



## Expression of functional leptin receptor (OB-Rb) mRNA in the sheep tissues

Abbas Abavisani<sup>1\*</sup>, Hesam Deghani<sup>1</sup> and Morteza Zahedi<sup>2</sup>

<sup>1</sup>Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine and Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran; <sup>2</sup>Department of Basic Sciences, Veterinary Faculty, Ferdowsi University of Mashhad, Mashhad, Iran

### Abstract

Leptin, a 167-amino acid hormone, is secreted mainly by fat tissue. Leptin signaling plays crucial roles on the regulation of metabolism and reproductive function through endocrine and probably paracrine mechanisms. So, detection of leptin receptor in various tissues is essential to an understanding of leptin physiology in sheep. The aim of the present study was to investigate expression pattern of functional ovine leptin receptor (OB-Rb) in adult tissues using reverse transcription and polymerase chain reaction analysis (RT-PCR). Ovine beta actin gene was chosen as an internal positive control as well as RNA purification marker. RT-PCR experiments confirmed the expression of functional leptin receptor mRNA in ovine tissues including fat, liver, rumen and lung, but not in kidney and hypophysis. Characterization of functional leptin receptor in different organs suggests that leptin has paracrine/autocrine effects on many target organs as well as their physiology in sheep which should be more investigated in the future.

**Keywords:** Sheep; leptin receptor mRNA (OB-Rb); tissues, RT-PCR

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### Introduction

Leptin, as a 16-kD protein is synthesized mainly by adipose tissue and is released into the blood (Zhang et al. 1994). Leptin is involved in the central and/or peripheral regulation of body homeostasis, energy intake, storage and expenditure, fertility and immune functions (Ahima and Flier, 2000). In humans and rodents, leptin inhibits food intake, reduces body weight, stimulates energy expenditure, and also plays an important role in reproduction (Chehab et al. 1997). There is strong evidence showing that the dominant action of leptin is to act as a 'starvation signal.' Leptin declines rapidly during fasting, and triggers a rise in glucocorticoids, and reduction in thyroxine (T<sub>4</sub>), sex and growth hormones (Ahima and Osei, 2004). It has been suggested that leptin have evolved as a signal linking adipose energy stores and the brain and peripheral targets, as a safeguard against the threat of

starvation. Reduced leptin levels promote energy intake and limit the high energy cost of reproduction, thyroid thermogenesis and immune response (Flier, 1998).

Six splice variants of the leptin receptor (LepR), "a" to "f", have been identified. LepR belongs to a family of class I cytokine receptors. LepR isoforms have a similar extracellular ligand-binding domain at the amino terminus, but differ at the intracellular carboxy-terminal domain. LepRa, LepRb, LepRc, LepRd and LepRf have transmembrane domains; however, only the 'long receptor,' LEPRb, has intracellular motifs necessary for activation of the JAKSTAT signal transduction pathway. LepRe lacks both transmembrane and intracellular domains and circulates as a 'soluble receptor' (Tartaglia, 1997). Komisarek and Dorynek (2006) reported that polymorphism in the bovine LepR gene probably affects milk fat content.

**Corresponding author:** Abavisani A, Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine & Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran  
Tel: +98 511 8803751; Fax: +98 511 8783651; abavisania@um.ac.ir

In ruminants, leptin gene was shown to be expressed in adipose tissue, fetal tissues, mammary gland, rumen, abomasum and pituitary gland (Dyer et al. 1997, Chilliard et al. 2001, Chilliard et al. 2005). Studies which shown its expression in various tissues suggest physiologic roles of leptin in many target organs through paracrine or autocrine action (Ahima and Osei, 2004). The levels of leptin mRNA and protein in adipose tissue and plasma are positively correlated to body fat and adipocyte size. Leptin is secreted in pulses that are inversely associated with ACTH and cortisol, and positively correlated to gonadotropins, estradiol and thyrotropin (Ahima and Osei, 2004).

Most of Fat-tailed breeds farmers aim to reduce adipose tissue in sheep carcass. However, information on feeding techniques for this purpose is limited. The utilization of hormones for modifying carcass composition and as predictors for production characteristics has been discussed over several years. It seems that leptin is a potential hormone for an assay based prediction of growth or carcass composition. Altmann et al. (2006) indicated that leptin concentration measured in the slaughter weight range has the greatest potential to assess body fat content of lambs, whereas an earlier prediction does not seem to be feasible. Plasma leptin is also linked to adipose tissue cellularity, body condition score and nutritional state in Holstein and in Charolais cows and also decreased by beta-agonists (Chilliard et al. 1998). Backfat thickness at slaughter was positively associated with the rate of the increase in plasma leptin concentrations from 11 to 14 months of age, However, plasma leptin levels seem to be weak index to predict final adiposity in Japanese Black steers (Kawakita et al. 2001).

Thus, understanding physiological effects of leptin can be useful for sheep breeding and modifying carcass composition. As a first step towards understanding the central and peripheral effects of leptin in sheep, it is necessary to demonstrate the expression of leptin receptors, especially OB-Rb in various tissues. The objective of this study was to determine the presence of LepR mRNA (OB-Rb) in some tissues in sheep.

## Materials and Methods

### Tissue preparation and RNA isolation

Tissue samples were obtained from 3 adult sheep. Tissues including liver, fat, lung, rumen, hypophysis and kidney were quickly removed after slaughtering. Freshly-dissected tissue segments were cut into small pieces and immediately transferred to the laboratory on ice and stored at  $-80^{\circ}\text{C}$ .

Total RNA of about 100 mg tissue samples was extracted according to manufacturer's instructions using the Tripure reagent (Roche, Germany) and

quantified by a Nanodrop. Only high quality samples were chosen for further analysis. In order to eliminate the possible residual genomic DNA from the RNA samples, one unit RNasefree DNase I (Roche, Germany) was added per each 10  $\mu\text{g}$  of RNA sample and incubated at  $37^{\circ}\text{C}$  for 30 min followed by adding 1  $\mu\text{l}$  25 mM EDTA (Fermentas, Germany) and heat inactivation of the enzyme at  $65^{\circ}\text{C}$  for 10 min.

### Reverse Transcription -Polymerase Chain Reaction

All used reagents were from Fermentas, Germany. The RT-PCR was carried out in a DNA thermocycler (Primus 25 advanced, Germany). 1  $\mu\text{g}$  of total RNA was reverse-transcribed into cDNA with 0.5  $\mu\text{g}$  oligo thymidine, 1 mM dNTP mix, 4  $\mu\text{l}$  5X reaction buffer to a final volume of 20  $\mu\text{l}$ , for 60 min at  $42^{\circ}\text{C}$  followed by heating at  $70^{\circ}\text{C}$  for 10 min to stop the reaction.

Oligonucleotide primer pairs specific for ovine functional leptin receptor (OB-Rb) and beta actin were designed based on the known sequences (table. 1). The beta-actin primer pairs were designed to span the junction of two exons to be RNA specific. Leptin receptor primer pairs were for amplification of 197 bp fragments and beta actin primer pairs were for amplification of 277 bp fragment of beta actin cDNA and 366 bp fragment of beta actin DNA.

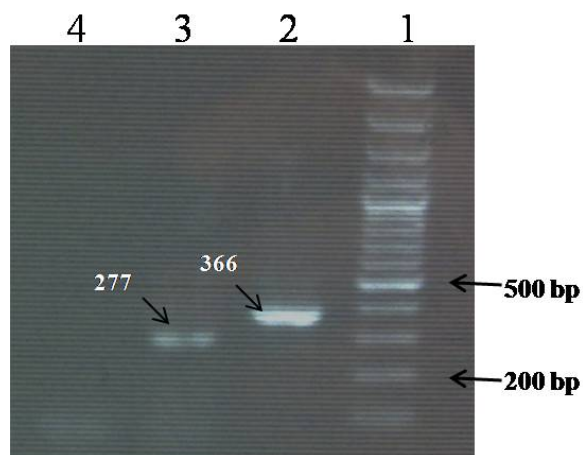
One  $\mu\text{l}$  of each RT reaction was used as template for PCR reactions in a final volume of 25  $\mu\text{l}$  with 0.2 mM dNTP mix, 20 pmol of each primer, 1.25 units SmarTaq DNA Polymerase, 1.5 mM  $\text{MgCl}_2$  and 5  $\mu\text{l}$  10X PCR buffer. The following amplification conditions were utilized:  $94^{\circ}\text{C}$  for 5 min followed by 40 cycles at  $94^{\circ}\text{C}$  for 45 s,  $66^{\circ}\text{C}$  for beta actin and  $55.5^{\circ}\text{C}$  for leptin receptor for 45 s, and  $72^{\circ}\text{C}$  for 45 s. After the last cycle, reactions were held at  $72^{\circ}\text{C}$  for 10 min. Primers specific for beta actin (a housekeeping gene) were used as positive internal controls for all samples to verify that the RT-PCR reactions were successful. Negative control reactions were performed similarly without addition of a template from the RT reaction. Also, the location of beta actin primer pairs on different exons was as a precaution against amplification of genomic DNA. Amplified cDNA were visualized by a 1.5% agarose gel electrophoresis and staining with ethidium bromide. The molecular sizes of the transcripts were determined by comparison with size markers run together with the cDNA product.

## Results

The beta-actin primer pairs were designed to span the junction of two exons to characterize genomic contamination. The fragment size of PCR products of ovine DNA and cDNA as template for PCR reaction with beta actin primer pairs was 366-bp and 277-bp fragments, respectively (Fig.1).

**Table 1: Nucleotide sequences of the primer sets used for RT-PCR**

Primers	Gene Bank Acc. No.	Forward	Reverse	Fragment size (cDNA) bp	Fragment size (DNA) bp
Beta actin	NM_001009784, U39357	CGGGAAATCGTCCGTGAC	CCGTGTTGGCGTAGAGGT	277	366
Leptin receptor	NM_001009763	AATTCTGACAGCGTTGGGT	ACTTTCCTCGGGGTTTGGTT	197	197



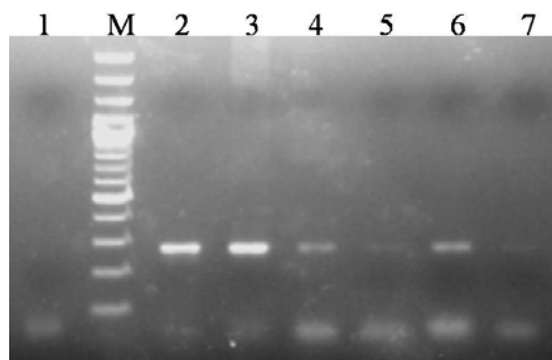
**Fig. 1:** A representative ethidium bromide-stained gel electrophoresis of beta actin DNA and cDNA fragments, amplified by PCR and RT-PCR, is presented. PCR product of ovine liver beta actin DNA (366 bp) and cDNA (277 bp) in lane 2 and 3, respectively. Lane 1 was 100 bp DNA marker and lane 4 was negative control.

#### Functional leptin receptor expression in ovine tissues

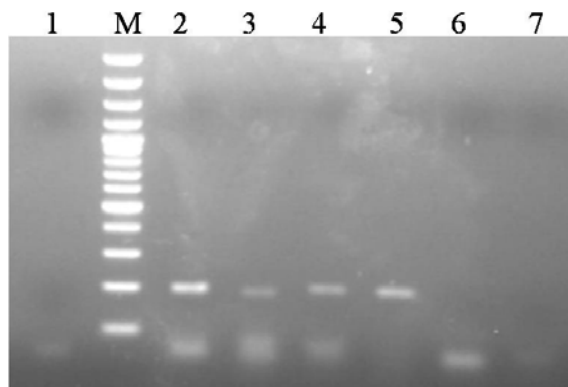
The mRNA for functional leptin receptor was detected in most of tissues surveyed. The mRNA for bovine beta actin acted as an internal positive control. So, we first assessed its amplification in all samples. Detection of only 277-bp fragment at all samples showed RNA purification as well as proper RT-PCR conditions along with no genomic contamination (Fig.2). Then, using leptin receptor primer pairs, leptin receptor mRNA transcripts of the predicted size (197 bp) were amplified and observed in tissues including fat, liver, rumen and lung but not in kidney and hypophysis (Fig.3). No amplification was observed in the negative controls.

#### Discussion

Many studies have shown the extensive expression of OB-Rb in various tissues of different species (Laud et al. 1999, Lin et al. 2000, Iqbal et al. 2001, Chelikani et al. 2003, Funahashi et al. 2003, Chilliard et al. 2005, Ogasawara et al. 2008, Pisani et al. 2008, Liu et al. 2010). Our results showed that the mRNA for the functional leptin receptor, OB-Rb, was detectable in fat



**Fig. 2:** Beta actin was used as positive internal controls for all samples to verify that the RT-PCR reactions were successful and confirm the absence of genomic contamination. Representative agarose gel demonstrating amplification of 277-bp ovine beta actin cDNA from liver (lane 2), fat (lane 3), rumen (lane 4), lung (lane 5), kidney (lane 6) and hypophysis (lane 7). Lane 1 was negative control and M was 100-bp DNA marker.



**Fig. 3:** Expression of ovine functional leptin mRNA was analyzed by reverse transcription PCR (RT-PCR) of RNA isolated from different tissues. Representative ethidium bromide-stained gel electrophoresis of leptin receptor cDNA fragments (197 bp) amplified by RT-PCR using leptin receptor primer pairs from liver (lane 2), fat (lane 3), rumen (lane 4), lung (lane 5), kidney (lane 6) and hypophysis (lane 7). Lane 1 was negative control. The sizes of the generated products were calculated by comparison with the mobility of 100 bp DNA step ladder (M).

tissue which confirms Dyer et al. (1997) finding. In addition, it was found in liver and lung in agreement with the data in bovine (Chelikani et al. 2003) and pig (Lin et al. 2000). In contrast to Chelikani et al. (2003) findings in bovine, OB-Rb mRNA was detectable in the rumen but was undetectable in kidney and hypophysis. This shows that the leptin receptor is not expressed in ovine kidney and hypophysis or its expression level is less than other tissues and have could not be detected by RT-PCR in this study. The latter is more reasonable as leptin stimulate LH and GH secretion by direct effects at the hypothalamic and adenohypophysis (Nagatani et al. 2000, Zieba et al. 2005). Moreover, the presence of LepR mRNA in ovine anterior pituitary have been previously reported (Dyer et al. 1997). Our new findings regarding the presence of Ob-Rb mRNA in liver, rumen and lung clear that leptin may be involved in digestion, respiration, and liver functions of the sheep.

Leptin is a key metabolic signal synthesized and secreted by fat cells that communicates information about body energy reserves, nutritional state, and metabolic shifts to the reproductive axis (Hausman et al. 2012). leptin integrate the animal's energy requirements, predictable food availability and potential for survival (Chilliard et al. 2005). Leptin contributions as hormone acting at multiple loci have now been clearly established, including effects at the hypothalamus, adenohypophysis, pancreas, adrenal and gonads (Zieba et al. 2005, Malendowicz et al. 2007, Zieba et al. 2007, Wójcik-Gładysz et al. 2009). All of these regulatory pathways involve activation of the leptin receptor. Leptin-activated LepRb regulates well-known insulin targets, such as IRS-1, MAP kinase, ERK, Akt, AMP kinase and PI3-kinase, raising the possibility that leptin pathways act in concert with insulin to control energy metabolism and other cellular processes (Porte et al. 2000). Leptin decreases insulin and glucocorticoid and stimulates growth hormone, catecholamine and thyroid hormone secretions, thus increasing tissue energy expenditure and adipose tissue lipolysis, and decreasing lipogenesis (Chilliard et al. 2005). In ruminants, exogenous leptin regulates LH and GH secretion in sheep which indicates its role in controlling neuroendocrine function (Nagatani et al. 2000).

In conclusion, our data on expression of the functional leptin receptor mRNA provides evidence for potential involvement of leptin in multiple physiological functions in various ovine systems. Quantification of transcript abundance of leptin receptor in various tissues in response to nutritional and hormonal manipulations should give useful information regarding functional significance of leptin receptor in various organs in sheep. Moreover, much additional work will be necessary to delineate fully the role of

leptin in the variety of physiological systems, and to develop pharmacological and genetic strategies for exploiting those roles in sheep breeding management as well as modifying carcass composition.

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