Leptin does not influence surfactant synthesis in fetal sheep and mice lungs

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Sato A, Schehr A, Ikegami M. Leptin does not influence surfactant synthesis in fetal sheep and mice lungs. Am J Physiol Lung Cell Mol Physiol 300: L498–L505, 2011. First published January 7, 2011; doi:10.1152/ajplung.00418.2010.—In the fetus, leptin in the circulation increases at late gestation and likely influences fetal organ development. Increased surfactant by leptin was previously demonstrated in vitro using fetal lung explant. We hypothesized that leptin treatment given to fetal sheep and pregnant mice might increase surfactant synthesis in the fetal lung in vivo. At 122–124 days gestational age (term: 150 days), fetal sheep were injected with 5 mg of leptin or vehicle using ultrasound guidance. Three and a half days after injection, preterm lambs were delivered, and lung function was studied during 30-min ventilation, followed by pulmonary surfactant components analyses. Pregnant A/J mice were given 30 or 300 mg of leptin or vehicle by intraperitoneal injection according to five study protocols with different doses, number of treatments, and gestational ages to treat. Surfactant components were analyzed in fetal lung 24 h after the last maternal treatment. Leptin injection given to fetal sheep increased fetal body weight. Control and leptin-treated groups were similar in lung function (preterm newborn lamb), surfactant components pool sizes (lamb and fetal mice), and expression of genes related to surfactant synthesis in the lung (fetal mice). Likewise, saturated phosphatidylcholine and phospholipid were normal in mice lungs with absence of circulating leptin (ob/ob mice) at all ages. These studies coincided in findings that neither exogenously given leptin nor deficiency of leptin influenced fetal lung maturation or surfactant pool sizes in vivo. Furthermore, the key genes critically required for surfactant synthesis were not affected by leptin treatment.

ALVEOLAR SURFACE IS OVERLAI'D with a pulmonary surfactant film, which reduces surface tension occurring on the alveolar surface (48). Low surface tension is critical for gas exchange, alveolar capillary blood flow (25), and alveolar macrophage function of phagocytosis (1). In the fetal lung, the synthesis of pulmonary surfactant dramatically increases at late gestation to prepare for transition to air breathing at birth. Increases in surfactant saturated phosphatidylcholine (Sat PC) pool size correlate with the increase in dynamic lung compliance in the developing lung (23), and deficiency of surfactant causes respiratory distress syndrome in the preterm newborn infant. Maternal corticosteroid treatment has been used to induce fetal lung maturation for high-risk pregnancies of premature delivery, and preterm newborn infants are routinely treated with surfactant, yet mortality rate of the preterm newborn infants still remains high. New therapy to induce fetal lung maturation and surfactant synthesis has been sought.

Leptin is a 16-kDa polypeptide hormone that is principally synthesized and secreted by adipose tissue in a number of organs, including lipofibroblast in the lung (18, 45, 46). Leptin has a wide range of physiological function, such as regulation of satiety, energy metabolism, bone formation, and reproduction. In the human and sheep fetus, leptin is secreted mainly by fetal adipose tissue (43) into the circulation that increases at late gestation (7, 12) and likely influences fetal organ development (18, 46). Increase in synthesis of surfactant lipid and surfactant proteins by the addition of leptin was demonstrated in vitro using fetal lung explant in culture (27, 44, 45). The only in vivo study of maternal leptin treatment for fetal lung maturation was on the rat, in which 14% increase in fetal lung weight relative to body weight was stated. Surfactant components were not quantitatively analyzed in this previous study (27). The efficacy of leptin treatment to increase surfactant lipid synthesis in the fetal lung is presently unknown.

Although the ob/ob mouse has a point mutation in the ob gene that results in the total absence of circulating leptin, ob/ob mice do not have any respiratory problems at birth. If leptin plays a critical role in fetal lung development in the mouse, deficiency of the leptin would cause lung development disorder and would have problems in a transition to air breathing at birth. It has been explained that fetal plasma contains enough leptin from the maternal heterozygous ob/+ mouse side (42) to induce normal lung maturation in ob/ob mice fetus. On the contrary, according to other studies, synthesis of leptin in placenta was minimal in mice (16), and the level of leptin in the ob/ob mouse fetal plasma was low or not detectable (13). The leptin in fetal plasma was higher in humans and sheep than mice, suggesting that there might be species differences in influence of leptin to surfactant synthesis and lung maturation in the fetal lung. The adult ob/ob mice showed depressed pulmonary function, such as hypercapnia, altered pressure-volume curve, and decreased alveolar space (36). Interestingly, these pulmonary abnormalities in adult ob/ob mice appeared before pronounced obesity and were partially restored by exogenous leptin treatment (34, 42). It is presently unknown whether pulmonary abnormalities in adult ob/ob mice are, at least in part, associated with altered surfactant homeostasis.

In the present study, we will evaluate the influence of leptin on lung maturation and surfactant synthesis in sheep and mice by the following experiments: 1) study the efficacy of fetal leptin treatment to induce preterm lamb lung maturation, 2) study the influence of maternal leptin treatment in mice on fetal pulmonary surfactant lipid pool size and expression of genes critical for surfactant synthesis, and 3) study whether surfactant homeostasis is altered in ob/ob mice.

METHODS

Protocols were approved by the Animal Care and Use Committee of the Cincinnati Children’s Research Foundation. Fetal leptin treatment of sheep. The effects of fetal leptin treatment on lung maturation were studied using date-mated pregnant ewes. Ewes carrying singletons at 122–124 days gestational age (GA; term: 150 days GA) were gently restrained in the shearing position. Recom-
binant human leptin (BioVision, Mountain View, CA), 5 mg in 1 ml of 0.9% NaCl, suspended according to the manufacturer’s instructions or 1 ml of vehicle, was injected into the shoulder of the fetus using a 20-gauge spinal needle with ultrasound guidance (24). Endotoxin level in recombinant human leptin was low and <0.1 ng/µg protein. Three and a half days after injection, preterm lambs were delivered by cesarean section and tracheostomized (24, 38). To induce surfactant secretion at birth and evaluate lung function, preterm newborn lambs were ventilated for 30 min with 100% O2, 4-cmH2O positive end-expiratory pressure (PEEP), and 40/min respiratory rate, and tidal volume (VT) was regulated at 6 ml/kg by changing peak inspiratory pressure (PIP) with maximum PIP set at 38 cmH2O, using pressure-limited infant ventilator (Sechrist Industries, Anaheim, CA). VT was monitored (BiCore Monitoring Systems, Anaheim, CA) continuously. A modified ventilation index (MVI) was calculated as (PIP monitored (BiCore Monitoring Systems, Anaheim, CA) continuously.

**Table 1. Mice maternal leptin treatment studies**

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I.P., intraperitoneal injection; E17.5, embryonic day 17.5.

and 10% dextrose infusion. At 30 min of age, lambs were given 100 mg of pentobarbital intravascularly, after which the endotracheal tube was clamped to permit oxygen absorption atelectasis. After the thorax was opened, the deflation limb of pressure-volume curve was measured (24, 38). Bronchoalveolar lavage fluid (BALF) was recovered from the left lung (28), and lung tissue after BAL was homogenized for analysis of surfactant Sat PC, total phospholipid, and total protein. Sat PC was isolated from aliquot of BALF and lung homogenates using osmium tetroxide and neutral alumina (22, 29), followed by phosphorus measurement (17), and Sat PC in the total lung was calculated. Phosphorus in extracted lipid from BALF and lung homogenate was analyzed by Western blot analyses using rabbit anti-bovine SP-A, SP-B, and SP-C (Chemicon International, Temecula, CA) and normalized to β-actin in the sample (25). The right upper lobe was inflated-fixed at 30 cmH2O for morphology. Lung tissue of the right lower lobe was frozen in liquid nitrogen for RNA isolation to evaluate surfactant protein mRNAs by quantitative RT-PCR (22).

**Maternal leptin treatment of mice.** In the fetal mouse lung, leptin and leptin receptor mRNAs, as well as most genes known to regulate surfactant synthesis, were detectable from embryonic day (E) 14.5 (4, 18, 22). In A/J strain mice, surfactant Sat PC increase dramatically in the fetal lung at late gestation from E18.5, and their term is E21.5. As indicated in Table 1, the pregnant A/J mice were divided into 5 studies indicated in Table 1, the pregnant A/J mice were divided into 5 studies indicated in Table 1, the pregnant A/J mice were divided into 5 studies indicated in Table 1, the pregnant A/J mice were divided into 5 studies. Treatment of fetus with leptin did not influence surfactant lipid pool sizes. *P < 0.05, **P < 0.005. E: relative expression of surfactant proteins SP-A mRNA, SP-B mRNA, and SP-C mRNA for control and +Leptin groups analyzed by RT-PCR. No changes were demonstrated in surfactant protein mRNAs by fetal treatment with leptin. F: likewise, SP-A and SP-B proteins in BALF analyzed by Western blot were similarly low for both control and +Leptin groups. Treatment of fetus with leptin did not influence surfactant lipid pool sizes. *P < 0.05, **P < 0.005. E: relative expression of surfactant proteins SP-A mRNA, SP-B mRNA, and SP-C mRNA for control and +Leptin groups analyzed by RT-PCR. No changes were demonstrated in surfactant protein mRNAs by fetal treatment with leptin. F: likewise, SP-A and SP-B proteins in BALF analyzed by Western blot were similarly low for both control and +Leptin groups. Treatment of fetus with leptin did not influence surfactant lipid pool sizes. *P < 0.05, **P < 0.005. E: relative expression of surfactant proteins SP-A mRNA, SP-B mRNA, and SP-C mRNA for control and +Leptin groups analyzed by RT-PCR. No changes were demonstrated in surfactant protein mRNAs by fetal treatment with leptin. F: likewise, SP-A and SP-B proteins in BALF analyzed by Western blot were similarly low for both control and +Leptin groups. Treatment of fetus with leptin did not influence surfactant lipid pool sizes. *P < 0.05, **P < 0.005. E: relative expression of surfactant proteins SP-A mRNA, SP-B mRNA, and SP-C mRNA for control and +Leptin groups analyzed by RT-PCR. No changes were demonstrated in surfactant protein mRNAs by fetal treatment with leptin. F: likewise, SP-A and SP-B proteins in BALF analyzed by Western blot were similarly low for both control and +Leptin groups. Treatment of fetus with leptin did not influence surfactant lipid pool sizes. *P < 0.05, **P < 0.005. E: relative expression of surfactant proteins SP-A mRNA, SP-B mRNA, and SP-C mRNA for control and +Leptin groups analyzed by RT-PCR. No changes were demonstrated in surfactant protein mRNAs by fetal treatment with leptin. F: likewise, SP-A and SP-B proteins in BALF analyzed by Western blot were similarly low for both control and +Leptin groups. Treatment of fetus with leptin did not influence surfactant lipid pool sizes.
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Bilateral mouse leptin (Sigma, St. Louis, MO) was suspended in 0.9% NaCl as suggested by the manufacturer, and 30 µg of leptin in 200 µl or same volume of vehicle was injected intraperitoneally to ~30-g body wt pregnant mouse at the preset gestational age. For high-dose study (study 2), 300 µg of leptin in 240 µl was used. The fetal mouse was delivered by cesarean section under anesthesia with isoflurane inhalation followed by exsanguination by cutting the abdominal aorta of the fetus, and lungs were harvested at the preset gestational ages. Body weight, lung weight, and sex were recorded. The fetal mouse lung was too small for BAL. The lungs were frozen in liquid nitrogen for surfactant Sat PC, total phospholipid analyses, and validation of mRNAs. Quantitative RT-PCR was used to validate surfactant protein mRNAs and several mRNAs related to pulmonary surfactant phospholipid synthesis using TaqMan gene expression assays (Applied Biosystems, Foster City, CA) (22).

Surfactant lipid in ob/ob mice. To clarify whether absence of circulating leptin in ob/ob mice influenced surfactant homeostasis, surfactant Sat PC and total phospholipid were analyzed as described above in the lung from fetal (E18.5), postnatal dev 2 newborn (PN2), and 7-wk-old (PN50) ob/ob and littermate control mice. BALF were recovered from PN50 mice for surfactant lipid analyses. Ten to twenty-one from four litters were studied for each study group.

Data analysis. Results were given as means ± SE. Comparisons between control and leptin-treated (+Leptin) groups were made with two-tailed unpaired t-tests. Significance was accepted at P < 0.05.

RESULTS

Fetal leptin treatment of sheep. Seven control (4 male, 3 female) and 5 fetal +Leptin lambs (3 male, 2 female) were studied. Cord blood pH of the preterm newborn lambs was normal (control 7.36 ± 0.04, +Leptin 7.35 ± 0.02), and the body weight and lung weight were not different between control and +Leptin groups with some variations among the lambs (body weight: control 3.1 ± 0.3 kg, +Leptin 3.5 ± 0.1 kg, lung weight: control 123 ± 12 g, +Leptin 120 ± 7 g). Total protein in the lung tissue homogenates was similar between groups (control 493 ± 41 mg, +Leptin 471 ± 20 mg). Nevertheless, fetal leptin treatment increased body weight-to-lung weight ratio compared with control (P < 0.03; Fig. 1A).

Although total protein (milligrams) in lung homogenate relative to lung weight (grams) was not influenced by leptin treatment (control 4.0 ± 0.1, +Leptin 4.0 ± 0.2), ratio of body weight to protein in lung homogenate was significantly increased by leptin treatment (P < 0.05; Fig. 1B), suggesting increase in body weight by fetal leptin treatment given 3.5 days before delivery. Surfactant Sat PC in BALF and lung homogenate after BALF from control and +Leptin lambs were similarly low (Fig. 1, C and D) and were 1/10 of that of the term fetus. The Sat PC pool size was typical of extremely immature 125–128 days GA preterm lamb lung with surfactant deficiency. The expression of surfactant protein mRNAs was similar for control and +Leptin mice (Fig. 1E). There were no significant differences in SP-A and mature SP-B proteins in BALF between control and +Leptin groups by Western blot (Fig. 1F). Because of the lung immaturity, SP-C was not detectable in BALF from both control and +Leptin groups. Thirty-minute ventilation was regulated according to the study protocol. To maintain target V T of 6 ml/kg (Fig. 2A), high PIP was required for these preterm newborn lambs. PIP was 37 ± 0.5 cmH2O (control) and 37 ± 0.9 cmH2O (+Leptin) at 10 min and 36 ± 0.7 cmH2O (control) and 36 ± 1.1 cmH2O (+Leptin) at 30 min of age, at which time lambs were killed. The lung compliance was extremely low for both control and +Leptin groups (Fig. 2B). The PO2 was high for both groups throughout 30 min of ventilation, and the final PO2 was 81 ± 9 mmHg (control) and 83 ± 15 mmHg (+Leptin) and resulted in very high MVI for both groups (Fig. 2C). The PO2 at 30 min were 74 ± 26 mmHg (control) and 64 ± 26 mmHg (+Leptin) with fraction of inspired oxygen (FiO2) = 1 and were not different between groups. Lung morphology was typical of preterm newborn lambs for both groups with patchy atelectasis and did not show any changes by leptin treatment (data not shown). Deflation limb of pressure-volume curve was similarly low for control and +Leptin groups, and volume at 40 cmH2O was only 15 ml/kg (Fig. 2D). Fetal body weight was increased by

Fig. 2. Three and a half days after leptin or vehicle injection to the fetus, preterm lambs at 125–128 days were delivered by cesarean section and ventilated for 30 min to evaluate lung function. A: tidal volume (VT) was well-regulated at target of 6 ml/kg by changing peak inspiratory pressure (PIP) and was similar between +Leptin and control groups. B: lung compliance during ventilation was low for both groups. Fetal leptin treatment did not improve lung function of the preterm newborn lamb. C: modified ventilation index (MVI) was calculated as ([VT × PIP × respiratory rate (fixed at 40/min)] ÷ 1000). MVI were similarly high for both control and +Leptin groups, demonstrating the lung immaturity at 125–128 days gestational age. D: the deflation limbs of pressure-volume curves were similarly low for both groups.
leptin treatment, although there was no influence of leptin treatment on pulmonary surfactant synthesis and lung function.

Maternal leptin treatment of mice. The pregnant mice were given leptin by intraperitoneal injection and studied according to the 5 protocols (Table 1). In our preliminary study of A/J strain mice, Sat PC pool size increased >2-fold from E18.5 to E19.5 and were born around E21.5 without any further changes in Sat PC. To detect the increase in surfactant pool size after leptin treatment, mice were studied on E18.5 or younger before surfactant pool size reached the maximum in the developing mouse lung. The number of preterm mice studied for each group, body weight, and lung weight are shown in Table 2. The body weight and lung weight were slightly increased for Leptin group mice in study 4 that were given maternal leptin treatment twice on E15.5 and E16.5 and studied on E17.5 (P < 0.05 vs. control). Leptin treatment did not influence surfactant Sat PC synthesis in all 5 studies regardless of gestational age and dose of leptin for treatment (Fig. 3A). Likewise, total phospholipids were similar in control and +Leptin groups for all 5 studies (Fig. 3B). In addition to the surfactant protein mRNAs (Fig. 4, A–D), the selected 5 genes, which were highly expressed in alveolar type II cells and are critically involved in surfactant synthesis, were analyzed by quantitative RT-PCR. Expression of mRNAs in +Leptin mice lungs relative to control mice were presented for each study. Slc34a2 (Fig. 4E) influenced phosphate transport associated with surfactant phospholipid synthesis (17, 22), LPCAT1 (Fig. 4F) had high affinity for palmitoyl-CoA and preferentially acylated lysoPC (5), Fasn (Fig. 4G) and Scd-1 (Fig. 4H) were fatty acid synthase (3), and C/EBPα (Fig. 6I) regulated many genes involved in surfactant biosynthesis. Leptin treatment did not show any influences on SP-A, SP-B, and SP-D mRNAs as well as the expression of mRNAs related to surfactant synthesis. For reasons presently unknown, SP-C mRNA was lower in +Leptin group of study 4, which was analyzed on E17.5.

Surfactant lipid in ob/ob mice. Sat PC was analyzed on ob/ob and control mice at the ages of E18.5, PN2, and PN50. The number of mice, body weight, and lung weight are shown in Table 3. The ob/ob mice at E18.5, PN2, and PN50 did not show any difference in body weight and lung weight compared with control mice. The increase in body weight of PN50 ob/ob mice was 35% over that of control, whereas the lung weight was only 10% heavier for ob/ob mice than control. Sat PC and phospholipid in the lung (all age groups) and BALF (PN50 group only) were similar between control and ob/ob mice (Fig. 5, A and B). Deficiency of leptin did not influence surfactant Sat PC pool size. Altered lung functions of ob/ob mice were unrelated to surfactant.

**DISCUSSION**

Pulmonary surfactant is synthesized in alveolar epithelial type II cells and increases as fetal lung matures to prepare for transition to air breathing at birth. Leptin, an adipose tissue-derived peptide hormone (50), regulates multiple physiological functions such as food intake, lipid metabolism, and energy metabolism (6, 15, 35, 47). Leptin was also thought to be one of the promoters of lung development and surfactant production because leptin in fetal plasma (human and sheep) and expression of leptin mRNA in placenta (human and rat) were increased with advancing gestation (7, 16, 18, 40, 49). Leptin increases surfactant proteins (SP-A, -B, and -C mRNAs and SP-A and -B proteins) in E17 fetal rat lung explants in vitro (45). Leptin was secreted from fibroblasts bound to its receptor on the alveolar type II cells and stimulates surfactant phospholipid de novo synthesis in fetal rat lung explants and type II cells in culture (44). The effects of exogenous leptin treatment to increase surfactant lipid synthesis in fetal lung have been
proposed but are as yet unknown. We intended to demonstrate beneficial effects of leptin treatment on fetal lung maturation, however, the results of all the in vivo studies were negative. The responses of fetal lung to the leptin treatment might vary by dose, duration, dosing interval, route, gestation age, and species. The doses of leptin used for the present studies were its physiological concentration (1 mg/kg) (20, 27), and these dose ranges of exogenous leptin restored phenotypes of ob/ob mice (8, 11). We carefully designed five studies of maternal leptin treatment to pregnant mice as well as direct fetal leptin injection to sheep. Furthermore, surfactant pool sizes were analyzed on fetus, newborn, and adult ob/ob mice. Neither exogenously given leptin under six treatment protocols for sheep and mice nor deficiency of leptin in ob/ob mice influenced fetal lung maturation or surfactant pool sizes. The key genes highly expressed in alveolar type II cells and critically required for surfactant synthesis were not affected by leptin treatment. This is the first study to demonstrate manifestly that there is no influence of leptin on surfactant synthesis in vivo, at least under our treatment protocols.

The maternal leptin treatment did not show any effects on surfactant pool size in preterm newborn mice, regardless of the 5 conditions under which we studied, including 10-fold higher dose, multiple treatments, and different durations of treatments. There are possible explanations why leptin does not influence surfactant synthesis in vivo. The following mechanisms did not occur in vitro, and surfactant synthesis in rat lung explants and type II cells in culture were increased by leptin (44, 45). One mechanism is the leptin level in fetal serum is controlled by placenta, and increase in maternal plasma leptin by maternal leptin treatment may not increase leptin in fetal plasma and may not induce fetal lung maturation. For example, well-feeding increased maternal plasma leptin in pregnant sheep, whereas fetal leptin concentration was unchanged and not influenced by high level of maternal plasma leptin (31). Another possible factor is that exogenously given leptin to the pregnant mice may be inhibited by soluble leptin-binding proteins (19). Soluble leptin receptor was considered to be one of the key leptin-binding proteins in humans and rodents. Circulating leptin-binding proteins were known to increase in

Fig. 4. Expression of surfactant protein genes SP-A (A), SP-B (B), SP-C (C), and SP-D (D) and genes associated with synthesis of surfactant phospholipids in lung from control and +Leptin mice were analyzed by RT-PCR: Slc34a2 (E), LPCAT1 (F), Fasn (G), Scd-1 (H), and C/EBPα (I). No differences were detected between control and +Leptin groups in any of the genes studied except SP-C, which was decreased by leptin treatment in study 4 (*P < 0.05).
maternal plasma after second trimester and bind to high-level leptin, which altered bioactivity and transport of leptin. Leptin-binding proteins also inhibited clearance of leptin from plasma by filtration in the kidney that resulted in 20-fold increase in both free and bound leptin in plasma (13, 32). High leptin inhibition at near-term increases maternal metabolic efficacy and food intake that was important for delivery and nursing. The majority of exogenously given leptin to pregnant mice may bind with leptin-binding proteins and lose its biological function in vivo. To avoid the above factors that might occur for maternal administration of leptin, we injected leptin directly to the fetus in sheep. Fetal leptin treatment also resulted in no changes in lung maturation or surfactant pool sizes.

Kirwin et al. (27) demonstrated increase in SP-A, -B, and -C mRNA as well as SP-A and -B protein by physiological concentration of leptin (1 and 10 ng/ml) in vitro using E17 fetal rat lung explant. In the same paper, they described additional in vivo study of maternal leptin treatment to pregnant rat with the major finding of a 14% increase in fetal lung weight relative to body weight. In our present study, the lung weight was increased only for E17.5 study (study 4) mice. Fetal lung weight is considerably influenced by fetal lung fluid volume remaining in the airways, and conclusions cannot be drawn on lung maturation from increased lung weight alone. Although increased immunohistochemistry of fetal rat lung for SP-B was included in the rat study, morphological lung maturation, especially larger alveolar space, was not seen in +Leptin group. In addition, lung weight, body weight, lung weight-to-body weight ratio, total protein in the lung tissue, surfactant phospholipids, and surfactant proteins were not presented or analyzed in their in vivo study.

Maternal glucocorticoid treatment has been routinely used for pregnant women with high-risk of preterm delivery. Maternal glucocorticoid treatment slightly increased surfactant phospholipid in rabbit and sheep fetuses, induced lung structural maturation, decreased protein leak, and improved preterm newborn lung function (26). Glucocorticoid response element was identified in the promoter region of the human leptin gene (14), and glucocorticoid was likely to target leptin gene transcription directly in utero. Increased leptin synthesis and secretion by glucocorticoid was demonstrated in fetal adipose tissue in vivo and in vitro. Antenatal glucocorticoid treatment of 130 days GA sheep fetus increased leptin >1.5-fold in fetal circulation, whereas exposure to triiodothyronine did not influence fetal plasma leptin (33). The present study suggested that increased leptin by glucocorticoid treatment may not be the mechanism of induction of lung maturation. On the other hand, increased leptin by glucocorticoid treatment played a role in regulation of body temperature of the preterm newborn (10). In contrast to the single treatment, prolonged multiple maternal glucocorticoid treatment had the opposite effect on fetal plasma leptin level, and whereas maternal plasma leptin was increased, leptin was decreased in fetal plasma (39, 41). Since leptin influenced fetal body weight, decreased leptin in fetuses after multiple doses of maternal glucocorticoid treatment caused retarded fetal growth (9, 41). Likewise, in the present study, body weight of preterm newborn lamb was significantly increased by antenatal fetal leptin treatment.

Newborn ob/ob mice do not have any respiratory problems in the transition to air breathing at birth. The explanation was that although ob/ob mice fetuses were unable to produce leptin, these fetuses were exposed to leptin by placental blood flow and lactational sources from the heterozygous ob/+ dams (2, 18, 37, 44). This explanation may be inappropriate because, unlike in humans, the source of the hyperleptinemia of late-gestation in the mouse fetus is mainly from adipose tissue, and the leptin produced by placenta is minimal in mice (16). Leptin levels in ob/ob mice fetuses were very low and not detectable in fetal serum and lung homogenate (13). We also could not detect any leptin by ELISA (Millipore, Billerica, MA) in E18.5

![Table 3. Body weight and lung weight of control and ob/ob mice](http://ajplung.physiology.org/)
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ob/ob fetal mice plasma (data not shown). Nevertheless, surfactant homeostasis was not influenced by deficiency of leptin in fetal and adult ob/ob mice lungs. Homozygous ob/ob mice exhibited subfertility. To make the complete absence of leptin in both the mother and fetus, homozygous ob/ob male and female mice were treated with exogenous leptin to mate in the previous study. The fetus in homozygous ob/ob dams developed normally and delivered with no signs of respiratory problems (30), suggesting that leptin deficiency does not influence fetal lung development. These homozygous ob/ob newborn mice did not survive over a day after delivery due to failure of the dam’s lactation. The adult ob/ob mice have depressed respiratory function before pronounced obesity, which was thought to be caused by decreased central respiratory control mechanisms and obesity as well (21, 34, 36). These respiratory problems in ob/ob mice were partially restored by exogenous leptin treatment (34, 42). Whether surfactant synthesis was altered in adult ob/ob mice and, at least partially, contributed to their abnormal pulmonary function is not currently known. The present study demonstrated that Sat PC pool sizes were normal in ob/ob mice at all ages studied, suggesting that leptin was not required for surfactant lipid homeostasis in mice. We conclude from this study that leptin alone does not play a critical role in surfactant synthesis, and exogenously given leptin does not influence fetal lung surfactant synthesis or preterm newborn lung function in vivo.

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DISCLOSURES

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this study.

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