Leptin role in the rat prostate ventral lobe

The involvement of leptin in prostate diseases is related to an increase in the gene expression of both a and b isoform leptin receptors, leptin itself, androgen receptor, and aromatase, as well as by a reduction in both estrogen isoform receptors. (Fertil Steril® 2011;95:1490-3. ©2011 by American Society for Reproductive Medicine.)

Key Words: Leptin, prostate, androgen receptor, estrogen receptor, aromatase

The prostate is an accessory sexual gland that produces key substances for the efficacy of sperm to fertilize eggs within the female reproductive tract. Its secretion is a thin milky fluid that contains calcium, citrate ion, phosphate ion, a clotting enzyme, and a profibrinolysin. The slightly alkaline characteristic of the prostatic fluid may be quite important for successful fertilization of the ovum (1). To accomplish this task, the prostate gland is finely regulated by neural and hormonal mechanisms, and possesses a complex histologic organization. It is located at the proximal region of the urethra, the prostatic urethra, as a well defined globular gland having two clearly distinctive regions, the dorsolateral prostate and the bilobulated ventral prostate, in rodent models.

The function of the prostate is regulated by a complex endocrine system. Androgens have a strong impact on the prostate in adult subjects regarding the maintenance of its morphology and secretory activity (2, 3), with the ventral prostate lobe being the main area that responds to androgen stimulation in rats (4). Although testosterone is the main androgen produced by the testes, it is converted into the more active hormone dihydrotestosterone by the 5α-reductase enzyme in the prostate (5). Prostate responses to androgens are mediated by the wide distribution of androgen receptors in epithelial cells, smooth muscle, and stroma cells (6).

Despite its direct action on the prostate, androgens can also be converted into estrogens by the enzyme aromatase (7). The role of estrogen within the prostate is complex and particularly dependent on local signaling mechanisms to maintain a balance between the effects of estrogen receptor (ER) α and ERβ. These receptors appear to play significantly different roles in the prostate, with ERα mediating the adverse and ERβ mediating the beneficial effects of estrogen (8). In rodents, ERβ is highly expressed in the adult prostate gland, whereas ERα is mainly expressed during neonatal periods (9, 10).

The mechanisms that control energy metabolism and body fat mass are inherently linked to those that govern fertility and stem from the evolutionary drive to survive in times of limited food supply. Adipose tissue functions as a highly specialized endocrine and paracrine organ producing an array of adipokines (11). Several adipokines, particularly leptin, ghrelin, and adiponectin affect processes involved in reproduction (12, 13).

Leptin, the most well characterized adipokine involved in reproduction, decreases caloric intake and increases energy expenditure (14). In relation to the reproductive function, leptin has direct effects in pituitary and gonads (15). Leptin exerts its effects by the leptin receptor (ObR), which is a transmembrane receptor found in many tissues, including the prostate (16). There are six known splice variants of the leptin receptor (ObRa to ObRf), all with the same extracellular domain but with differing intracellular domains. Of these isoforms, only the long isoform, ObRb, contains all intracellular parts able to activate the signaling pathways being expressed mainly in the hypothalamus. Among the short isoforms, ObRa is highly expressed in the peripheral tissues (17).

It is well established that androgen plays an important role in prostate disease development (18). Estrogens are recognized as carcinogens by the International Agency for Research on Cancer, and induce tumors in various organs of a number of different species (19). An increasing body of evidence suggests the involvement of leptin in human prostate growth and the development of prostate cancer, a strong correlation existing between the volume of body fat (i.e., obesity) and prostate cancer progression and mortality (20).

Based on the relationship between leptin, androgen, estrogen, and prostate diseases, the aim of the present study was to evaluate how leptin can regulate its gene expression as well as the gene expression of androgen, estrogen, and leptin receptors and aromatase enzyme in the ventral lobe of the rat prostate.

The study design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro. The prostate ventral lobe of male adult rats was dissected under sterile conditions and divided into two parts, both

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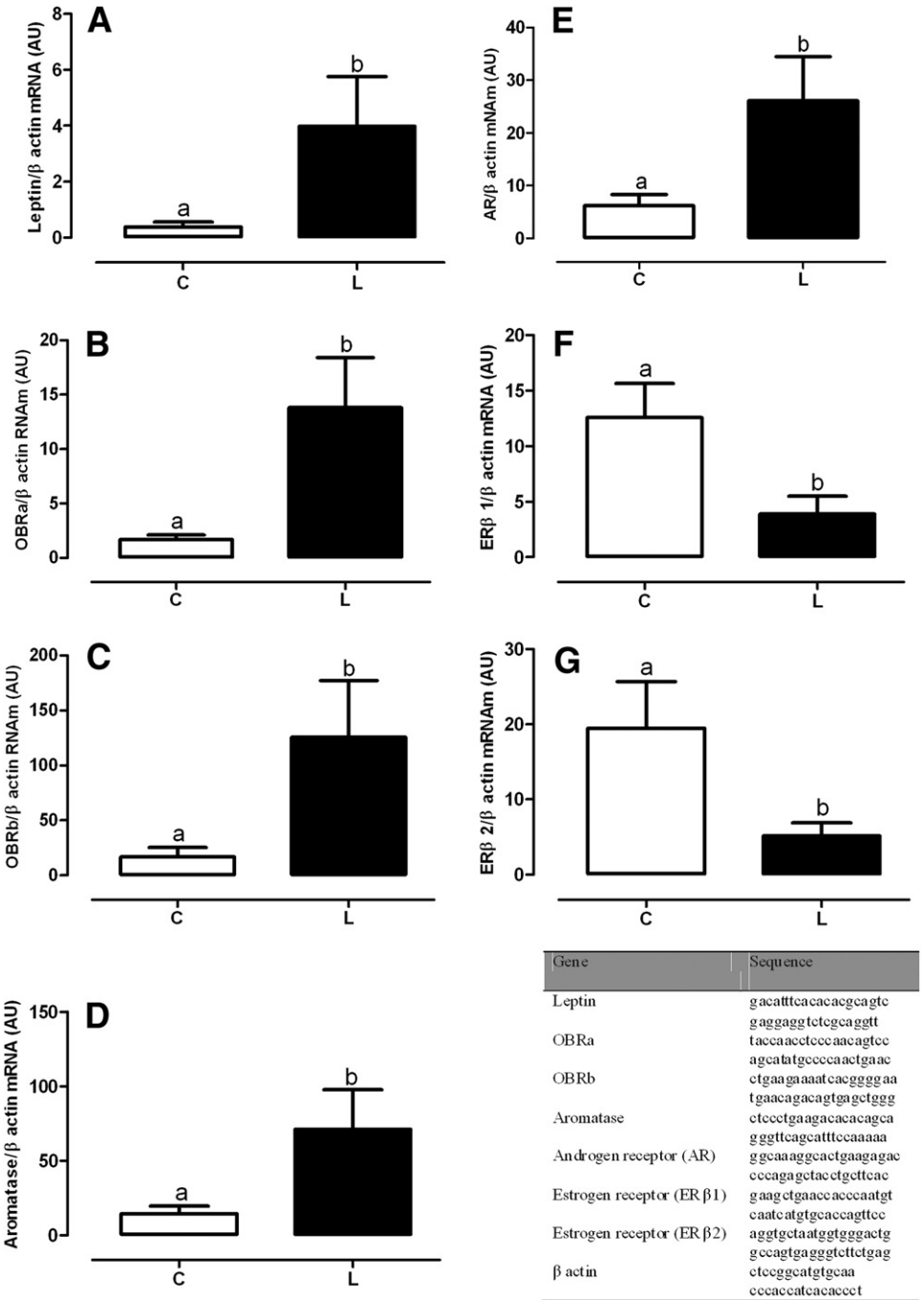
FIGURE 1

Gene expression of (**A**) leptin, (**B**) short isoform leptin receptor (ObRa), (**C**) long isoform leptin receptor (ObRb), (**D**) aromatase, (**E**) androgen receptor (AR), (**F**) estrogen receptor (ER) β1, and (**G**) ERβ2 in the rat prostate ventral lobe after leptin treatment for 3 hours (L) or not (C). β-Actin was used as an internal control. Primer sequences are listed in the table. Data are represented as mean ± SEM of 15 animals. Different letters mean statistically significant difference.



Gene	Sequence
Leptin	gacatttcacacacgcagtc gaggaggtctcgcaggtt
OBRa	taccaacctcccaacagtcc agcatatgccccaactgaac
OBRb	ctgaagaaaatcacgggggaa tgaacagacagtgagctggg
Aromatase	ctcccctgaagacacacagca gggttcagcatttccaaaaa
Androgen receptor (AR)	ggcaaaggcactgaagagac cccagagctacctgcttcac
Estrogen receptor (ER β1)	gaagctgaaccaccccaatgt caatcatgtgcaccagttcc
Estrogen receptor (ER β2)	aggtgctaatggtgtgggactg gccagtgagggtcttctgag
β actin	ctccggcatgtgcaa cccaccatcacaccct

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maintained in Dulbecco's Modified Eagle medium supplemented with 10% fetal bovine serum and 1 ng/mL of gentamycin. After 1 hour of incubation, the medium was changed by the same medium as above supplemented (L) or not (C) with 16 ng/mL leptin (15). To determine the best time of leptin treatment, ventral lobes of two rats were initially treated with leptin for 3, 24, and 48 hours. The ObRa gene expression was evaluated and showed a significant increase ($P<.01$) after 3 hours of leptin treatment compared with

24 and 48 hours ([3 hours: C 1.9 ± 0.5, L 19.5 ± 5.6; AU] [24 hours: C 0.9 ± 0.1, L 1.5 ± 0.4; AU] [48 hours: C 1.4 ± 0.4, L 7.5 ± 2.3; AU]).

Thereafter, the ventral lobes of 15 male adult rat prostates were submitted to the conditions above and treated with leptin for 3 hours. At the end of the incubation time, RNA was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Then 1 μg RNA sample was used in a 20-μL cDNA reaction using oligo-dT and the superscript III cDNA synthesis system (Invitrogen) according to the manufacturer's protocol. The gene expression of leptin, ObRa, ObRb, aromatase, androgen, ERβ1, and ERβ2 were evaluated by real-time polymerase chain reaction (primer sequences listed in Fig. 1).

The data were reported as mean ± SEM. Statistical significance of experimental observations was determined by Student t test. The level of significance was set at $P<.05$.

The results showed that leptin treatment for 3 hours led to an increase in the gene expression of ObRa, ObRb, leptin itself, androgen receptor, and aromatase. However, there was a significant reduction in ERβ1 and ERβ2 gene expression (Fig. 1).

Despite the strong evidence suggesting the involvement of leptin in human prostate growth and development of prostate cancer (20), studies regarding leptin expression and its role on prostate tissue remain scarce. It has been shown that leptin could stimulate proliferation and migration in prostate cell lines (21). The results observed in the present study suggest that the effect of leptin in the prostate could be reached by the up-regulation of leptin and its main receptor isoforms, ObRa and ObRb.

It is well established that androgens play an important role in prostate disease development. A higher expression of androgen receptor has been reported in cancer (18). Therefore, the up-regulation of androgen receptors by leptin treatment could be a strong association between obesity and prostate diseases.

It has been established that a high aromatase expression is important in the development and progression of breast cancer (22). It has also been shown that aromatase expression in the prostate is altered in cancer, further supporting a role for estrogen in this organ (23). The up-regulation of aromatase by leptin treatment suggests that this could be another step of interaction between obesity and prostate disease.

There is a progressive loss of ERβ expression in prostatic hyperplasia and, to a greater extent, invasive cancer, which may promote cell proliferation and possibly carcinogenesis (24). The reduction of ERβ1 and ERβ2 gene expression by leptin treatment observed in the present study raises another possibility by which leptin can influence human prostate growth and development and be associated with prostate cancer.

Based on the results that are reported for the first time in this paper, we can hypothesize that leptin may have an important positive effect regarding developing prostate diseases. The involvement of leptin in those diseases may be related to an increase in the gene expression of both ObRa and ObRb, leptin itself, androgen receptor, and aromatase, as well as by a reduction in both ER isoforms. An effort to reduce body weight and, as a result, leptin serum levels, could be important to improve the efficacy of prostate disease treatments. A flowchart with the interactions among the various hormones, receptors and substances in the study and their interaction in the prostate is shown in Supplemental Figure 1 (available online at www.fertstert.org).

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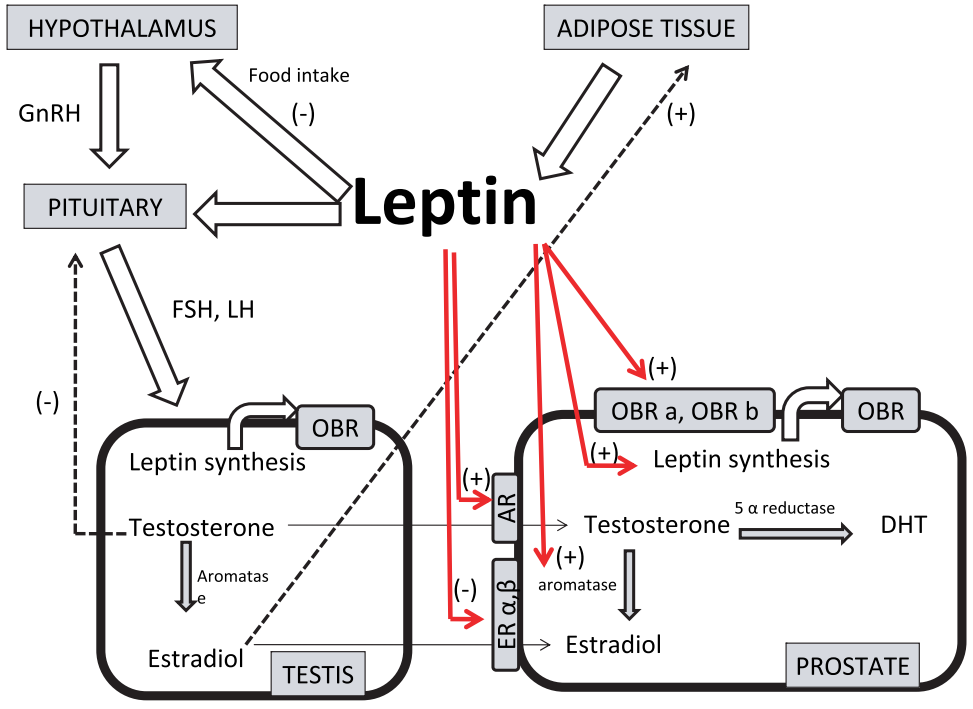
SUPPLEMENTAL FIGURE 1

Leptin is a molecular signal from adipose tissue that regulates the food intake, presumably through neuropeptide Y actions (14). Leptin is related to the hypothalamus-pituitary-gonad axis function (15). It is also synthesized in the reproductive tissues (16). Leptin exerts its effects by the leptin receptor (ObR), which is a transmembrane receptor found in many tissues, including testis (25) and prostate (16). There are six known splice variants of the leptin receptor (ObRa to ObRf), all with the same extracellular domain but with differing intracellular domains (17). Besides regulating the function of the reproductive organs by acting via ObR, leptin may also regulate E_2 synthesis by regulating aromatase enzyme (26). E_2 concentration could also influence leptin synthesis (27). Despite their direct action on the prostate via androgen receptors (ARs), androgens can also be converted to estrogens by the aromatase enzyme (7) and to dihydrotestosterone (DHT) by 5α-reductase (5). The role of estrogen within the prostate is complex and is particularly dependent on local signaling mechanisms to maintain a balance between the adverse effects of estrogen receptors (ER) α and the beneficial effects of ERβ (8). The red arrows indicate the effects of leptin treatment reported in the present study.



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