Leptin receptor expression in fetal lung increases in late gestation in the baboon: a model for human pregnancy

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Abstract

Leptin produced by both adipose tissue and the placental trophoblast, has been proposed to regulate numerous aspects of human conceptus development. Although recent animal studies have suggested an additional role for the polypeptide in fetal lung maturation, no evidence has been reported in primates. Therefore, we employed the baboon (*Papio* sp.), a well-characterized primate model for human pregnancy, to determine the presence and ontogeny of leptin receptor in fetal lung with advancing gestation. Lungs were collected from fetal baboons, early in gestation (days 58–62, $n = 4$), at mid gestation (days 98–102, $n = 4$), and late in gestation (days 158–165, $n = 4$) (term 184 days). mRNA transcripts for leptin (LEP) and both long and short intracellular domain isoforms of the leptin receptor (LEP-R_L and LEP-R_S) were assessed by RT-PCR. Leptin receptor protein was evaluated by immunoblotting and cell types expressing leptin receptor were identified in late pregnancy by immunohistochemistry. Fetal serum leptin concentrations, determined by RIA, remained relatively unchanged at 5.7 ± 1.1 ng/ml (mean $\pm$ S.E.M.) in mid pregnancy and 8.4 ± 3.0 ng/ml in late pregnancy ($P > 0.05$). Although leptin were detectable in fetal lung, no changes in transcript abundance were apparent with advancing gestation. However, transcripts for both LEP-R_L and LEP-R_S receptor isoforms increased several-fold ($P < 0.05$) in fetal lung between mid and late gestation, while leptin receptor protein was detectable only in late pregnancy. Leptin receptor was localized in distal pulmonary epithelial cells, including type II pneumocytes. In conclusion, leptin is present in the fetal baboon and its receptor is enhanced during late gestation in cells responsible for the synthesis of pulmonary surfactant. Collectively, these and past findings may suggest a modulatory role for the polypeptide in pulmonary development and/or may identify leptin receptor as a physiological marker of primate fetal lung maturity.

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Introduction

During human pregnancy leptin is produced by maternal and fetal adipose tissues, as well as by the placental trophoblast, and is measured in significant concentrations in the maternal, fetal and umbilical circulations (Henson & Castracane 2000). In addition to modulating adipose stores and maintaining energy homeostasis via satiety (Friedman & Halaas 1998, Ahima & Flier 2000), leptin has also been implicated as a direct regulator of conceptus development. Proposed roles for this multifaceted polypeptide include regulating trophoblast invasion and endocrinology, fetal and placental growth, angiogenesis, hematopoiesis, fetal bone development, and

the maturation of the fetal immune system. Indeed, acting via autocrine and/or paracrine mechanisms, leptin may be an important factor in the maintenance of pregnancy itself (Bajoria *et al.* 2002, Henson & Castracane 2002, 2003, Sagawa *et al.* 2002).

In addition to these roles, leptin has also been associated with the regulation of pulmonary development and respiration. Thus, in the mouse, leptin receptor mRNA has been detected in the lungs of fetuses (Hoggard *et al.* 1997). Specific leptin-binding sites in the pulmonary parenchyma and bronchiolar epithelium bind purified radio-labeled analog and leptin receptor expression has been demonstrated between birth and adulthood (Dal Farra *et al.* 2000). Receptors are also expressed in both normal

human lung and the SQ-5 human lung squamous cancer cell line. SQ-5 cell proliferation was enhanced following co-incubation with recombinant human leptin, suggesting a receptor-mediated effect (Tsuchiya *et al.* 1999). In the *ob/ob* mouse, respiratory anomalies that are common with the obese phenotype (tachypnea, decreased lung compliance and aberrant respiratory muscle adaptations) were attenuated following prolonged leptin administration (Tankersley *et al.* 1998). Similarly, in wild-type mice that were obese merely as a consequence of their high-fat diet, respiratory depression was alleviated by leptin treatment (O'Donnell *et al.* 1999).

Insufficient maturation of the fetal lungs, a condition that can be characterized by the production of inadequate amounts of pulmonary surfactant by epithelial type II cells, is a leading cause of human neonatal morbidity and mortality following premature birth. In the term infant, an increase in cortisol prompts the differentiation of type II cells and consequently, surfactant synthesis (Bolt *et al.* 2001). However, in preterm infants, surfactant levels are insufficient and pulmonary insults lead to acute lung injury and in some cases, chronic lung disease. Surfactant production is also modulated by soluble growth factors that mediate epithelial mesenchymal interactions. In this capacity, Torday *et al.* (2002), reported that leptin was expressed by fibroblasts and that leptin receptor was expressed by type II cells in fetal rat lung, suggesting a paracrine signaling mechanism in developing pulmonary tissue. They later concluded that leptin plays a direct role in enhancing surfactant production (Torday & Rehan 2002), a potential recently reiterated by Vlahakis & Hubmayr (2003). Leptin receptor was also identified in fetal rabbit type II cells and additional evidence supported correlations between leptin, maturation of the pulmonary epithelium and surfactant production (Bergen *et al.* 2002). Evidence associating leptin with human pulmonary development has yet to be reported, necessitating the need for the current study, which to the best of our knowledge is the first to investigate the potential inter-relationship of leptin with fetal pulmonary development in a primate model.

Significant differences exist between humans and non-primate species with respect to the endocrinology of pregnancy and the development of a functional maternal-fetoplacental unit (Pepe & Albrecht 1995). Therefore, in the current study we employed the baboon, a nonhuman primate that is an excellent model for human pregnancy with regard to both general gestational endocrinology (Castracane & Goldzieher 1986, Henson 1998) and LEP-related mechanisms (Henson *et al.* 1999, Green *et al.* 2000, O'Neil *et al.* 2001). Our primary objectives were to determine: (i) fetal serum leptin concentrations; (ii) leptin receptor ontogeny, via quantitative assessment of mRNA transcripts for leptin (LEP) in fetal lungs in early, mid and late pregnancy; (iii) the presence of leptin receptor protein in fetal pulmonary tissue; and (iv) the identities of specific cell types in fetal lung that express leptin receptor.

Materials and Methods

Animals

Animals were maintained and used in accordance with USDA regulations and the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23). The protocol was approved by the Institutional Care and Use Committee of the Tulane National Primate Research Center. Female baboons (*Papio* sp.) were quartered with males for mating in indoor/outdoor enclosures at the anticipated time of ovulation, as determined by daily menstrual cycle history and visible turgescence of external sex skin. Females were later housed individually in stainless steel cages, as we have previously described (Henson *et al.* 1999, Shi *et al.* 1999). Twelve-hour light photoperiods (0600–1800 h) were maintained in air-conditioned rooms and animals received a maintenance ration with fresh fruit daily and water freely available.

Collection of tissues

Lung tissue was obtained from fetal baboons upon cesarean delivery, under isofluorane anesthesia, after sedation with ketamine HCl/atropine, as we have previously described (Henson *et al.* 1999). Surgeries were performed early in gestation (days 58–62, $n = 4$), at mid gestation (days 98–102, $n = 4$), or late in gestation (days 158–165, $n = 4$) (term 184 days). Fetal blood samples were collected via cardiac puncture, prior to killing (sodium pentobarbital) and the collection of fetal lung tissue. Serum was separated by centrifugation (1000 *g*, 10 min) and frozen at -20°C for RIA. Tissue was flash-frozen in liquid nitrogen and stored at -70°C , with random sections utilized for later RT-PCR and immunoblotting, or fixed in 10% formalin for immunohistochemistry.

RNA extraction and RT-PCR

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), according to Chomczynski & Sacchi (1987) and Chirgwin *et al.* (1979), as we have adapted for use in our laboratory (Henson *et al.* 1998, Shi *et al.* 1999). All samples were treated with DNase (Invitrogen) to eliminate DNA contamination and reprecipitated with sodium acetate and 100% ethanol. Oligonucleotide primers were synthesized (Midland Reagent Company, Midland, TX, USA) for leptin (Wabitsch *et al.* 1996), long (LEP- R_L) (Kielar *et al.* 1998) and short (LEP- R_S) (Glasow *et al.* 1998) intracellular domain isoforms of the leptin receptor, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Henson *et al.* 1998). Sequence analysis of PCR products (Biotech Core, Palo Alto, CA, USA) confirmed that primers specifically amplified regions of leptin and leptin receptor cDNAs. cDNAs were synthesized from 2 μg total RNA using the SuperScript kit (Invitrogen) and PCR was performed using HotStarTaq DNA Polymerase (Qiagen Inc., Valencia, CA,

USA) in a GeneAmp PCR System 2400 (Perkin Elmer Applied Biosystems, Foster City, CA, USA) as we have described (Henson *et al.* 1998).

Conditions for PCR were adapted from Green *et al.* (2000) and O'Neil *et al.* (2001). They were as follows: LEP 30 cycles for amplification, denaturation at 94 °C for 60 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s; LEP-R_s, 30 cycles for amplification, denaturation at 94 °C for 30 s, annealing at 51 °C for 45 s, extension at 72 °C for 30 s; LEP-R_L, 35 cycles for amplification, denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s; GAPDH, 24 cycles for amplification, denaturation at 94 °C for 30 s, annealing at 51 °C for 45 s, extension at 72 °C for 30 s. PCR products were visualized under u.v. light on 2% agarose gels 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 (absence of reverse transcriptase) was used as a control for DNA contamination, with no PCR products being evidenced in subsequent validation experiments. Following semiquantitative assessment of mRNA transcripts by comparison of PCR products with GAPDH, quantitative competitive RT-PCR was performed using the PCR MIMIC methodologies we have described (Henson *et al.* 1998, 1999). In this methodology, one set of primers is used to amplify both the target gene cDNA and the mimic, a non-homologous DNA fragment engineered to contain specific target gene sequences necessary for recognition by the gene specific primers for the gene of interest. The mimic DNA competes with the target cDNA for the same primers, thereby serving as an internal standard. Products were resolved by 2% agarose gel electrophoresis, and band intensities analyzed using the Alpha Imager 2000 digital analysis system (Alpha Innotech, San Leandro, CA, USA). Concentrations were expressed as attomoles/μg RNA (1 attomole = 6×10^5 molecules). All PCR experiments are subjected to replication, with the subsequent documentation of similar results required.

Immunoblotting

Total protein was extracted from fetal lung tissue using a homogenization buffer containing: 40 mmol/l KH₂PO₄, 10 mmol/l sucrose, 50 mmol/l KCl, 30 mmol/l EDTA, 2 mmol/l EGTA, 25 μg/ml aprotinin, 25 μg/ml leupeptin and 1 mmol/l polymethylsulfonylfluoride. The tissue homogenate was then centrifuged, supernatant collected and protein concentration quantified according to Bradford (1976). Fifty micrograms of protein were denatured, reduced and then electrophoresed on a pre-cast 4–12% gradient Bis-Tris gel (Invitrogen), before transfer to nitrocellulose for immunoblot detection. Standards of known molecular mass (BioRad, Hercules, CA, USA) were run in adjacent lanes. Leptin receptor was detected with goat polyclonal anti-human leptin receptor (N-20; Santa Cruz

Biotechnology Inc., Santa Cruz, CA, USA) at a dilution of 1:500. This antibody detects the extracellular domain of the leptin receptor, i.e. both long (LEP-R_L) and short (LEP-R_s) isoforms. A secondary antibody, donkey polyclonal anti-goat IgG, labeled with horseradish peroxidase (Santa Cruz) was then detected using the ECL Western blotting detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) utilizing chemiluminescence film (HyperFilm; Amersham). A specific blocking peptide (Santa Cruz) for the leptin receptor antibody was used effectively as a control, successfully blocking the receptor signal.

Immunohistochemistry

Immunohistochemistry was performed according to the technique of Liu *et al.* (1997), as adapted for use in our laboratory. Fetal baboon lung was fixed in 10% buffered formalin, paraffin imbedded and 5 μm sections cut. The sections were deparaffinized, antigen unmasked in citrate buffer and endogenous peroxidase activity quenched in 0.3% hydrogen peroxide diluted in methanol. Sections were then incubated in PBS, 0.2% fish skin gelatin (FSG) and 0.1% Triton X-100 for 20 min. Immunohistochemistry was facilitated using the Goat ABC Staining System (Santa Cruz) in a humidified chamber. Tissues were incubated in normal blocking serum for 1 h, then incubated with primary antibody for either human leptin receptor (Ob-R [N-20]; Santa Cruz) or pulmonary surfactant A (SP-A [N-19]; Santa Cruz) at a 1:25 dilution in normal blocking serum for 1 h at room temperature. Sections were again washed with PBS/FSG, then incubated with biotinylated secondary antibody. Sections were then washed with PBS/FSG then incubated with AB enzyme reagent. Hybridization was then detected using peroxidase substrate. Sections were counter-stained using Gill's hematoxylin (2 × formulation) and mounted. Controls included sections without: (i) primary antibody and (ii) primary and secondary antibody.

RIA

Leptin concentrations were determined by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA), employing an antibody against human leptin. Inter- and intraassay coefficients of variation ($n = 5$) for leptin were less than 9.0 and 5.0% respectively, with a sensitivity of 0.5 ng/ml. The human assay had been previously validated for the baboon using a pregnant serum pool, for which serial dilutions yielded concentrations that were linear and parallel to those in human serum (Henson *et al.* 1999).

Statistical analysis

ANOVA, with standard post-hoc Student–Newman–Keuls tests, was employed using SigmaStat statistical analysis software, version 5.0 (SPSS, Richmond, CA, USA), to establish statistical significance between groups. Significant differences were understood to exist when $P < 0.05$.

Results

Leptin and leptin receptor mRNA transcripts

Leptin mRNA transcripts were detected by RT-PCR in fetal lung tissue, although no apparent rise or fall in transcript abundance could be ascertained with advancing gestation (data not shown). However, leptin receptor transcript abundance (both long and short isoforms), as assessed by basic RT-PCR in respect to constitutively expressed GAPDH, appeared to increase dramatically in fetal lung tissue following mid gestation (Fig. 1). Indeed, as quantified by competitive RT-PCR and depicted in Fig. 2A, LEP-RL transcript abundance (*n* = 4) increased from 0.011 ± 0.004 attomoles/ μ g total RNA (mean $\pm$ S.E.M.), in early gestation, to 0.017 ± 0.004 in mid gestation, to 0.119 ± 0.013 in late gestation. Therefore, although LEP-RL transcript abundance in early and mid gestation was quite low, approaching the level of non-detection, concentrations in late gestation were 10- and 7-fold greater (*P* < 0.05) respectively. Overall, specific mRNA transcripts for LEP-Rs were of greater abundance than those for LEP-RL, although a similar rate of increase was observed with respect to advancing gestation. Thus, as illustrated in Fig. 2B, LEP-Rs transcript abundance (*n* = 4) increased from 0.069 ± 0.028 attomoles/ μ g total RNA in early gestation, to 0.139 ± 0.032 in mid gestation, to 0.550 ± 0.057 in late gestation. Therefore, LEP-Rs transcript abundance in late gestation was approximately 4-fold greater (*P* < 0.05) than at mid gestation and 8-fold greater (*P* < 0.05) than in early gestation.

Figure 1 Representative expression of mRNA transcripts for LEP-RL (A) and LEP-Rs (B) isoforms and GAPDH (C) in fetal lung tissues collected from two baboon fetuses in early (lanes 1 and 2) pregnancy, as well as in mid (lanes 3 and 4) and late (lanes 5 and 6) pregnancy.

Figure 2 LEP-RL (A) and LEP-Rs (B) mRNA transcript abundance, as determined by competitive RT-PCR in fetal lung tissues collected in early (*n* = 4 fetuses), mid (*n* = 4 fetuses), and late (*n* = 4 fetuses) baboon pregnancy. Different lowercase letters indicate significant differences between means (ab, *P* < 0.01).

Leptin receptor protein

An immunoblot, which is representative of lung tissue collected from baboon fetuses at early (*n* = 2), mid (*n* = 2) and late (*n* = 2) gestation, appears as Fig. 3. Leptin receptor protein could not be detected by immunoblotting in fetal lungs collected in early and mid gestation, but was detected (approximately 130 kDa, as determined by molecular mass standards – not shown) in tissues collected in late gestation.

Localization of leptin receptor in fetal lung

As depicted in Fig. 4A–D, leptin receptor protein in lungs from late gestation fetuses was localized by immunohistochemistry in distal pulmonary epithelial cells, including type II cells (Fig. 4C). This cell type is known to actively

Figure 3 Excerpt from a representative immunoblot depicting relative LEP receptor abundance (130 kDa) in fetal lung tissues collected in early (*n* = 2 fetuses, lanes 1 and 2), mid (*n* = 2 fetuses, lanes 3 and 4), and late (*n* = 2 fetuses, lanes 5 and 6) baboon pregnancy.

Figure 4 Photomicrographs of lung tissue from fetal baboons in late gestation depicting the results of (A) hematoxylin-eosin staining ($\times 200$) and immunohistochemical localization of (B) surfactant protein A ($\times 400$) or (C) LEP receptor ($\times 400$) protein in pulmonary epithelial cells. A negative (without primary antibody) immunohistochemical control for LEP receptor ($\times 400$) is included (D). Bars in panels A ($\times 200$) and B ($\times 400$) are 50 μm . Arrows denote pulmonary type II cells.

produce pulmonary surfactant, as evidenced by specific immunohistochemical staining of surfactant protein A (Fig. 4B) in alternate sections.

Fetal serum leptin concentrations

Fetal serum leptin levels ranged from 5.7 ± 1.1 ng/ml at mid gestation to 8.3 ± 3.0 ng/ml in late gestation, concentrations that were not significantly different ($P > 0.05$) with respect to gestational age.

Discussion

The fetal lung develops the capacity for gas exchange relatively late in human pregnancy, as with increasing maturity surface tension declines at the alveolar air-liquid interface in response to the production of surfactant glycerophospholipids and proteins (Pepe & Albrecht 1995). Fetal epithelial type II cells in the pulmonary alveoli produce this material, which is stored in lamellar bodies and then secreted into the air spaces via exocytosis. Although some type II cells are present in terminal alveolar sacs at mid gestation, the production of abundant surfactant does not begin until nearer term, a trait shared by humans with nonhuman primates (Feijen *et al.* 1979). Insufficient surfactant production commonly results in respiratory distress syndrome, a condition characterized by alveolar collapse, the overdistention of remaining compliant alveoli, pulmonary edema and focal pulmonary hemorrhages. It is a major cause of morbidity and mortality in premature infants (<32 weeks gestation, <1200 g body weight). The maturation of type II cells, along with their potential to produce adequate surfactant, is principally regulated by

the enhanced concentration of fetal cortisol inherent at term, a mechanism that in cases of impending premature delivery can be elicited by the administration of synthetic glucocorticoids.

In addition to the effects of cortisol, an array of growth factors, including transforming growth factor- $\beta 3$, epithelial growth factor, tumor necrosis factor and vasointestinal peptide are involved in pulmonary development (Bolt *et al.* 2001). Indeed, in fetal baboons in which cortisol levels were suppressed *in utero* via estrogen deprivation, lung maturation proceeded unabated, suggesting the need for further studies to identify and characterize associated regulatory factors (Pepe *et al.* 2003). Therefore, although it is known that the maturation of type II cells is modulated by a number of soluble, low molecular weight peptides, the identity of a specific fibroblast pneumocyte factor (FPF) remains elusive. This protein is proposed to be induced by glucocorticoids, promote surfactant production, and be down-regulated by androgens (Torday *et al.* 2002). A number of potential commonalities exist between leptin and this putative modulator. Thus, it is interesting to note that lung development in male fetuses is somewhat delayed when compared with females in a variety of species, a phenomenon that reflects an inhibition by androgens (Provost *et al.* 2002). This relationship is mirrored by a similar androgen inhibition of leptin biosynthesis, an effect that is definitively linked to gender (Casabiell *et al.* 2001, Morash *et al.* 2001). Other regulatory parallels include the effects of glucocorticoids, which in addition to up-regulating the putative FPF in the lung (Bolt *et al.* 2001), increase leptin synthesis and release by human adipocytes (Leal-Cerro *et al.* 2001), and enhance leptin levels in preterm infants

(Ng *et al.* 2002). Moreover, the co-culture of human placental trophoblast cells with glucocorticoids has been observed to enhance leptin release (Coya *et al.* 2001), while maternal glucocorticoid treatment resulted in an up-regulation of placental leptin receptor protein in rat pregnancy (Sugden *et al.* 2001). Therefore, with respect to: (i) the effects of androgens and glucocorticoids; (ii) the common tissues of origin of both leptin and the putative regulator; and (iii) the similarities in the molecular masses of leptin and the proposed FPF (Bergen *et al.* 2002, Torday & Rehan 2002), leptin has been suggested by Torday *et al.* (2002) as a logical FPF candidate.

Fetal leptin, originating in the placental trophoblast (Chen *et al.* 2000) or in fetal adipose tissues (Lepercq *et al.* 2001), is firmly associated with conceptus growth and development in a number of species, including humans (Bajoria *et al.* 2002, Henson & Castracane 2002, 2003, Sagawa *et al.* 2002). Effects are mediated in a variety of tissues via a specific leptin receptor, which is a member of the cytokine class I receptor superfamily. It exists as multiple alternatively spliced isoforms, each differing in the length of their intracellular domains (Tartaglia 1997). The long isoform (LEP-R_L) is detected in various tissues, but is predominant in the hypothalamus, where it functions in the regulation of satiety and is capable of signal transduction through the activation of Janus kinase and the signal transducers and activators of transcription. A shorter isoform (LEP-R_S) is predominant in peripheral tissues such as the lung and, as reported by Bjorbaek *et al.* (1997, 2001), contributes to leptin signaling via mitogen-activated protein kinase. In the present study, we have determined the ontogeny of leptin receptor in fetal lung during pregnancy in the baboon, a species that is a proven investigational model for the endocrinology of human pregnancy (Castracane & Goldzieher 1986, Pepe & Albrecht 1995, Henson 1998), as well as for fetal (Kotas & Kling 1979, Coalson *et al.* 1998, Pepe *et al.* 2003) and neonatal (Janssen *et al.* 2002) lung maturation. Indeed, the choice of model may be of even greater importance with respect to those mechanisms specifically linked to leptin dynamics, as significant differences in leptin and leptin receptor regulation may exist in pregnancy-associated tissues (fetus, placenta, amniochorion, decidua, etc.) in primates (human and non-human) vs those in nonprimate species (Henson & Castracane 2000, 2002, 2003, Bajoria *et al.* 2002, Sagawa *et al.* 2002).

With regard to the potential for leptin to modulate pulmonary development and function, cumulative evidence identifies both fetal and adult lungs as leptin-responsive tissues (Chen *et al.* 1996, Hoggard *et al.* 1997, Tsuchiya *et al.* 1999, Dal Farra *et al.* 2000), and clearly implies roles for the polypeptide in helping to stimulate pulmonary cell proliferation (Tsuchiya *et al.* 1999) and enhancing stretch-induced surfactant production (Torday & Rehan 2002, Torday *et al.* 2002, Vlahakis & Hubmayr 2003). In evidence of these effects, genetically obese (*ob/ob*) mice are unable to produce leptin, but are exposed to leptin

in utero by virtue of their propagation in *+/+* or *ob/+* (leptin-sufficient) dams. However, following birth, lung volume and pulmonary compliance in neonates became attenuated if not supplemented with exogenous leptin (Tankersley *et al.* 1998). With respect to mice that were leptin receptor deficient due to genetic mutation, Tsuchiya *et al.* (1999) reported that the rate of tracheal epithelial cell proliferation was only one-quarter ($P < 0.01$) that of their unaffected littermates, illustrating a potentially important supplementary role for leptin in pulmonary development. Other studies further suggest a significant impact of leptin on general pulmonary health in rodents (O'Donnell *et al.* 1999), as well as on specific respiratory disorders in humans (O'Donnell *et al.* 2000). Intriguingly, obesity-related respiratory anomalies are characteristically exacerbated in males (Kapsimalis & Kryger 2002), perhaps reflecting again the gender dichotomy that is typical with respect to circulating leptin concentrations (Casabiell *et al.* 2001).

In conclusion, we have reported that leptin originating in the placental trophoblast and/or fetal adipose tissue exists in significant concentrations in the circulation of the fetal baboon, attesting to its physiological availability. Although we previously determined that leptin receptor transcripts in both the placental trophoblast and maternal adipose tissues were constitutively expressed throughout baboon pregnancy (Green *et al.* 2000), our current findings indicate a significant divergence with respect to fetal lung. Thus, transcripts for two isoforms of the leptin receptor were ontogenetically up-regulated in fetal pulmonary tissue with advancing gestation, an effect that was mirrored by the synthesis of leptin receptor protein near term. Immunoblotting of leptin receptor revealed a protein of approximately 130 kDa, similar to that previously reported in other species (Hoggard *et al.* 1997, Tartaglia 1997, Bergen *et al.* 2002). Although the determination of a specific cause and effect relationship was beyond the scope of the present study, leptin receptor expression was observed largely in type II epithelial cells, which are responsible for the production of pulmonary surfactant. These constitute the first such observations in a primate. Ontogenetic regulation of the receptor may suggest a role for the polypeptide in modulating general pulmonary development or optimizing surfactant production, and/or may identify leptin receptor as a physiological marker of primate fetal lung maturity.

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