Preliminary Communication

Decrease of the Obese Gene Expression in Bovine Subcutaneous Adipose Tissue by Fasting

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The expression of mRNA of leptin, the product of the obese gene, in bovine adipose tissue was analyzed by a lysate RNase protection assay. The mRNA level was significantly decreased by food deprivation for two days and partially recovered after 3 hr of refeeding, indicating that obese gene expression in the ruminant was regulated by feeding.

Key words: leptin; obese gene; bovine; starvation; adipose

Leptin is the product of the obese gene which is expressed in adipose tissue and is known to be a satiety factor since it represses food intake and thence reduces body fat.1,2) Studies on single-stomached species have shown that expression of the obese gene was repressed by starvation and induced by feeding.3,4) The obese gene was first cloned in rodents,5,6 and studies on leptin have so far been conducted mainly on rodents and humans. The role of leptin has not been elucidated in ruminants which are physiologically far different from single-stomached species and have unique eating behavior. In the present study, we established leptin mRNA determination in cows, and the effect of food deprivation on leptin mRNA was analyzed.

A partial cDNA fragment of bovine leptin was obtained by a reverse transcription-polymerase chain reaction. Template RNA was obtained from the subcutaneous fat tissue of two Holstein cows. The sequences of the sense and antisense primer corresponded to nucleotide numbers 1190–1209 and 3255–3274, respectively (GenBank accession number U50365; the sequence contains an intron, 1252–3005). An antisense RNA probe was synthesized and used for measuring leptin mRNA. Leptin mRNA was detected by a lysate protection assay as previously described.7) In addition to the ease and high sensitivity of the method, the assay gives the mRNA content per wet weight of tissue, thereby omitting the necessity for measuring internal standard mRNA. A discrete band of expected size (331 nucleotides) was obtained when the RNA preparation was from white fat tissues including subcutaneous, pericardial and perinephric fat tissues (Fig. 1). In non-adipose tissues, including the kidney, heart and muscle, leptin mRNA was under a detectable level (data not shown). The result shows that leptin mRNA was specifically expressed in adipose tissues in cows as it is in rodents and humans.

The effect of food deprivation on leptin mRNA expression was studied by using female Japanese Black cattle reared for reproduction, aged three to eleven and weighing 380 to 516 kg. Five head of cattle were fed enough of both concentrate and roughage until 4 p.m., and then 2 to 5 grams of subcutaneous fat tissues at between the seventh and the tenth rib were surgically obtained after sedating with xylazine (0.2 mg/kg) and local infiltration anesthesia by 2% lidocaine. The tissues were snap-frozen and applied for the leptin mRNA determination. The cows were then food-deprived for 48 hrs, and fat tissues were taken from the corresponding position on the other side of the previous surgery. Concentrate and roughage were then freely given for 3 hrs and adipose tissues were again taken. As is shown in Fig. 2, 48 hrs of starvation caused a significant decrease in leptin mRNA to 47% of the well-fed condition. This shows that obese gene expression in the cow is regulated...

Fig. 1. Bovine Leptin mRNA in Adipose Tissues detected by the Lysate Protection Assay.

Adipose tissues from pericardial (lane 1), perinephric (2) and endoabdominal (3) fat tissue, and subcutaneous fat tissue from a forelimb (4), hindlimb (5) and shoulder (6) were taken from a Holstein cow and snap-frozen in liquid nitrogen. Leptin mRNA contained in the same amount of wet tissues was detected by a lysate RNase protection assay and visualized by an imaging analyzer (MacBAS 2500, Fuji Film). The positions of the molecular weight markers are shown on the left.

(bases)

400

300

Marker 1 2 3 4 5 6

331

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Abbreviations: mRNA, messenger ribonucleic acid; RNase, ribonuclease
by feeding. Short refeeding for 3 hrs only partially restored the adipose leptin mRNA level to 67% of the well-fed control level, suggesting that a brief exposure to food is not sufficient and that reaching satiety or an alteration in metabolic status is necessary to restore obese gene expression. The feeding-dependent response seen here is similar to that in rodents, with the discrepancy that short refeeding of rodents fully restored its expression since these species may reach the 'satiated' condition quickly. The slow response observed in the present study is in accordance with the case of human. Interestingly, two of the cows used in the experiment were apparently more fatty than the others, and adipose tissue from those individuals contained a higher leptin mRNA level than the others (data not shown). Taking these results together, it is suggested that the changes in obese gene expression are involved in regulating the eating behavior of cows as with rodents and humans.

In conclusion, we developed a lysate RNase protection assay for determining the leptin mRNA level in a biopsied sample of bovine fat tissue and showed that obese gene expression in bovine adipose tissue is regulated by feeding.

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References