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11-β Hydroxysteroid Dehydrogenase Type 2 in Human Adult and Fetal Lung and Its Regulation by Sex Steroids

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ABSTRACT: 11-β hydroxysteroid dehydrogenase type 2 (HSD2) oxidizes the biologically active glucocorticoid (GC), cortisol, to inactive cortisone. We characterized HSD2 gene expression and activity in human adult and fetal lung tissues and in cultured fetal lung explants, and examined the potential regulation of HSD2 in the fetal lung by sex steroids. Human adult lung, fetal lung, and cultured fetal lung explant tissues contained similar amounts of HSD2 mRNA. However, higher levels of HSD2 protein were detected in human fetal lung tissue than in adult lung, with expression being restricted to a subset of epithelial cells in the fetal lung tissue. Differentiated fetal lung explants maintained in culture expressed higher levels of HSD2 protein and enzymatic activity than undifferentiated fetal lung tissues. Finally, HSD2 protein levels were decreased in male, but not female, fetal lung explants treated with 17-β estradiol. In contrast, 5-α dihydrotestosterone did not significantly affect HSD2 levels. These data indicate that HSD2 protein and activity levels increase in parallel with the differentiation of alveolar type II epithelial cells *in vitro*, and that HSD2 protein levels are regulated by 17-β estradiol in male fetal lung tissue. **(*Pediatr Res* 62: 26–31, 2007)**

In humans, the biologically active GC, cortisol, has a hydroxyl group at the 11-β position of the steroid ring, whereas the biologically inactive GC, cortisone, has a ketone group at this position (1). The interconversion between cortisol and cortisone is catalyzed by the HSD, HSD1 and HSD2 (1). HSD1 is an 11-β reductase that converts the 11-keto GC, cortisone, to the 11-hydroxy GC, cortisol (Fig. 1). In contrast, HSD2 is an 11-β dehydrogenase that converts cortisol to cortisone (Fig. 1). Thus, the HSD1 and HSD2 enzymes can increase or decrease the local concentrations of biologically active GC in peripheral tissues (1).

Glucocorticoids affect lung fluid transport, cell division, differentiation, pulmonary surfactant production, and inflammation (2,3). Cortisol levels increase in amniotic fluid during pregnancy and a surge in fetal GC levels near term is necessary for fetal lung maturation in preparation for extrauterine life (2,4). Synthetic GCs, such as dexamethasone and prednisone, are used to treat inflammatory lung diseases in children and adults, and to promote lung maturity in fetuses at risk for preterm delivery (2,3,5). However, prenatal exposure to GC has been associated with immune suppression, hypertension, altered nutrient metabolism, and decreased growth hormone levels (2,6). Additionally, antenatal administration of GC to prevent or treat neonatal lung disease has been linked to decreased somatic growth, altered immune system reactivity, and an increased risk for developing hypertension and insulin resistance later in life (7).

Sex-specific differences in fetal lung maturation have been observed in several species, including humans, with lung development delayed in males compared with females (8). Androgens, such as 5-α dihydrotestosterone, counteract the maturation-promoting effects of GC in human fetal lung explants, whereas estrogens, such as 17-β estradiol, accelerate fetal lung maturation (9,10).

The human fetal lung actively oxidizes cortisol to cortisone (indicative of HSD2 activity) and this activity decreases as the fetus approaches term, potentially allowing for an increase in the local concentration of biologically active cortisol in the maturing lung (11). HSD2 mRNA and protein have been detected in human adult and fetal lung tissues, as well as in a number of human lung epithelial cell lines (12,13). In contrast, the oxidoreduction of cortisone to cortisol (suggestive of HSD1 activity) is negligible in intact cultured lung explants and undetectable in human fetal lung *in vivo* (11,14,15).

Explants of undifferentiated, mid-trimester human fetal lung tissue maintained in culture for 5–7 d contain newly differentiated type II alveolar epithelial cells that produce pulmonary surfactant characteristic of the fetal lung near term (16). This model system provides a unique opportunity to study, *in vitro*, developmental events that normally take several months to occur *in vivo* (16,17). In the present study, we compared HSD2 expression in undifferentiated human fetal lung tissue and in cultured human fetal lung explants following type II alveolar epithelial cell differentiation. We also compared HSD2 expression in human adult lung *versus* human fetal lung tissues, and looked for sex-specific differences in HSD2 expression in human fetal lung. Finally, we evaluated the regulation of HSD2 protein in human fetal lung by the sex steroids, 17-β estradiol and 5-α dihydrotestosterone.

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Abbreviations: GC, glucocorticoid; **HSD,** 11-β hydroxysteroid dehydrogenases

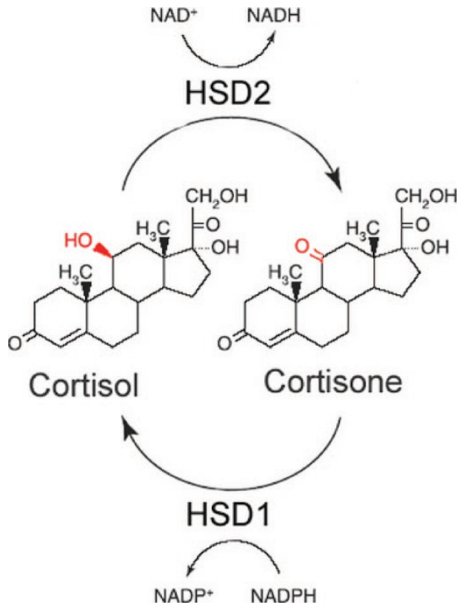

Figure 1. Cortisol metabolism by the 11-β hydroxysteroid dehydrogenase system. Local concentrations of biologically active GC are increased by HSD1-mediated oxidoreduction of cortisone to biologically active cortisol. HSD2 decreases the local concentration of biologically active GC *via* the oxidation of cortisol to inactive cortisone.

METHODS

Human lung tissues. Human adult lung tissues were obtained using a protocol approved by The University of Iowa Human Subjects Review Committee. Adult lung tissues taken from unused donor lungs were obtained from Dr. Michael Welsh, Department of Internal Medicine, University of Iowa. The lungs were maintained in cold isotonic Ringer's solution until processing, which occurred within 24 h of clamping of donor circulation. Lung tissues were dissected free of major blood vessels and airways, frozen in liquid N_2 and stored at −80°C.

Human fetal lung explants. Human fetal lung tissues were obtained from mid-trimester abortuses using a protocol approved by The University of Iowa Human Subjects Review Committee. Human fetal lung explants were cultured as described previously, with or without 5-α dihydrotestosterone (500 ng/mL = 1.7 μM) or 17-β estradiol (500 ng/mL = 1.8 μM) (16). The explants were incubated at 37°C and media changed daily as previously described (18). Starting tissue (fetal lung tissue before culture) and the harvested cultured explants were frozen in liquid N_2 and stored at −80°C.

HSD2 enzyme activity assays. Intact control and hormone-treated explants were washed with PBS and then incubated at 37°C in 1 mL of serum-free media that contained 1 nM 1,2,4-[^{3}H]-cortisol (41 Ci/mmol, American Radiolabeled Chemicals, St. Louis, MO) for 24 h on either d 1 (starting tissue) or d 6 (cultured explants). Labeled steroids were then isolated from the media and the production of tritiated cortisone was assessed *via* thin layer chromatography as previously described (12). HSD2 oxidase activity was determined by calculating the percent conversion of [^{3}H]-cortisol to [^{3}H]-cortisone. Parallel assays were performed with no lung tissue to control for spontaneous interconversion between 11-hydroxy and 11-dehydro GC. Upon completion of the assay, the explant tissues were homogenized in 100 μL of lysis buffer (1 nM phenylmethylsulfonyl fluoride in PBS) and the protein content in the supernatant was determined using Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). Protein levels were used to control for slight variations in tissue content between experiments. The HSD2 activity was expressed as picomoles of cortisol converted per microgram of protein.

Protein isolation and immunoblot analysis. Lung tissues were homogenized in lysis buffer, centrifuged at 600 × *g* to pellet debris, and the supernatant collected. Supernatant protein concentrations were quantified as described above. Immunoblotting was performed using a rabbit primary antibody specific for human HSD2 (2 μg/mL) as previously described (12,19). HSD2 protein levels were normalized to β-actin levels, which were determined on membranes that were stripped and reprobed with a β-actin MAb (1:7500, Chemicon International, Temecula, CA). Total homogenate proteins from human kidney (15 μg) (BioChain, Hayward, CA) were used as a positive control for immunoreactive HSD2 protein.

RNA isolation and semi-quantitative real-time reverse-transcriptase PCR. Lung tissue was homogenized in 1 mL of TRIzol reagent (Invitrogen, Carlsbad, CA) and total RNA was isolated and quantified as described previously (12). RNA integrity was monitored by examining the ethidium bromide stained 28S and 18S rRNA bands after electrophoresis on agarose gels. Real-time reverse-transcriptase (RT) PCR was performed in reaction tubes containing 10 ng of total RNA, the Brilliant 1-Step QRT-PCR master mix (Stratagene, La Jolla, CA) and Assays-On-Demand gene expression reagents for either human HSD2 or β-actin mRNA. Analysis was performed using an Mx3000P QPCR instrument (Stratagene). HSD2 mRNA levels were normalized to those of β-actin in the same sample.

Immunohistochemistry. Fetal lung tissues were fixed in formalin (10% formaldehyde in PBS) overnight and embedded in paraffin. Sections were prepared, mounted on glass slides, and immunostaining performed as previously described using a Vectastain Elite kit (Vector Laboratories, Burlingame, CA) and the HSD2 antibody (200 ng/mL) (18).

Sex determination of human fetal tissues. Total DNA was isolated from fetal lung tissues, resuspended in water, and quantified by determining the absorbance at 260 nm. One microgram of the DNA was used in PCR reactions with primers specific for a homologous region of the X and Y chromosomes, as previously described (20).

RESULTS

HSD2 protein localization in fetal lung tissue and in cultured fetal lung explants. HSD2 protein was present in epithelial cells at the distal tips of prealveolar lung ducts in uncultured human fetal lung tissue (Fig. 2*A*). HSD2 protein was cytoplasmic and localized to the apical region of the cells (Fig. 2 *B*). HSD2 staining was not observed in more proximal epithelial cells of the lung ducts or in connective tissue. Following 6 d in explant culture, HSD2 protein was detected in all of the differentiated type II alveolar epithelial cells lining the prealveolar lung ducts (Fig. 2*C*). Additionally, immunoreactive HSD2 protein was distributed throughout the cytoplasm and nuclei of these epithelial cells (Fig. 2*D*).

HSD2 gene expression in human adult and fetal lung. Semi-quantitative real-time RT-PCR using total RNA isolated

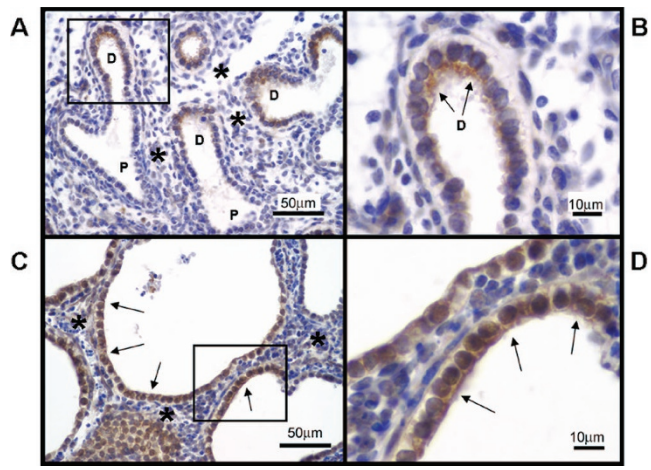

Figure 2. Tissue distribution and subcellular localization of HSD2 protein in human fetal lung tissue. (*A*) HSD2 protein in the uncultured human fetal lung tissue was localized in epithelial cells at the distal (*D*) tips of the prealveolar ducts. HSD2 was not present in more proximal (*P*) epithelial cells of the lung ducts or in the connective tissue (asterisk). (*B*) Enlargement of boxed area in *A* illustrating localization of HSD2 to the apical cytoplasm of the epithelial cells. (*C*) Immunoreactive HSD2 protein was detected in all of the differentiated type II epithelial cells (*arrows*) in the cultured human fetal lung explants. (*D*) Enlargement of boxed area in *C* illustrating the localization of HSD2 protein in the cultured fetal lung epithelial cells. Images in *A* and *C* were taken at 40× magnification.

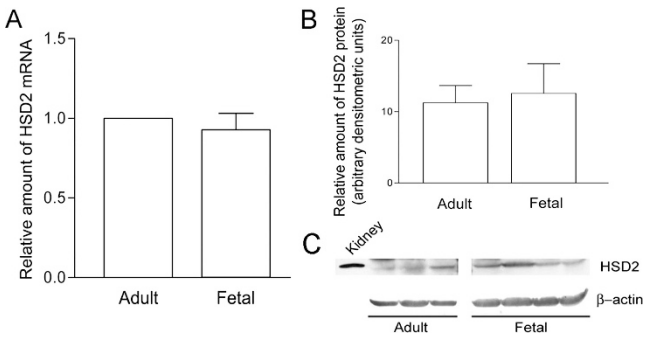

Figure 3. HSD2 mRNA and protein in human adult and fetal lung tissues. (*A*) Relative amounts of HSD2 mRNA present in fetal lung ($n = 4$) and in adult lung ($n = 4$), which were normalized to one. (*B*) The relative amount of HSD2 protein in fetal lung ($n = 4$) and in adult lung ($n = 3$). (*C*) Representative immunoblots for HSD2 and β-actin proteins.

from adult ($n = 4$) and fetal lung tissues ($n = 4$) revealed similar levels of HSD2 mRNA (Fig. 3*A*). Immunoblot analyses for HSD2 protein demonstrated that HSD2 protein levels in human adult ($n = 3$) and fetal lung tissues ($n = 4$) were not significantly different when normalized to those for β-actin (Fig. 3*B*).

HSD2 gene expression and activity in starting and cultured fetal lung explants. HSD2 mRNA levels in cultured fetal lung tissues were not significantly different from those in starting fetal lung tissues (Fig. 4*A*). However, immunoblot analyses revealed significantly higher amounts of HSD2 protein in cultured fetal lung explant tissue compared with levels in uncultured fetal lung tissue (Fig. 4*B*).

As shown in Figure 4 *C*(*a*), the cultured fetal lung explants had higher levels of HSD2 oxidase activity than the starting fetal lung tissues. The increase in HSD2 enzyme activity in the cultured fetal lung explants was similar in magnitude to the increase in HSD2 protein observed in the same tissues (Fig. 4, *B* and *C*), although the increase did not reach statistical significance ($n = 4$; $p = 0.14$, paired t test). A representative scan showing separation of labeled cortisol and cortisone *via* thin layer chromatography is shown in Figure 4 *C*(*b*). Labeled cortisol is the first major peak, while the more polar compound, cortisone, migrates further and is the second peak.

HSD2 protein levels in male versus female fetal lungs and regulation by sex steroids. We performed immunoblot analyses for HSD2 protein on total homogenate proteins from male and female fetal lung tissues. The gender of the fetal lung tissue was determined by PCR (Fig. 5*A*). The relative amounts of HSD2 protein in male ($n = 3$) and female ($n = 5$) uncultured fetal lung tissues were similar (Fig. 5*B*). Immunoblot analyses for HSD2 protein were also performed on fetal lung explants that had been cultured in the presence of either 5-α dihydrotestosterone (DHT) (500 ng/mL) or 17-β estradiol (500 ng/mL). There was a statistically significant down-regulation of HSD2 protein levels in fetal lung explants ($n = 11$) treated with 17-β estradiol, whereas DHT had no effect (Fig. 6*A*). When the data were analyzed on the basis of sex ($n = 5$, males and $n = 6$, females), there was a significant down-regulation of HSD2 protein levels in male, but not female, fetal lung explants treated with 17-β estradiol (Fig. 6*B*). DHT did not have a significant effect on

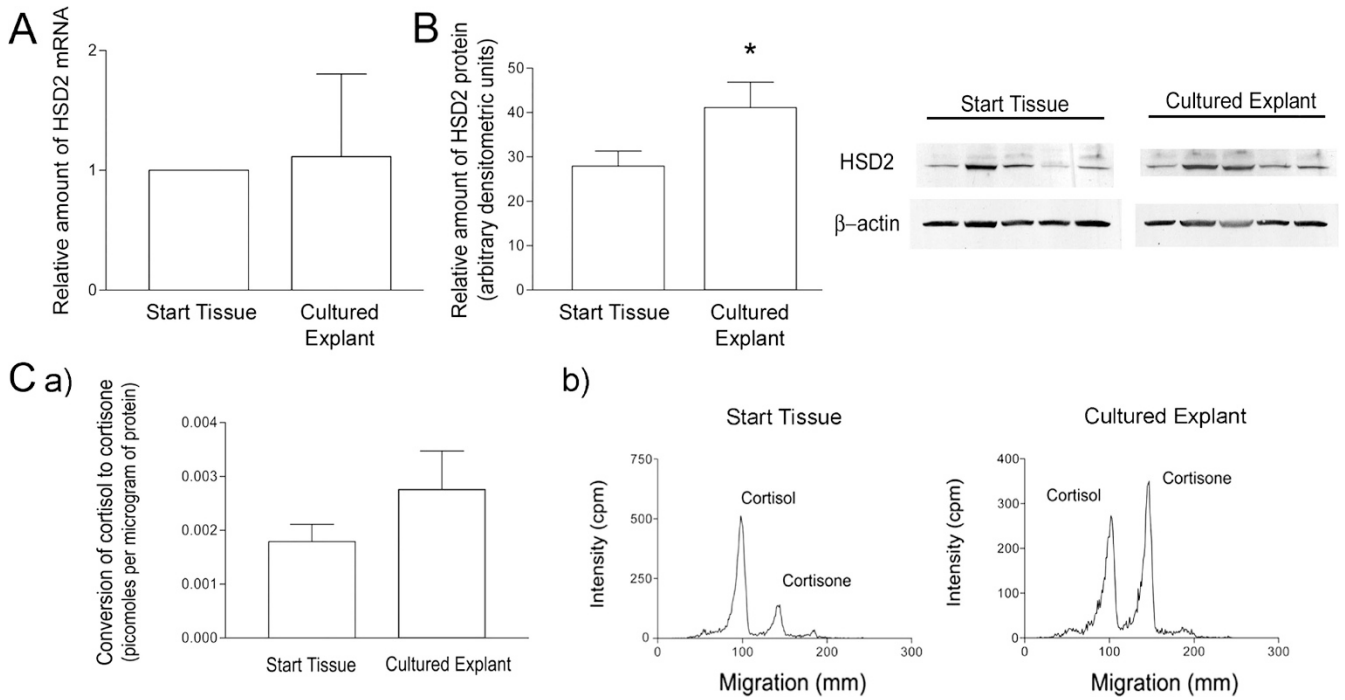

Figure 4. HSD2 mRNA, protein, and enzyme activity in starting fetal lung tissue *vs* cultured fetal lung explants. (*A*) Relative amounts of HSD2 mRNA in the starting fetal lung ($n = 4$) and in the cultured fetal lung explants ($n = 4$). (*B*) HSD2 protein levels in the fetal lung explants before and after 6 d in culture. Data are from 11 fetal lung samples (starting and cultured fetal lung explant tissues were from same lung). Representative immunoblots for HSD2 and β-actin are shown on the right. $^*p < 0.05$ using a paired t test. (*C*) Summary of HSD2 activity assays in starting human fetal lung tissues and in corresponding cultured explants ($n = 5$, $p = 0.14$) (*a*); scan of representative plate illustrating the separation of labeled steroid metabolites *via* thin layer chromatography (*b*).

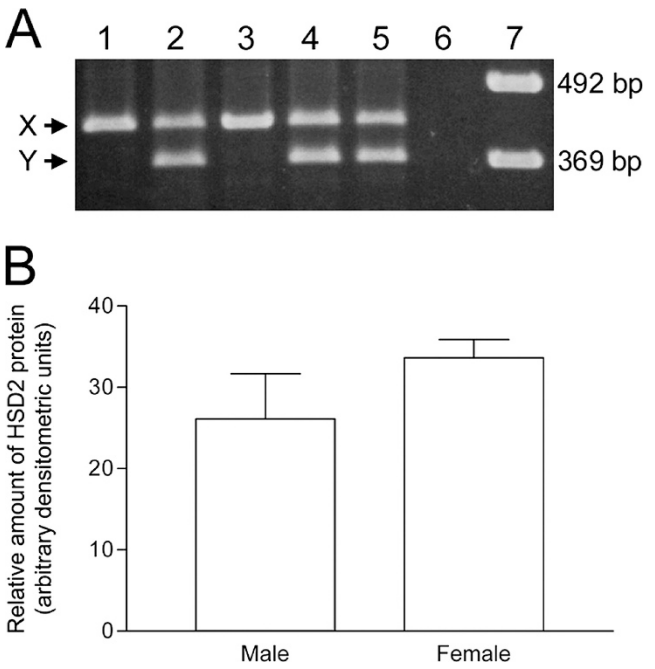

Figure 5. Comparison of the relative amounts of HSD2 protein in male *vs* female human fetal lung explants. (*A*) Representative PCR for X and Y chromosome sequences for sex determination of human fetal lung. Female tissues (*lanes 1,3*) show amplification of only X chromosome sequences, whereas male tissues show amplification of both X and Y chromosome sequences (*lanes 2,4,5*). (*B*) Immunoblot analysis for HSD2 protein in male ($n = 5$) and female ($n = 3$) fetal lung tissues.

HSD2 protein levels in either male or female fetal lung explants (Fig. 6*B*).

DISCUSSION

The final maturation of the human fetal lung is mediated by a surge in endogenous fetal cortisol levels near term (2). Synthetic GC, *e.g.* betamethasone and dexamethasone, are routinely administered to pregnant women at risk for preterm delivery to accelerate fetal lung maturation (2). These treatments are effective in reducing the incidence of neonatal lung disease and neonatal mortality (2). GC are also often used in the treatment of asthma and other inflammatory lung diseases (3).

The human fetal lung actively oxidizes the biologically active GC, cortisol, to inactive cortisone, which is indicative of HSD2 activity (14,15). HSD2 gene expression has been detected in human fetal lung *via* immunohistochemistry, RT-PCR, and immunoblot analyses (12,13). In contrast, HSD1 protein is undetectable in human adult and fetal lung tissues (12,21,22). Additionally, no conversion of cortisone to cortisol (indicative of HSD1 activity) has been observed in human fetal lung *in vivo* (15).

The human fetal lung explant system has been used to study type II alveolar cell differentiation (16), the hormonal regulation of gene expression (17,23), the effects of GC on lung structure (17), as well as the metabolism of GC (24). We observed that HSD2 is expressed at discrete sites in the undifferentiated human fetal lung tissue. Specifically, HSD2 protein was restricted to epithelial cells in the distal regions of the prealveolar ducts where epithelial cell proliferation facilitates the branching morphogenesis required to achieve normal lung structure. The presence of HSD2 in these areas may serve to limit cortisol exposure, which could inhibit proliferation and promote the premature differentiation of these epithelial cells (2).

In contrast, in cultured lung explants containing differentiated alveolar type II cells, HSD2 protein levels appeared to be elevated when compared with levels in the starting fetal lung tissue and the HSD2 protein appeared to be distributed throughout the cytoplasm and in the nucleus. These findings may reflect a nuclear function for HSD2 within differentiated alveolar epithelial cells. We observed an increase in HSD2 protein levels in cultured fetal lung explants. This increase paralleled the differentiation of type II alveolar epithelial cells in the explants (16). HSD2 protein has previously been detected in alveolar type II cells of the human adult lung (25). Similar increases in HSD2 expression have been observed concomitant with the differentiation of human colonic epithelial cells and placental trophoblast cells, suggesting that the expression of this enzyme is influenced by cellular differentiation (26,27).

HSD2 protein in mid-trimester human fetal lung tissue was localized to the apical region of the undifferentiated alveolar epithelial cells. The subcellular localization of this microso-

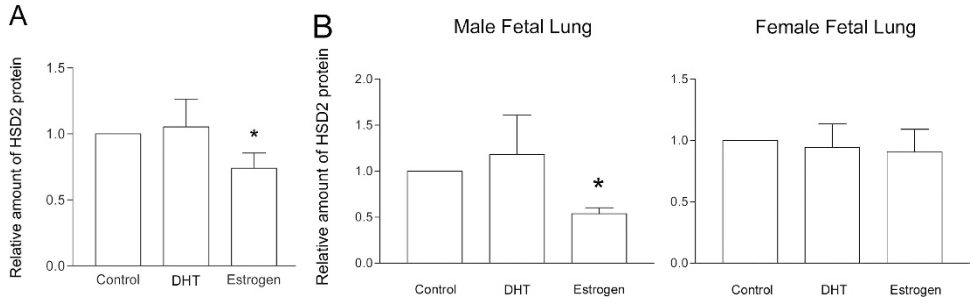

Figure 6. Effects of sex steroids on HSD2 protein levels in cultured fetal lung tissue. Explants were cultured for 6 d in the presence of either no steroids (control), dihydrotestosterone (DHT), or 17-β estradiol (estradiol). (*A*) Effects of DHT and estradiol on HSD2 protein levels in human fetal lung tissues. 17-β estradiol treatment decreased HSD2 protein levels by 26.1 ± 11% ($n = 11$, $^*p < 0.05$ using a paired *t* test *vs* untreated controls, which were normalized to one). (*B*) 17-β estradiol treatment decreased HSD2 protein levels in male fetal lung explants by 47 ± 6% ($n = 5$, $^*p < 0.05$ using a paired *t* test *vs* untreated control tissues, which were made equal to one), but did not affect HSD2 in female fetal lung explants ($n = 6$). DHT exposure did not significantly affect HSD2 protein levels in either male or female fetal lung explants.

mal enzyme may be related to the location of endoplasmic reticulum in the undifferentiated human lung epithelial cells (28). In contrast, the cultured fetal lung explant tissues expressed HSD2 protein throughout the cytoplasm of the epithelial cells lining the prealveolar ducts, with many cells also exhibiting nuclear localization of HSD2. Nuclear localization of HSD2 protein has been observed in the epithelial cells of other human tissues including the kidney, colon, salivary gland, and endometrium, data suggestive that the metabolism of GC by HSD2 may also be a nuclear event in these cells (29–31).

We did not observe a statistically significant difference in the levels of HSD2 mRNA and protein in adult *versus* fetal lung tissues. In addition, undifferentiated, mid-trimester human fetal lung tissues and cultured, differentiated human lung explant tissues expressed similar amounts of HSD2 mRNA. However, the cultured fetal lung explants contained higher levels of HSD2 protein and HSD2 enzymatic activity than the starting fetal lung tissues. Thus, HSD2 protein turnover may be decreased in differentiated type II epithelial cells or the HSD2 gene may be subject to posttranscriptional regulation, as has been reported in other cell types (32). Interestingly, HSD2 activity decreases in the human fetal lung *in vivo* late in gestation, presumably to allow maximal amounts of biologically active cortisol to promote lung differentiation (11).

There are sex-specific differences in human fetal lung maturation (8). Males have a higher incidence and severity of neonatal lung disease (33). Furthermore, prenatal GC therapy is more effective in preventing respiratory distress syndrome (RDS) in female infants than male infants (34). Androgen exposure has been linked to delayed fetal lung maturation in humans (9). Conversely, it has been reported that 17-β estradiol treatment accelerates fetal lung maturation, and that estrogen levels are low in human infants with RDS (10,35). Immunoblot analyses of HSD2 protein in mid-gestation male and female fetal lung tissues revealed no significant differences in the baseline levels of HSD2 protein. However, HSD2 levels were significantly decreased by 17-β estradiol (50% decrease) in male fetal lung explants, whereas HSD2 levels in female fetal lung explants were not affected by either 17-β estradiol or DHT treatment. The concentration of 17-β estradiol used in this study (500 ng/mL, 1.8 μM) is similar to the levels of estradiol in fetal plasma (36). The lack of an effect of sex steroids in the female fetal lung explants may be related to the concentration of androgen or estrogen receptors in the fetal lung tissue. The androgen receptor (AR) has been detected in human fetal lung epithelial cells by immunohistochemistry, with lower levels of AR in female than in male fetal lung tissues (37). Human fetal lung tissue specifically binds estrogen, and mRNA transcripts for the β- isoform of the estrogen receptor (ER) have been detected in human fetal lung tissues (38,39). ER expression has been reported to be similar in male and female human adult lung (40); however, the relative amount of ER in male *versus* female human fetal lung has not been established.

As pregnancy progresses, the levels of estrogens in fetal plasma and amniotic fluid rise, with amniotic fluid levels of 17-β estradiol levels higher in mid-gestation pregnancies with female than with male fetuses (36,41). As a result, female fetuses are exposed to higher levels of estrogen at earlier gestational ages than males. Thus, fetal lung tissues obtained from female fetuses may be less sensitive to exogenous estrogen because there may be fewer unliganded ER that can bind additional estrogen and/or because ER levels can be down-regulated by estradiol in human cells (42). Indeed, late gestation male fetal rat lung tissues have been shown to bind significantly more (50%) 17-β estradiol than age-matched female fetal rat lung tissues (10).

In summary, we characterized the relative levels of HSD2 mRNA, protein, and enzyme activity in human adult and fetal lung tissues, and in cultured human fetal lung explant tissues undergoing differentiation *in vitro*. We have examined the relative levels of HSD2 protein in male and female human fetal lung tissues, as well as the regulation of HSD2 by sex steroids. Our data demonstrate that the expression of HSD2 protein in human fetal lung is dynamic and affected by the differentiation state of alveolar epithelial cells. Additionally, we have shown that the expression of HSD2 protein is regulated by 17β-estradiol in male, but not female, human fetal lung tissues.

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