Leptin Receptor Transcripts Are Constitutively Expressed in Placenta and Adipose Tissue with Advancing Baboon Pregnancy (44502)

AMY E. GREEN,*·§ JENNIFER S. O'NEIL,*·§ KENNETH F. SWAN,* RUDOLF P. BOHM, JR.,¶ MARION S. RATTERREE,¶ AND MICHAEL C. HENSON*·†·‡·§¶·[1]

*Departments of *Obstetrics/Gynecology, †Physiology, ‡Anatomy, and §The Interdisciplinary Program of Molecular and Cellular Biology, Tulane University School of Medicine, New Orleans, Louisiana 70112–2699; and ¶Tulane Regional Primate Research Center, Covington, Louisiana 70433–8915*

Abstract. **The baboon (*Papio* sp.) is an accepted nonhuman primate model for the study of the endocrinology of human pregnancy. To further characterize this model with regard to leptin function, messenger RNA transcripts for both long (Ob-RL) and short (Ob-RS) leptin receptor isoforms were identified in maternal tissues at various stages of gestation. Thus, placental villous, subcutaneous and omental adipose tissues were collected upon cesarean delivery at early (Days 60–62), mid (Days 98–102) and late (Days 159–164) pregnancy (term ≈ 184 days). Additionally, amniochorion, decidua, and corpus luteum were collected in late gestation. Expression of Ob-RL and Ob-RS transcripts was determined in relation to constitutively expressed glyceraldehyde-3-phosphate dehydrogenase *via* reverse transcriptase-polymerase chain reaction, and transcripts were localized within specific placental cell types by *in situ* hybridization. Ob-RL and Ob-RS transcripts were present in amniochorion, decidua, and corpus luteum at term and appeared constitutively expressed throughout gestation in placenta and adipose tissues. Ob-RS was expressed in greater ($P < 0.02$) abundance than Ob-RL in all tissues. Within the placenta, receptor isoforms were localized predominantly to the syncytiotrophoblast. The expression of leptin receptor transcripts in maternal adipose tissues, as well as in the syncytiotrophoblast, amniochorion, decidua, and corpus luteum, suggests the potential for autocrine/paracrine roles for the polypeptide in the endocrinology of primate pregnancy. These are the first such observations in a nonhuman primate and support the use of the baboon as a model for the study of leptin in human pregnancy.** [P.S.E.B.M. 2000, Vol 223:362–366]

Leptin, a 16-kDa polypeptide produced by both adipocytes and the placenta, functions in the regulation of energy homeostasis *via* interaction with a specific hypothalamic receptor (1). Human leptin receptor transcripts have been identified in adipose tissue (2), placenta (3–5), and ovary (2, 6), suggesting that leptin may exert direct effects on a number of peripheral tissues. In the human placenta, leptin, Ob-RL, and Ob-RS transcripts have been localized to the syncytiotrophoblast, suggesting potential regulatory roles for the polypeptide in pregnancy (5). The leptin receptor is a member of the Cytokine Class 1 Receptor Superfamily and is homologous to the gp130 subunit of cytokine receptors, such as interleukin-6 (IL-6). It exists as five alternatively spliced isoforms, each differing in the length of their intracellular domains (7). The long isoform (Ob-RL) is detected predominantly in the weight-regulating regions of the hypothalamus and has a 302-amino acid intracellular domain, capable of signal transduction through the activation of Janus kinase and the signal transducers and activators of transcription (JAK/STAT). Three

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[1] To whom requests for reprints should be addressed at Tulane University School of Medicine, Department of Obstetrics and Gynecology, 1430 Tulane Avenue, New Orleans, LA 70112–2699. E-mail: michael.henson@tulane.edu

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of the shorter isoforms, including Ob-RS, have cytoplasmic domains ranging from 32–40 amino acids and differential signaling capabilities, possibly involving mitogen activating protein kinase (MAPK) (8). Although the functions of the shorter isoforms have yet to be determined, they may be involved in either leptin transport across the blood-brain barrier or leptin clearance from the body, or they may act as circulating leptin binding proteins (7, 9).

The baboon (*Papio* sp.) is a proven model for the study of human pregnancy (10) and we have previously reported on the similarities in maternal leptin profiles between human (5) and baboon (11) pregnancy. Because the further development of animal models in which leptin regulation and function can be studied will prove valuable to a better understanding of leptin in human gestation, the goal of the current study was to characterize the ontogeny of leptin receptor transcripts with advancing baboon pregnancy.

Materials and Methods

Animals. Baboons were maintained in accordance with USDA regulations and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86–23). The experimental protocol was approved by the Institutional Care and Use Committee of the Tulane Regional Primate Research Center. Fifteen female baboons, weighing ≈ 14–17 kg, were individually housed in stainless steel cages. As we have previously described, a 12-hr photoperiod (0600–1800 hr) was maintained in air-conditioned rooms, and animals were fed a primate maintenance ration with fresh fruit daily and water provided *ad libitum* (11). Females were quartered with males for mating within indoor/outdoor enclosures (4–5 days) coinciding with the estimated occurrence of ovulation, as determined by daily menstrual cycle records and the observance of external sex skin turgescence (10–12).

Tissue Collection. Baboon placental villous, amniochorion, decidua, corpus luteum, omental, and subcutaneous adipose tissues were collected at cesarean delivery under isofluorane anesthesia, as we have previously reported (11, 12). Tissues were obtained at early (60–62 days), mid (98–102 days) and late (159–167 days) gestation. Normal term in the baboon was ≈ 184 days. Samples designated as "early" were collected ≈ 30–35 days following the luteal-placental shift. Placental tissue was harvested from multiple villous trees and pooled for each animal, as we have previously described (13). Samples of abdominal subcutaneous adipose tissues were collected at the site of the initial abdominal incision, with samples of omental adipose tissues collected from the peritoneal cavity. Amniochorion, decidua, and corpus luteum were collected in late gestation. Tissue samples were flash-frozen in liquid nitrogen for storage at −80°C for reverse transcriptase-polymerase chain reaction (RT-PCR) or fixed in Histochoice (Amresco, Solon, OH) for *in situ* hybridization.

RNA Extraction and RT-PCR. Total RNA was extracted from all tissues using TRIzol reagent (Life Technologies, Grand Island, NY) according to Chomczynski and Sacchi (14) as adapted in our laboratory (5). All samples were treated with DNase (Gibco, Life Technologies Inc., Gaithersburg, MD) to eliminate DNA contamination and reprecipitated with sodium acetate and 100% ethanol. Oligonucleotide primers were synthesized (Midland Reagent Company, Midland, TX) for Ob-RL (2), Ob-RS (15), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5). Primer sequences, Genbank accession numbers, and PCR product sizes are listed in Table I. Sequence analysis of PCR products (Biotech Core, Palo Alto, CA) confirmed that primers specifically amplified regions of Ob-RL and Ob-RS complimentary DNAs (cDNA) (11). Complimentary DNAs were synthesized from 2 μg total RNA (placental, amniochorion, decidua, and corpus luteum) or 1 μg of total RNA (adipose tissues) using SuperScript Kit (Life Technologies), and PCR was performed in Temp-Tronic Thermocyclers (Barnstead/Thermolyne, Dubuque, IA) as we have previously described (5). Conditions for PCR were as follows: Ob-RL (32 cycles for amplification, denaturation at 94°C for 60 sec, annealing at 60°C for 60 sec, extension at 72°C for 90 sec) (2), Ob-RS (32 cycles for amplification, denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec, extension at 72°C for 40 sec) (15), GAPDH (24 cycles for amplification, denaturation at 94°C for 30 sec, annealing at 58°C for 60 sec, extension at 72°C for 60 sec) (5). PCR products were viewed under UV light on 2% agarose gels

Table I. Sequences of Ob-R_L (Long Isoform), Ob-R_S (Short Isoform), and GAPDH Primers Used in RT-PCR

Gene (gen bank accession no.)	Primer sequence	Product size (base pairs)
Ob-R_L (U43168)	5′ primer 5′-TTGTGCCAGTAATTATTTCCTCTT-3′ 3′ primer 5′-CTGATCAGCGTGGCGTATTT-3′	439
Ob-R_S (U52914)	5′ primer 5′-ATTCAATTGGTGCTTCTGTT-3′ 3′ primer 5′-CATTGGGTTCATCTGTAGTG-3′	573
GAPDH(M33197)	5′ primer 5′-TGATGACATCAAGAAGGTGGTGAAG-3′ 3′ primer 5′-TCCTTGGAGGCCATGTAGGCCAT-3′	240

with ethidium bromide. PCR reactions were accompanied by the following controls: GAPDH affirmed consistent cDNA synthesis; sterile water blanks served as a reagent control; and RNA that had not been transcribed into cDNA was used as a control for DNA contamination.

Linear ranges for each receptor isoform and GAPDH were determined to ensure that all reactions were analyzed during the exponential phase of amplification. Amplification efficiencies for each receptor isoform and GAPDH were found to be consistent between individual reactions, ensuring minimal interassay variability (16). Band intensities were analyzed using the Alpha Imager 2000 digital analysis system (Alpha Innotech, San Leandro, CA), as we have previously described (5, 11). Semiquantitative assessment of mRNA transcripts was made *via* a ratio between leptin receptor isoforms and GAPDH.

***In Situ* Hybridization.** Paraffin-embedded tissue sections (6 μm) were floated onto Superfrost Plus microscope slides (Labcraft, Fisher Scientific, Norwalk, GA). The slides were fixed overnight at 37°C and baked at 65°C prior to deparaffinization. *In situ* hybridization was performed using 5′ biotinylated probes for both Ob-RL and Ob-RS, as we have described previously (5).

Statistical Analysis. A one-way analysis of variance (ANOVA) and *t*-tests were performed to establish statistical significance between groups. Significant differences were understood to exist when $P < 0.05$.

Results

Gestational Profile for Leptin Receptor Isoforms. Ob-RL and Ob-RS expression was determined *via* RT-PCR in placental villous, amniochorion, decidua, corpus luteum, omental, and subcutaneous adipose tissues (Fig. 1). Expression of Ob-RL and Ob-RS mRNA transcripts was semiquantitatively assessed through comparison with constitutively expressed GAPDH. No significant differences ($P > 0.05$) in Ob-RL or Ob-RS transcript abundance were observed as a consequence of advancing gestation in placenta, omental adipose tissue, or subcutaneous adipose tissue (Fig. 2). Thus, both isoforms appear to be constitutively expressed throughout baboon pregnancy. Also as illustrated in Figure 2, Ob-RS transcripts were consistently expressed in greater abundance than Ob-RL transcripts in placenta ($P < 0.002$), omental adipose tissue ($P < 0.02$), and mid- and late subcutaneous adipose tissue ($P < 0.01$). There were no statistically significant differences between Ob-RS and Ob-RL in subcutaneous fat collected at the early time point.

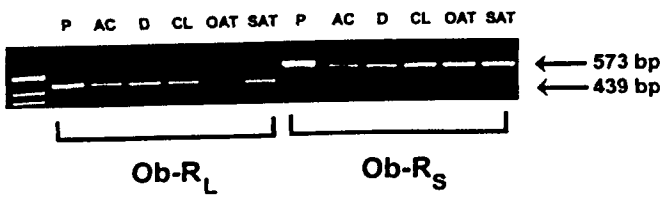

Figure 1. Expression of Ob-RL and Ob-RS mRNA transcripts in peripheral tissues. Ob-RL (439 bp) and Ob-RS (573 bp) mRNA transcript expression was demonstrated by RT-PCR in placental villous tissue (P), amniochorion (AC), decidua (D), corpus luteum (CL), omental adipose tissue (OAT), and subcutaneous adipose tissue (SAT).

Placental Histology. To determine specific cellular localization of Ob-RL and Ob-RS transcription, sections of placental villous tissue were evaluated by standard light

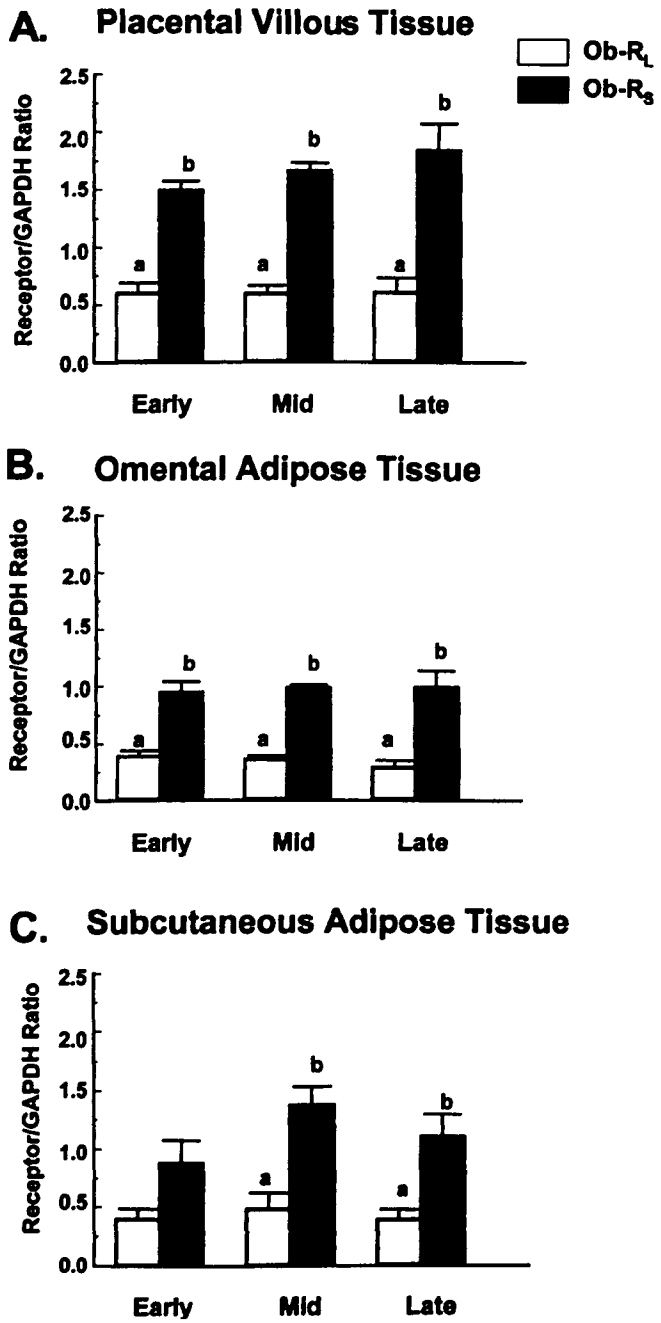

Figure 2. Ob-RL and Ob-RS mRNA transcripts in peripheral tissues. Ratios of band intensities of Ob-RL and Ob-RS receptor isoforms to GAPDH at early (Days 60–62), mid- (Days 98–102), and late (Days 159–167) gestation in (A) placental villous tissue [$n = 5$], (B) omental adipose tissue [$n = 4$], and (C) subcutaneous adipose tissue [$n = 4$]. Each bar represents the mean ± standard error. Open bars denote Ob-RL, and closed bars denote Ob-RS. Different lowercase letters denote statistically significant differences in transcript abundance: placental tissue (*a* compared with *b*, $P < 0.002$), omental adipose tissue (*a* compared with *b*, $P < 0.02$), subcutaneous adipose tissue (*a* compared with *b*, $P < 0.01$).

microscopy and *in situ* hybridization. As depicted in Figure 3A, hematoxylin and eosin staining revealed multinucleated syncytiotrophoblasts surrounding placental villi (black arrowhead). Ob-RS and Ob-RL mRNA transcripts were predominantly expressed within the syncytiotrophoblast encasing the villous trees (Figs. 3B & 3C). The 5′ sense strand was used as a negative control for both receptor isoform hybridizations. Figure 3D is representative of a negative control, using the 5′ sense strand for Ob-RL.

Discussion

The leptin receptor is a key component in the regulation of leptin action at the tissue level. Thus, leptin receptors are expressed in a variety of organs, and *in vitro* evidence suggests that, in addition to effects on the hypothalamus, leptin may also exert direct effects on a number of peripheral tissues, including the placenta (5), ovary (6), adrenal gland (15), and adipose tissue (17). Although a number of regulatory roles for leptin have been implied (1, 4, 11, 15, 18), little is known about leptin receptor ontogeny during primate pregnancy. Therefore, to more fully understand pregnancy as a unique physiological state that affects whole-body metabolism and energy expenditure, six distinct tissues were examined in the current study for expression of two signaling competent isoforms of the leptin receptor. The presence of both receptor isoforms in the placenta, amniochorion, decidua, corpus luteum, and adipose tissue attests to the potential significance of leptin as a reproductive hormone. Physiological roles for leptin at these peripheral sites are yet undefined, but we might hypothesize from our findings (5, 11) and those of others (19) that leptin has the potential to initiate, *via* receptor-mediated mechanisms, signaling cascades ultimately affecting placental endocrine function, conceptus growth, and/or the maternal hypothalamic-pituitary axis.

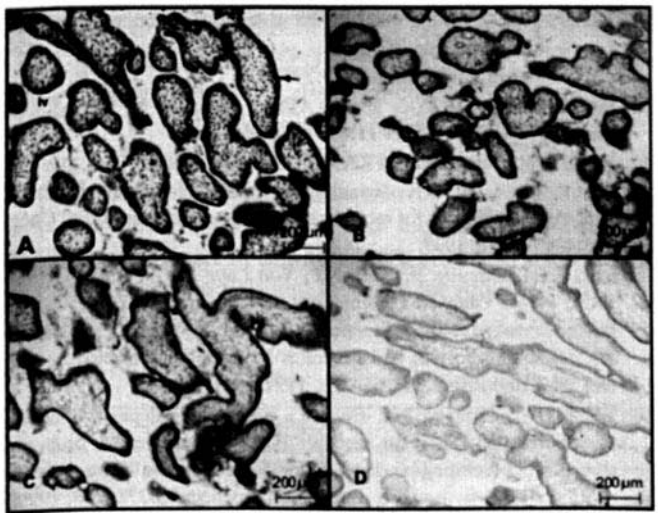

Figure 3. *In situ* hybridization of Ob-RL and Ob-RS in placenta. (A) Hematoxylin and eosin stain of villous placental tissue from early in gestation. The syncytiotrophoblast (black arrowhead) and the intravillous space (iv) are denoted. Positive *in situ* hybridization of (B) Ob-RS and (C) Ob-RL, using the 3′ antisense mRNA. (D) *In situ* hybridization of Ob-RL using the 5′ sense mRNA as a negative control.

Although all tissue samples were collected after the luteal-placental shift, we report that long and short isoforms of the leptin receptor are constitutively expressed in placenta, as well as in two distinct adipose depots, throughout most of baboon pregnancy. This finding contrasts with a recent report, which documented an increase in placental leptin receptor mRNA transcripts with advancing gestation in the rat (20). However, certain differences may be inherent between primate and rat pregnancy. In this capacity, leptin transcripts may (21) or may not (22) be present in rat placenta, a feature that may be in contrast to both the baboon and the human, species that exhibit similar profiles for placental leptin mRNA transcripts during pregnancy (5, 11). Because of this and other reproductive similarities, we have proposed the baboon to be an excellent model for the study of leptin and leptin receptor biology during human pregnancy (11).

In the baboon, Ob-RS mRNA transcripts were expressed in greater abundance than Ob-RL, suggesting the potential for divergent signaling capacities. Leptin receptor signaling involves the dimerization of the cytoplasmic domains of the receptor chains. This action is essential for signal transduction and is proposed to bring JAKs, which are associated with intracellular motifs, into the necessary proximity for cross-phosphorylation and activation of their catalytic domains (23). Upon JAK activation, STAT proteins are recruited, dimerized, and translocated to the nucleus to modulate the transcription of target genes. Ob-RS and Ob-RL are the two most prominent isoforms of the leptin receptor, and they have differing signaling capabilities, reflected in the varying lengths of their intracellular domains. Ob-RL has a fully intact intracellular domain, which enables it to activate the JAK/STAT pathway, whereas Ob-RS is unable to recruit STAT proteins due to the absence of certain intracellular protein motifs (24). Despite this, Ob-RS still has the capacity to transduce signals through JAK or MAPK activation (8).

This study demonstrated a greater abundance of placental and adipose Ob-RS mRNA transcripts than Ob-RL, suggesting an increased presence of the membrane-bound shorter isoform. Precedent established from other homodimerizing cytokine receptors attests to strong dominant-negative repression, by dimerization, of inefficient signaling isoforms with signaling competent ones (25). This implies that Ob-RS may repress the signaling capacity of Ob-RL by forming a heterodimer. Despite this supposition, Ob-RL is only minimally affected by dominant negative repression by Ob-RS heterodimer formation (24, 26) and is capable of signal transduction in light of a greater abundance of Ob-RS (26). In peripheral tissues, Ob-RS, though incapable of activating STAT proteins, may be of greater importance than once thought in transducing leptin signals to the nucleus. The greater abundance of Ob-RS may facilitate an increased activation of other signaling pathways.

The syncytiotrophoblast is that portion of the placenta responsible for the manufacture of many of the steroid and polypeptide hormones normally associated with pregnancy in the human and nonhuman primate (10, 13). In the current study, two leptin receptor isoforms were localized to this cell population within the baboon placenta. Previously, we have identified transcripts for leptin in baboon syncytiotrophoblast (11), and both leptin and two leptin receptor isoforms in human syncytiotrophoblast (5). Collectively therefore our results suggest that leptin has the capacity to act in an autocrine/paracrine fashion within this endocrinologically active tissue. We also report that Ob-RL and Ob-RS mRNA transcripts are constitutively expressed in baboon placenta and adipose tissues, with Ob-RS expressed in relatively greater abundance. Both isoforms were also found to be expressed in amniochorion, decidua, and corpus luteum. This study further emphasizes the potential importance of leptin to regulatory mechanisms in the primate maternal-fetoplacental unit and to the utility of the baboon as an endocrine model for human pregnancy. However, further investigations are needed to address the physiological roles of leptin and its receptor throughout primate pregnancy.

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