

Cloning and expression pattern of bovine adipogenin isoform

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The generation of new adipocytes results from differentiation mediated by several transcription factors identified as master regulators for adipogenesis. Recently, we reported that adipogenin, a new adipose-specific gene, was highly expressed in adipose tissues and up-regulated during adipocyte differentiation in bovine and mice. In the process of analyzing the expression of adipogenin in adipose tissues from cattle, we found an isoform of adipogenin cDNA, generated by alternative splicing. Therefore, the objective of this study was to isolate of bovine adipogenin isoform and analyze its expression on adipose tissue and differentiated adipocytes. The putative complete coding sequence of the bovine adipogenin isoform gene is 384 bp in length. The splicing event results a new ORF that could generate an isoform of 127 amino acids. Alignment analysis using BLAST program (NCBI) with bovine genomic (chromosome 13 contig NW_001493160) and cDNA adipogenin isoform sequences indicates that flanking nucleotides of divergence point among sequences of this 133 bp fragment and adipogenin cDNA perfectly match with exonic sequences from exon 1/intron 1 and intron 2/exon 3 junctions, confirming that entire exon 2 is spliced in the another fragment. To amplify specifically the adipogenin isoform, we realize RT-PCR analysis using adipogenin-specific forward primer derived from exon 1/exon 3 junction. Total RNA was extracted from bovine tissues and cultured preadipocytes and differentiated adipocytes in 6-well culture dishes, and cDNA synthesis and PCR reactions were performed. The expression of bovine adipogenin isoform mRNA in adipose tissues was significantly higher ($P < 0.05$) than that in non-adipose tissues examined. The expression of adipogenin isoform was significantly highly expressed in adipocytes ($P < 0.05$) compared to stromal-vascular cells. The levels of bovine adipogenin isoform mRNA significantly increased ($P < 0.05$) throughout the 10-day of adipocyte differentiation. In conclusion, this spliced form of adipogenin is another factor in the regulation of adipocyte gene expression and in the adipogenic process.