTTCGCCAGCAAGAAGGGGAGAGTCGCCTGAATTTGGTGCAAAGGAACGTTAACGTCTTCAAGTTC  468
 K  I  V  V  V  T  A  G  V  R  Q  Q  E  G  E  S  R  L  N  L  V  Q  R  N  V  N  V  F  K  F   120
ATCATTCCTCAGATCGTCAAGTACAGTCCTGCCTGCATCATCATTGTGGTTTCCAACCCAGTGGATATTCTCACATATGTTACCTGGAAA  558
 I  I  P  Q  I  V  K  Y  S  P  A  C  I  I  I  V  V  S  N  P  V  D  I  L  T  Y  V  T  W  K   150
CTAAGTGGATTACCCAAGCACCGTGTGATTGGGAGTGGATGTAACCTGGATTCTGCTAGATTTCGCTACCTTATGGCTGAAAAACTTGGC  648
 L  S  G  L  P  K  H  R  V  I  G  S  G  C  N  L  D  S  A  R  F  R  Y  L  M  A  E  K  L  G   180
ATTCATCCCAGCAGCTGCCACGGATGGATTTTGGGGGAACATGGCGACTCAAGCGTGGCTGTGTGGAGTGGAGTGAATGTGGCAGGCGTT  738
 I  H  P  S  S  C  H  G  W  I  L  G  E  H  G  D  S  S  V  A  V  W  S  G  V  N  V  A  G  V   210
TCTCTCCAGGAACTGAATCCAGAAATGGGAACAGACAATGATAGTGAAAATTGGAAGGAAGTGCATAAGATGGTGGTTGAGAGTGCCTAT  828
 S  L  Q  E  L  N  P  E  M  G  T  D  N  D  S  E  N  W  K  E  V  H  K  M  V  V  E  S  A  Y   240
GAAGTCATCAAGCTAAAAGGATATACCAACTGGGCTATTGGATTAAGTGTGGCTGATCTTATTGAATCCATGTTGAAAAATCTATCGAGG  918
 E  V  I  K  L  K  G  Y  T  N  W  A  I  G  L  S  V  A  D  L  I  E  S  M  L  K  N  L  S  R   270
ATTCACCCAGTGTCAACAATGGTGAAGGGCATGTATGGCATTGAGAATGAAGTCTTCCTGAGCCTTCCGTGTATCCTGAATGCTCGAGGG 1008
 I  H  P  V  S  T  M  V  K  G  M  Y  G  I  E  N  E  V  F  L  S  L  P  C  I  L  N  A  R  G   300
TTAACCAGTGTTATCAACCAGAAGCTGAAGGATGAAGAGGTTGCTCAACTCAAGAAAAGTGCAGACACCCTCTGGGGCATCCAAAAGGAC 1098
 L  T  S  V  I  N  Q  K  L  K  D  E  E  V  A  Q  L  K  K  S  A  D  T  L  W  G  I  Q  K  D   330
CTGAAGGACCTGTGACTTCCGGCTGCAAGGCTGTAGACACTTAGCAACTACAGTGTGATTAACCACAAGCCTTTAGTTTGCATCCATGTA 1188
 L  K  D  L  *                                                                              334
CATGGAGCACAGTTCGCTTTTGTCTTCCTTAAGTCTGTGAATCTGGGCTCCCAGAATCAAAGCCCATGCTTGCTTTAATGCTTGCAAGAT 1278
GAGTCCTTGAACAAATCAAATAAACCACCTAGTGTAGTGTG                                                   1319
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Figure 1. Nucleotide sequences and deduced amino acid sequences of bovine L-lactate dehydrogenase H chain cDNA. The amino acids are aligned with the second nucleotide of each codon. Asterisk (*) marks stop codon. The polyadenylation signal is underlined. The nucleotide sequence data are in the EMBL, GenBank, and DDBJ nucleotide sequence databases with the accession number AJ401268.

vitro packaging and transfected into the XL1-Blue cells. The unamplified titers were 5.7×10^6 plaque-forming units per milliliter (pfu/ml). The insert size distribution of the library determined by PCR was 0.4 ~ 1.9 kb.

Differential screening and sequencing

To identify the fasting-induced genes in bovine adipose tissues, a differential screening method was used as previously described (Kim et al., 2000; Lee et al., 1996, 2001). Briefly, the library was plated with XL1-Blue cells, and phage DNA was transferred to the membrane in duplicate. Differential hybridization of the membrane was done with ^{32}P-labeled cDNA that had been prepared from mRNAs of fed tissues and fasted tissues, respectively. After comparing the signals from two films, the fasting-induced plaques were identified and characterized by sequence analysis.

Partial sequencing of the clone was done by the dideoxy nucleotide chain- termination method using the Sequenase DNA Sequencing Kit (USB, USA) and T3 primer. For the full-sequencing, the Pst I- and Hind III-digested fragments were subcloned into pGEM vector and the fragments was sequenced by the dideoxy chain termination method using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The sequences of the clone were compared to the sequence data of GenBank in NIH, USA.

Northern analysis

For northern analysis, twenty micrograms of total RNA were electrophoresed on a 1% agarose gel containing formaldehyde, and blotted onto a membrane. The lambda DNA containing the cDNA insert was converted into the pBK-CMV phagemid by *in vivo* excision. The plasmid was digested with EcoR I and Xho I, and the insert was obtained

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Bovine : MATLKEKLIAPVAEEETTIPVNKITVVGVGQVGMACAISILGKSLTDELALVDVLEDKLKGEMMDLQHGSLFLQTPKIVA  80
Swine  : ................................................................................
Human  : ..............A.V.......................A.............................................

Bovine : DKDYSVTANSKIVVVTAGVRQQEGESRLNLVQRNVNVFKFIIPQIVKYSPACIIIVVSNPVDILTYVTWKLSGLPKHRVI 160
Swine  : ...............................................D.....................................
Human  : ...............................................D.....................................

Bovine : GSGCNLDSARFRYLMAEKLGIHPSSCHGWILGEHGDSSVAVWSGVNVAGVSLQELNPEMGTDNDSENWKEVHKMVVESAY 240
Swine  : ..................V.............................................................
Human  : ................................................................................

Bovine : EVIKLKGYTNWAIGLSVADLIESMLKNLSRIHPVSTMVKGMYGIENEVFLSLPCILNARGLTSVINQKLKDEEVAQLKKS 320
Swine  : ....................................Q.............V..............D......N.
Human  : ................................................................D.............

Bovine : ADTLWGIQKDLKDL 334
Swine  : ..............
Human  : .....D........
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Figure 2. Comparison of the deduced amino acid sequences of bovine LDH-B with those of other species. Dot denotes the amino acid residue identical to that of bovine species.

after low melting agarose gel electrophoresis. The insert of cDNA clone was labeled using a Prime-It Random Primer Labeling Kit (Stratagene, USA). The membrane was hybridized with the ^{32}P-labeled insert of the cDNA clone.

RESULTS AND DISCUSSION

Differential screening and cloning of lactate dehydrogenase H chain cDNA in adipose tissues of Korean cattle

Fasting-induced genes were identified from adipocyte cDNA library by differential screening method. One clone was selected for sequencing. The clone had a total length of 1,319 nucleotides coding for 334 amino acids (figure 1). By a search for sequence similarity in GenBank databases, it was identified as one encoding L-lactate dehydrogenase H chain (LDH-B). Comparison of the nucleotide sequences of bovine LDH-B with those of pig (Tsuji et al., 1994) and human (Sakai et al., 1987) showed 91% and 90% identity, respectively. Comparison of the deduced amino acid sequences of bovine LDH-B with those of pig (Tsuji et al., 1994), human (Sakai et al., 1987), rat (Tsuji et al., 1994), and mouse (Hiraoka et al., 1990) showed 98%, 98%, 97%, and 96% identity, respectively (figure 2).

Expression of lactate dehydrogenase H chain cDNA in adipose tissues of Korean cattle

The effects of fasting on the expression of LDH-B gene were examined by northern analysis using total RNA extracted from the pooled adipose tissues containing equal amount of abdominal, perirenal, subcutaneous and intramuscular tissues of Korean cattle. Tissues were isolated from fed-, 48 h fasted-, and 6 h refed- bulls. Northern analysis revealed that the mRNA levels of LDH-B were higher in fasted animals compared to those in fed and refed animals (figure 3A). We also examined whether there was a regional difference in LDH-B gene expression among several adipose tissue sites in response to fasting. Expression levels were similar among several fat depots (abdominal, perirenal, intramuscular and subcutaneous tissues). Major induction of LDH-B gene expression by fasting was detected only in subcutaneous tissues, but fasting did not influence the expression in the abdominal, perirenal and intramuscular tissues (figure 3B).

Lactate dehydrogenase (LDH) catalyzes the reversible oxidation of lactate to pyruvate. LDH occurs in vertebrate tissues as at least 5 different isozyme (A4, A3B, A2B2, AB3, and B4; Holbrook et al., 1975). All LDH isozymes contain four polypeptide chains, but isozymes contain different ratios of two kinds of polypeptides. The A chains (designated M for muscle) and B chains (designated H for heart) are encoded by two different genes (Stock et al., 1997). Prior and Jacobson (1979) have demonstrated that the major (97%) LDH isozyme in bovine adipose tissue is the heart-type isozyme. Cold acclimation resulted in increase activity levels of LDH in adipose tissues of rats (Terblanche et al., 1998). Previously, protein malnutrition increased heart type LDH activity (H-subunits) in rat uterus

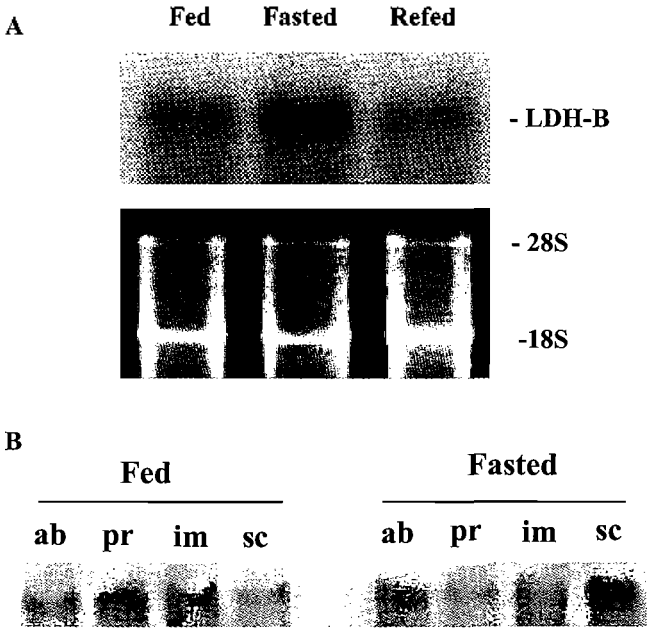

Figure 3. Northern analysis of LDH-B gene in bovine adipose tissues of fed-, 48 h fasted-, and 6 h refed- Korean bulls. **A.** The 40 μg of pooled total RNA isolated from abdominal, perirenal, intramuscular and subcutaneous adipose tissues were separated on a 1% formaldehyde/ agarose gel, and transferred onto the membrane by capillary reaction. The blot was hybridized with the ^{32}P-labeled cDNA clone. That similar amounts of RNA were present in each lane was checked by the intensities of 28S and 18S bands as shown. **B.** Total RNA (40 μg) isolated from abdominal (ab), perirenal (pr), intramuscular (im) and subcutaneous (sc) adipose tissues were analyzed by the northern method.

with a concomitant decrease in muscle-type LDH activity (Singh and Roy, 1980). Mulhausen and Moore (1969) reported that fasting increased synthesis of LDH H subunits in the adipose tissues of rats. LDH isozymes A4 favor rapid reduction of very low concentration of pyruvate to lactate in anaerobic tissues (muscle), whereas those of enzyme B4 tend to favor rapid oxidation of lactate to pyruvate in aerobic tissues (heart). Thus, our results demonstrate that induction of LDH-B gene during fasting may represent a metabolic shift from anaerobic state to aerobic predominance in fasted adipose tissues.

Our study demonstrates that major induction of LDH-B gene expression by fasting was observed only in subcutaneous tissues, but not in abdominal, perirenal and intramuscular adipose tissues. This is the first report showing regional differences of LDH-B gene expression by fasting among several fat depots. Reason for the different responses to fasting among several adipose tissues is not clear. The differential expression of the LDH-B gene may be dependent on different hormonal sensitivity between subcutaneous and intramuscular fat. Fasting has increased levels of glucagon, epinephrine and norepinephrine, inducing a predominant catabolic state (Diaz-Munoz et al., 2000; Hojlund et al., 2001). Further studies are required to assess the physiological importance in the regional differences of LDH-B gene expression in response to fasting.

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