

# Real-time PCR analysis of leptin and leptin receptor expression in the rat prostate, and effects of leptin on prostatic acid phosphatase release

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**Abstract.** We have recently demonstrated the expression of leptin and leptin receptor (Ob-R) isoforms a, b, c, e and f in the rat seminal vesicles and prostate. The aim of the present study was to provide a semiquantitative real-time PCR estimation of leptin/Ob-R isoform mRNA expression in the seminal vesicles and individual components of rat prostate, and to ascertain the *in vitro* effects of leptin on prostate acid phosphatase release. The highest expression of the leptin and Ob-R genes was in the seminal vesicles and lateral prostate lobe, respectively. Of the various isoforms, Ob-Rb displayed the highest and Ob-Re the lowest expression. Leptin ( $10^{-8}$  and  $10^{-6}$  M) enhanced acid phosphate release from seminal vesicles, and ( $10^{-6}$  M) decreased it from the coagulating lobe. Taken together, our findings support the contention that leptin may be involved in the autocrine-paracrine functional regulation of rat seminal vesicles and prostate. The physiological relevance of the marked heterogeneity of the different prostate lobes in both their leptin/Ob-R expression and functional response to leptin remains to be addressed.

## Introduction

Despite evidence strongly suggesting the involvement of leptin in the regulation of growth and function of the prostate and prostate tumors (1-7), investigations on the expression of leptin and its receptor isoforms in the human normal and tumorous prostate are very scarce (4,8-10). Using reverse transcription (RT)-polymerase chain reaction (PCR), Western blotting and immunocytochemistry, we have recently demonstrated the expression of leptin and leptin receptor (Ob-R) isoforms

(a,b,c,e and f) in the seminal vesicles and coagulating, dorsal, lateral and ventral lobes of the rat prostate (11).

The structural and functional heterogeneity of the individual lobes of the rat prostate is well known (12), especially as far as the epithelial-cell responses to androgens and other hormones are concerned (13-16). There are also clear-cut differences in the level of expression of non-gastric  $H^+/K^+$ -ATPase- $\alpha$  and endothelin receptor density (17,18), and in the secretion of prostate secretory protein (PSP94) and protease inhibitors (19,20). Moreover, angiographic studies demonstrated that the prostate ventral lobe is better irrigated than the dorsal one (21).

Based on these considerations, it seems worthwhile to investigate by semiquantitative real-time PCR the mRNA expression of leptin and Ob-R isoforms in the rat seminal vesicles and individual prostate lobes, as well as to examine the effects of leptin on the specialized function of their epithelial cells (i.e. acid phosphatase release).

## Materials and methods

**Animals and reagents.** Adult male rats (200-250 g body weight), bred in our laboratory facilities, were kept under a 14:10-h light:dark cycle (illumination onset at 6:00 a.m.) at 23°C, and maintained on a standard diet with free access to tap water. Rats were decapitated, and their prostates with seminal vesicles were promptly removed. The study protocol was approved by the local ethics committee for biomedical studies. Recombinant murine leptin(1-147) was purchased from Prepro-Tech EC (London, UK), and Iscador Qu (mistletoe preparation) from Weleda Co. (Schwabisch Gmund, Germany). RPMI-1640 medium, and all other chemicals and reagents were provided by Sigma-Aldrich Corp. (St. Louis, MO).

**Real time-PCR.** Total RNA was extracted from seminal vesicles and prostate coagulating, dorsal, lateral and ventral lobes (22,23), and reverse transcribed, as previously detailed (24,25). Real-time PCR was carried out in a Roche LightCycler 2.0 with software version 4.0 (26,27), using the following program: denaturation step (95°C for 10 min), and 45 cycles of three step amplification (denaturation, 95°C for 10 sec; annealing, 58°C for 5 sec; and extension, 72°C for 10 sec).

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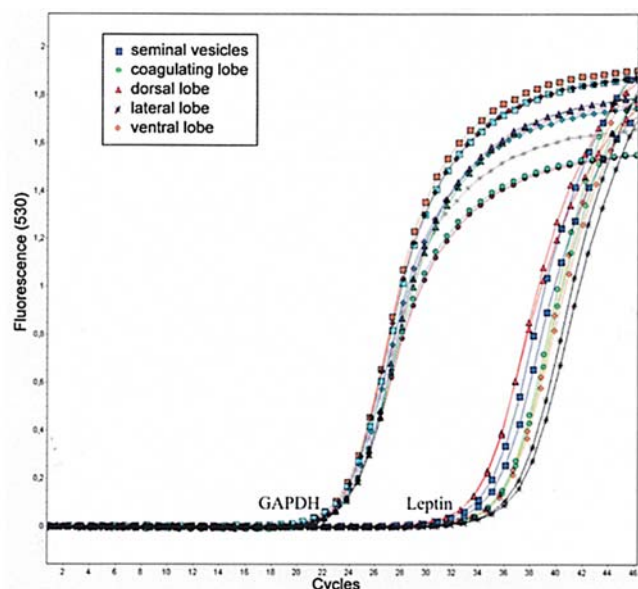


Figure 1. Amplification curves of leptin cDNA obtained from mRNA isolated from seminal vesicles and prostate lobes of an exemplary adult rat.

Subsequently, melting curve (60–90°C with a heating rate of 0.1°C/sec) was performed to check the specificity of amplification and the presence of byproducts. All samples were amplified in duplicate, and the glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) gene was used as a reference to normalize data. The primer sequences were those reported previously (11).

**Acid phosphatase release.** Seminal vesicles and prostate were squeezed to remove their content, and carefully dissected and sliced. Fragments (60–100 mg) of seminal vesicles and prostate ventral and coagulating lobes were incubated in 1 ml RPMI-1640 medium for 10 h at 37°C in an atmosphere of 5% CO<sub>2</sub>-95% air and 100% humidity. The medium was discarded, and new medium, containing Iscador Qu (1 mg/ml) or leptin (10<sup>-8</sup> and 10<sup>-6</sup> M), was added. Fragments were incubated for 120 min. Prostatic acid phosphatase activity in the incubation medium was measured by a commercial kit (Pointe Scientific, Canton, MI). In this assay,  $\alpha$ -naphthylphosphate was added to the medium, and the reaction of the released  $\alpha$ -naphthyl with Fast Red TR was measured at 405 nm wavelength in a microplate autoreader (EL-13; Bio-Tek Instruments, Winooski, VT). Data were expressed as the mean  $\pm$  SEM of 8 independent experiments, and their statistical comparison was made by the unpaired Student's t-test.

## Results

Real time-PCR confirmed (11) the expression of the leptin gene in both rat seminal vesicles and prostate lobes (Fig. 1). Semiquantitative estimation showed the highest expression of leptin mRNA in the seminal vesicles and dorsal lobe, and the lowest expression in the coagulating, lateral and ventral lobes (Fig. 2).

The level of expression of Ob-R isoforms in the various tissues examined was as follows: i) Ob-Ra, ventral lobe > lateral lobe >> seminal vesicles and coagulating and dorsal

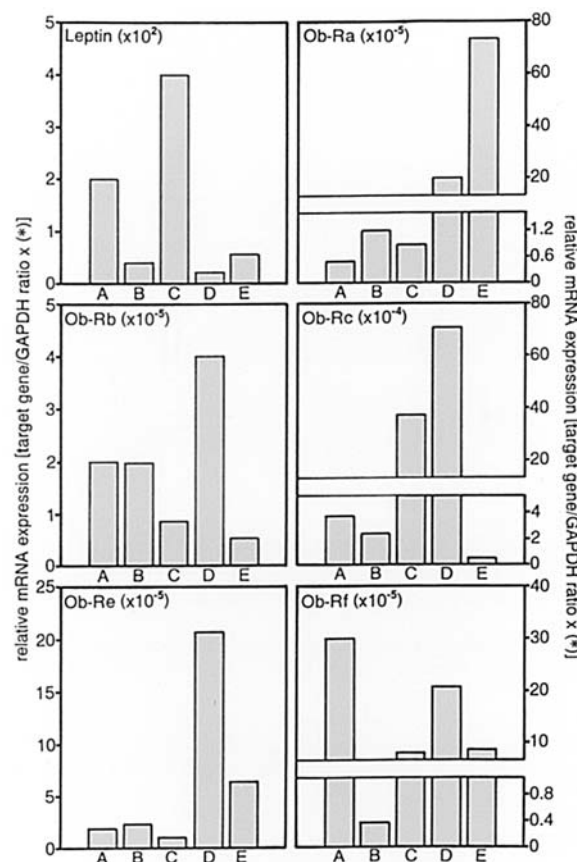


Figure 2. Real-time PCR semiquantitative analysis of leptin and Ob-R gene expression in the seminal vesicles (A), and coagulating (B), dorsal (C), lateral (D) and ventral lobes (E) of the prostates of adult rats. Bars are means of two independent estimations.

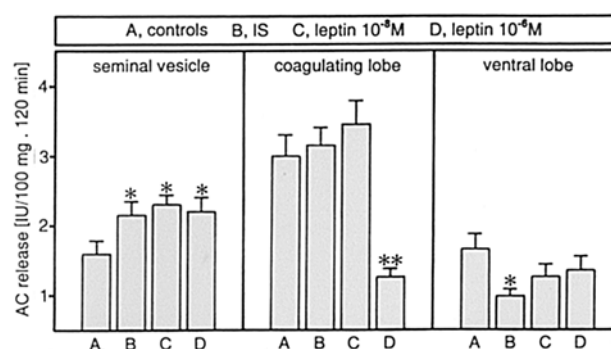


Figure 3. Effects of Iscador Qu (IS) and leptin on acid phosphatase (AC) release from seminal vesicles and prostate lobes of adult rats. Bars are means  $\pm$  SEM of 8 independent experiments. \*P<0.05 and \*\*P<0.01 from the respective control group.

lobes; ii) Ob-Rb, lateral lobe > seminal vesicles and coagulating lobe > dorsal and ventral lobes; iii) Ob-Rc, lateral lobe >> seminal vesicles and coagulating lobes > ventral lobe; iv) Ob-Re, lateral lobe > ventral lobe > seminal vesicles and coagulating and dorsal lobes; and v) Ob-Rf, seminal vesicles and lateral lobe > dorsal and ventral lobes >> coagulating lobe (Fig. 2).

Iscador Qu lowered acid phosphatase release from the prostate ventral lobe, but not from the coagulating lobe, and

raised that from seminal vesicles. Leptin did not affect acid phosphatase release from the ventral lobe and enhanced that from seminal vesicles. At the higher concentration ( $10^{-6}$  M), leptin decreased acid phosphatase release from the coagulating lobe (Fig. 3).

## Discussion

Our present findings confirm and expand our previous observations (11), and show a notable heterogeneity among seminal vesicles and various prostate lobes as far as the level of leptin/Ob-R expression and effects of leptin on the specialized function of their epithelial cells are concerned.

The highest relative expression of the leptin gene was found in the seminal vesicles and dorsal prostatic lobe, while that of Ob-R was observed in the lateral lobe. It is well known that only Ob-Rb, i.e. the long isoform of Ob-R which possesses an intracytoplasmic domain of 302 amino acids, contains all motifs (among which Box 1 and Box 2) necessary for the complete activation of JAK-STAT and MAPK cascades, while Ob-Re does not possess a transmembrane domain and only circulates as a soluble receptor. Other Ob-R isoforms (Ob-Ra, Ob-Rc and Ob-Rf), due to their truncated intracytoplasmic domain, are able to activate only parts of the leptin signaling pathways (28-36). On these grounds, it appears of great interest that our real-time PCR assay showed that, of the Ob-R isoforms studied in seminal vesicles and prostate, Ob-Rb displays the highest and Ob-Re the lowest expression.

The effects of leptin on acid phosphatase release from rat seminal vesicles and prostate have been examined and compared to those of Iscador Qu. Mistletoe (*Viscum album* L) extract Iscador Qu is commonly used in experiments on the prostate (37), because it contains high concentrations of lectins, which are known to bind and release prostatic acid phosphatase (37,38). Although both leptin and Iscador Qu enhance acid phosphatase release from seminal vesicles, their effects on prostate are notably different, inasmuch as the two compounds lower the enzyme release from the coagulating and the ventral lobe, respectively. Unfortunately, our findings do not allow us to correlate, even tentatively, the functional response to leptin of the various prostate lobes with their level of expression of Ob-Rs.

Despite these disappointing limitations, our study provides evidence that leptin may be involved in the autocrine-paracrine regulation of the biological activity of epithelial cells of the rat seminal vesicles and prostate. Although the possibility that locally synthesized leptin may be secreted into the lumen of the glands remains an open issue, the identification of free leptin in human seminal plasma appears to support this contention (39). Moreover, our investigation clearly demonstrates a striking heterogeneity among the various prostate lobes in both leptin/Ob-R expression and functional response to leptin, whose physiological relevance remains to be assessed.

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