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► To cite this version:

Despina D Briana, Dimitrios Gourgiotis, Maria Boutsikou, Stavroula Baka, Antonios Marmarinos, et al.. Clara Cell Protein in Full-term Pregnancies: The Influence of Intrauterine Growth Restriction. *Pediatric Pulmonology*, 2010, 45 (12), pp.1186. 10.1002/ppul.21305 . hal-00566723

HAL Id: hal-00566723

<https://hal.science/hal-00566723>

Submitted on 17 Feb 2011

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Journal:	<i>Pediatric Pulmonology</i>
Manuscript ID:	PPUL-09-0400.R2
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	13-Apr-2010
Complete List of Authors:	Briana, Despina; Athens University Medical School Gourgiotis, Dimitrios; Athens University Medical School Boutsikou, Maria; Athens University Medical School Baka, Stavroula; Athens University Medical School Marmarinos, Antonios; Athens University Medical School Liosi, Sofia; Athens University Medical School Hassiakos, Dimitrios; Athens University Medical School Malamitsi-Puchner, Ariadne; Athens University Medical School
Keywords:	Clara cell protein, lung, intrauterine growth restriction, fetus, neonate

**Clara Cell Protein in Full-term Pregnancies: The Influence
of Intrauterine Growth Restriction**

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Short title: Clara cell protein in IUGR

Summary

Background: Clara cell protein (CC16) is an immunomodulatory/anti-inflammatory broncho-alveolar-derived molecule and a biomarker of pulmonary epithelial cells maturity and alveolo-capillary membrane injury. Intrauterine-growth-restricted- (IUGR) neonates may present with structural lung immaturity, impaired immunocompetence and increased risk for respiratory infections and chronic obstructive lung disease in later life. **Objectives:** To investigate circulating CC16 concentrations in maternal, fetal and neonatal samples from IUGR and appropriate-for-gestational-age-(AGA) pregnancies. **Methods:** Serum CC16 concentrations were determined by EIA in 40 mothers and their 20 IUGR and 20 AGA singleton full-term fetuses-neonates on postnatal day 1-(N1) and 4-(N4). **Results:** No significant differences in CC16 concentrations were observed between IUGR and AGA groups. In both groups, maternal CC16 concentrations were lower compared to N1 and N4 ones ($P<0.001$ in each case). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ in each case). In the AGA group, N1 CC16 concentrations were significantly higher than N4 ones ($P<0.001$). Combining groups, N1 CC16 concentrations positively correlated with gestational age ($r=0.364$, $P=0.021$). Finally, the effect of gender, parity and maternal age on CC16 concentrations was not significant. **Conclusions:** The lack of differences in CC16 concentrations between IUGR and AGA groups possibly suggests that the lung immaturity and later respiratory diseases, associated with the former, may not be related to early CC16 deficiency. CC16 concentrations increase with advancing gestational age and peak on the first day of life, possibly indicating a vital role of the protein in fetal lung maturation and extrauterine pulmonary adaptation.

Key words: Clara cell protein, lung, intrauterine growth restriction, fetus, neonate

Introduction

Clara cell protein (known as CC16, CCSP, CC10 or uteroglobin), one of the major secretory proteins of the lungs, is produced by the nonciliated Clara cells, lining the bronchial and bronchiolar epithelium.¹ CC16 has been implicated in fetal lung growth² and has been ascribed many anti-inflammatory/immunomodulatory properties, including inhibition of pro-inflammatory cytokine production and neutrophil infiltration.^{1,3,4} Thus, CC16 prevents and ameliorates lung injury in adult mice and humans^{5,6} and protects the respiratory tract from life-threatening immunological reactions elicited by infectious agents.^{3,6}

CC16 concentrations in bronchoalveolar lavage fluid (BALF) depend on the number and integrity of Clara cells.¹ BALF CC16 concentrations are increased in patients with respiratory distress syndrome and decreased in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis.^{4,7,8} On the other hand, serum CC16 concentrations reflect alveolo-capillary membrane permeability.⁴ Lower serum CC16 concentrations have been detected in adults with chronic obstructive pulmonary disease,⁹ in children with asthma¹⁰ and preterm neonates with respiratory distress and bronchopulmonary dysplasia.^{11,12} Furthermore, serum CC16 is considered a sensitive and specific marker of lung injury in adults.^{1,4,9}

Recent epidemiological and experimental evidence in humans and animals indicates that the lung can be detrimentally affected by an adverse intrauterine environment, resulting in persistent alterations in lung structure and impaired respiratory function during postnatal life.¹³ In this respect, intrauterine growth restriction (IUGR) has been associated with respiratory compromise and higher incidence of lower respiratory tract infections and chronic obstructive airway disease in later life.¹⁴⁻¹⁶ However, data

assessing the impact of IUGR on fetal lung development are limited and predominantly derive from animal experimentation.¹⁷⁻¹⁹

This study was based on the hypothesis that circulating CC16 concentrations may differ between IUGR and appropriate for gestational age (AGA) fetuses and neonates, since the former may present with structural lung immaturity, impaired immunocompetence and increased risk for developing respiratory infections and chronic obstructive lung disease in later life.¹⁴⁻²⁰ Therefore, we aimed to investigate, for the first time to our knowledge, serum CC16 concentrations in IUGR and AGA pregnancies at crucial perinatal time-points and correlate them with several maternal and fetal anthropometric/clinical variables.

Materials and Methods

The Ethics Committee of our teaching hospital approved the study protocol. All included mothers provided signed informed consent before recruitment. Forty parturients giving consecutively birth, either to 20 AGA or 20 asymmetric IUGR full-term singleton infants (birth weight $\leq 5^{\text{th}}$ customized centile) were included in the study. The Gestation Related Optimal Weight computer-generated program²¹ was used to calculate the customized centile for each pregnancy, taking into consideration significant determinants of birth weight, such as maternal height and booking weight, ethnic group, parity, gestational age and gender.²¹ Gestational age was estimated using the date of the last menstrual period and early antenatal ultrasound. Birth weight was measured with an electronic scale.

Ten of the 20 mothers with IUGR offspring were smoking >10 cigarettes/day during the whole duration of pregnancy. The remaining 10 mothers presented with preeclampsia²² (6 cases), hypothyroidism (2 cases), iron-deficient anemia (1 case), and gestational diabetes mellitus (1 case).

Doppler studies were performed in the IUGR group every 10-15 days, starting from the 32nd gestational week. During each Doppler velocimetry evaluation, three consecutive measurements of the pulsatility index (PI) of the studied vessel (uterine, umbilical, cerebral arteries) were done and the mean value was recorded. Concerning uterine and umbilical arteries,^{23,24} mean PI values were progressively found to be in the upper physiological limits for the corresponding gestational age in 10 cases (ranging between the 90th and the 95th percentile), while in the remaining 10 cases PI values showed increased impedance to flow, being above the 95th percentile for gestational age. Regarding middle cerebral arteries,²⁵ Doppler studies showed resistance to be in the lower physiological limits for gestational age, indicating the

initiation of blood flow redistribution process, in order to spare vital organs (brain, heart and adrenals). Nevertheless, amniotic fluid was diminished in all IUGR cases. For the evaluation of the amniotic fluid, the largest fluid column on the vertical plane was assessed and was defined as diminished, if <2 cm. Placental weights were reduced²⁶ ranging from 240 to 450g.

In the AGA group, mothers were healthy and were either non-smokers or abstained from smoking during pregnancy. Placentas were normal in appearance and weight.²⁶

Tests for congenital infections were negative in all women of both groups, and their offspring had no symptoms of intrauterine infection or signs of genetic syndromes. One- and five-minute Apgar scores were in all cases and controls ≥ 8 . All neonates were breastfed. Demographic data of participating subjects are listed in Table 1.

Blood was collected in pyrogen-free tubes from: i) the mothers during the first stage of labor, or before receiving anesthesia in cases of elective caesarean section ii) the umbilical cords after double clamping, reflecting fetal state, and iii) the neonates before feeding on postpartum day 1 (N1) and 4 (N4), characterizing transition and stabilization to extrauterine life, respectively. Serum was separated by centrifugation after clotting and was kept frozen at -80°C until assay.

The determination of serum CC16 concentrations was performed by enzyme immunoassay (EIA) (Biovendor, GmbH, 69120 Heiderberg, Germany). The minimum detectable concentration, intra- and interassay coefficients of variation were 20 pg/mL, 5.7% and 4.1%, respectively.

Statistical analysis

CC16 data were normally distributed (Kolmogorov- Smirnov test); thus, parametric tests (Anova for repeated measures, paired samples t-test with Bonferroni correction for multiple comparisons) were applied in the analysis. Spearman's or Pearson's

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correlation coefficient, where appropriate, were used to detect any positive or negative correlations. A $P < 0.05$ was considered statistically significant.

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Results

Determined circulating CC16 values are shown in Table 2 (mean $\pm$ SD), as well as in Figure 1 (mean, [95% confidence intervals]). No significant differences in CC16 concentrations were observed between IUGR cases and AGA controls.

In the AGA group, maternal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ and $P=0.004$, respectively). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P=0.001$ in each case), while N1 CC16 concentrations were significantly higher compared to N4 ones ($P=0.008$). Fetal CC16 concentrations positively correlated with N1 and N4 ones ($r=0.619$, $P=0.004$, $r=0.680$, $P=0.001$, respectively). N1 CC16 concentrations positively correlated with N4 ones ($r=0.549$, $P=0.012$).

In the IUGR group, maternal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ and $P=0.03$, respectively). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ($P<0.001$ and $P=0.005$, respectively) and positively correlated with N1 and N4 ones ($r=0.592$, $P=0.006$ and $r=0.481$, $P=0.032$, respectively). N1 CC16 concentrations positively correlated with N4 CC16 ones and gestational age ($r=0.585$, $P=0.007$ and $r=0.510$, $P=0.022$, respectively).

In a combined group, N1 CC16 concentrations positively correlated with gestational age ($r=0.364$, $P=0.021$, respectively). Finally, the effect of infants' customized centiles, gender, parity, maternal age, as well as smoking on CC16 concentrations was not significant in both groups.

Discussion

The results of this study indicate a lack of significant differences in serum CC16 concentrations between IUGR cases and AGA controls. Serum CC16 concentrations can be affected by lung CC16 production, as well as by CC16 effusion from the lungs across the alveolo-capillary membrane.¹ In human adults, BALF CC16 levels correlate well with serum concentrations, and, therefore, the latter have been suggested as a reliable indicator of the total amount of protein synthesized in the respiratory tract.²⁷

CC16 production seems to be vital in postnatal pulmonary adaptation. However, in animal models, there is no evidence to suggest that the postnatal absorption of lung fluid is dependent on pulmonary perfusion, indicating that lung liquid absorption is dependent on the pulmonary epithelium and not on the pulmonary vasculature.²⁸

Furthermore, CC16 plays an important role in the development of lung injury associated with lung maturity.^{2,11} Thus, low cord blood CC16 concentrations in preterm infants¹², due to reduced production, consequent to Clara cells immaturity,²⁹ independently predict the development of bronchopulmonary dysplasia,¹² while intra-tracheal administration of recombinant human CC16 in preterm infants with respiratory distress syndrome reduces the neutrophil counts, which is indicative of CC16 anti-inflammatory properties.³⁰ Furthermore, the importance of CC16 as an anti-inflammatory pneumoprotein places neonates with CC16 deficiency at possible risk of postnatal respiratory injury.¹¹

IUGR has been proposed to induce permanent changes in lung structure and pulmonary defense mechanisms.¹³ In this respect, increased incidence of respiratory distress and lower respiratory tract infections has been documented in low birth weight, even full-term, infants.^{14,31} On the other hand, it is well established that stress

can lead to maturation of the fetal lung, mediated, at least in part, by an increase in fetal cortisol.³² Thus, IUGR-characterized by hypercortisolaemia³³ - has been previously suggested to be associated with enhanced fetal lung maturation.^{33,34} However, this association remains controversial on the basis of human and animal observations, as well as histological data.^{13,34}

In this respect, increased amniotic fluid indices of lung maturation have been documented in cases with presumed placental insufficiency.³⁴ However, prolonged intrauterine exposure to glucocorticoids in IUGR could have an inhibitory effect on alveolar formation, resulting in an emphysematous state of the lung.³⁵ Moreover, in a series of studies, the lungs of IUGR fetuses appeared to be structurally impaired¹³ with reduced weight, DNA and protein content,³⁶ and immature alveolar wall and air-blood barrier.³⁷ Nevertheless, in our study, the lack of differences in CC16 concentrations between IUGR cases and AGA controls possibly assumes that the lung immaturity and later respiratory diseases associated with the former may not be related to early CC16 deficiency.

This study confirms the presence of a gestational influence on CC16 production, which has also been suggested by previous reports concerning amniotic fluid,^{2,38} BALF of mechanically ventilated preterm neonates³⁹ and umbilical cord blood of both preterm and term infants.¹¹ CC16 is detectable in amniotic fluid from the 15th week of gestation² and increases with advancing gestational age, possibly due to continuous fetal CC16 production, secondary to fetal airway growth and/or lung maturation.^{2,38} Furthermore, in accordance with a previous report,¹¹ our results indicate that serum CC16 concentrations peak on the first day of life. This production surge at birth has been demonstrated to be more pronounced in term compared to preterm infants, conferring the former with superior extrauterine pulmonary protection.¹¹ Thus, term

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infants who are better equipped for extrauterine life had highest serum and lung fluid CC16 on day 1, indicating that CC16 production at birth may be an important part of postnatal adaptation.¹¹

Additionally, our data suggest that neonatal CC16 concentrations are higher than maternal ones. Similarly, a recent study demonstrated increased CC16 concentrations in newborns compared to healthy adults.¹¹ Such concentrations may be necessary for postnatal lung adaptation.

Furthermore, we were not able to confirm the reported circadian alterations of serum CC16, with on average a 20% decrease at the end of the day.⁴⁰

Finally, the absence of a gender effect in circulating CC16 concentrations is in line with previous reports in healthy adults.^{41,42}

In conclusion, circulating CC16 concentrations are similar between IUGR and AGA groups, possibly suggesting that the lung immaturity and later respiratory diseases associated with the former may not be related to early CC16 deficiency. Moreover, serum CC16 concentrations increase with increasing gestational age and peak on the first day of life, possibly indicating a vital role of this protein in fetal lung maturation and extrauterine pulmonary adaptation, respectively. Future research is necessary to elucidate the role of CC16 in fetal lung development and postnatal respiratory adaptation in both normal and complicated pregnancies.

Acknowledgments

No conflict of interest is declared.

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Table 1. Demographic data for AGA and IUGR neonates and their mothers

	AGA	IUGR	<i>P</i>
	Mean (SD)	Mean (SD)	
Birthweight (g)	3280 (333)	2511 (261)	<0.001
Birthweight centile	46 (24.7)	2.95 (1.9)	<0.001
Gestational age (weeks)	39.2 (0.83)	38.5 (1.2)	NS
Gender			NS
Male	10 (50%)	7 (35%)	
Female	10 (50%)	13 (65%)	
Maternal age (years)	30 (4.8)	32.7 (4.3)	NS
Parity			NS
Primigravida	14 (70%)	11 (55%)	
Other	6 (30%)	9 (45%)	
Mode of delivery			NS
Vaginal	15 (75%)	10 (50%)	
Cesarean section	5 (25%)	10 (50%)	

NS: non significant

Table 2. Mean±standard deviation (SD) values (ng/ml) of CC16 in maternal (MS), fetal (UC), postnatal day 1 (N1) and 4 (N4) serum in appropriate for gestational age (AGA) and intrauterine growth restricted (IUGR) groups.

GROUP		MS	UC	N1	N4
AGA	Mean±SD	2.48±0.53	2.67±0.58	8.76±5.98	4.43±1.97
IUGR	Mean±SD	2.54±0.68	2.94±0.69	7.39±3.70	5.52±3.15

Figure legend

Fig. 1. Mean (95% CI) serum CC16 concentrations in mothers (MS), fetuses (UC) and neonates on day 1 (N1) and 4 (N4) postpartum in appropriate for gestational age (AGA) and intrauterine growth restricted (IUGR) groups.

Clara Cell Protein in Full-term Pregnancies: The Influence of Intrauterine Growth Restriction

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Short title: Clara cell protein in IUGR

Summary

Background: Clara cell protein (CC16) is an immunomodulatory/anti-inflammatory broncho-alveolar-derived molecule and a biomarker of pulmonary epithelial cells maturity and alveolo-capillary membrane injury. Intrauterine-growth-restricted- (IUGR) neonates may present with structural lung immaturity, impaired immunocompetence and increased risk for respiratory infections and chronic obstructive lung disease in later life. **Objectives:** To investigate circulating CC16 concentrations in maternal, fetal and neonatal samples from IUGR and appropriate-for-gestational-age-(AGA) pregnancies. **Methods:** Serum CC16 concentrations were determined by EIA in 40 mothers and their 20 IUGR and 20 AGA singleton full-term fetuses-neonates on postnatal day 1-(N1) and 4-(N4). **Results:** No significant differences in CC16 concentrations were observed between IUGR and AGA groups. In both groups, maternal CC16 concentrations were lower compared to N1 and N4 ones ($P<0.001$ in each case). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ in each case). In the AGA group, N1 CC16 concentrations were significantly higher than N4 ones ($P<0.001$). Combining groups, N1 CC16 concentrations positively correlated with gestational age ($r=0.364$, $P=0.021$). Finally, the effect of gender, parity and maternal age on CC16 concentrations was not significant. **Conclusions:** The lack of differences in CC16 concentrations between IUGR and AGA groups possibly suggests that the lung immaturity and later respiratory diseases, associated with the former, may not be related to early CC16 deficiency. CC16 concentrations increase with advancing gestational age and peak on the first day of life, possibly indicating a vital role of the protein in fetal lung maturation and extrauterine pulmonary adaptation.

Key words: Clara cell protein, lung, intrauterine growth restriction, fetus, neonate

Introduction

Clara cell protein (known as CC16, CCSP, CC10 or uteroglobin), one of the major secretory proteins of the lungs, is produced by the nonciliated Clara cells, lining the bronchial and bronchiolar epithelium.¹ CC16 has been implicated in fetal lung growth² and has been ascribed many anti-inflammatory/immunomodulatory properties, including inhibition of pro-inflammatory cytokine production and neutrophil infiltration.^{1,3,4} Thus, CC16 prevents and ameliorates lung injury in adult mice and humans^{5,6} and protects the respiratory tract from life-threatening immunological reactions elicited by infectious agents.^{3,6}

CC16 concentrations in bronchoalveolar lavage fluid (BALF) depend on the number and integrity of Clara cells.¹ BALF CC16 concentrations are increased in patients with respiratory distress syndrome and decreased in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis.^{4,7,8} On the other hand, serum CC16 concentrations reflect alveolo-capillary membrane permeability.⁴ Lower serum CC16 concentrations have been detected in adults with chronic obstructive pulmonary disease,⁹ in children with asthma¹⁰ and preterm neonates with respiratory distress and bronchopulmonary dysplasia.^{11,12} Furthermore, serum CC16 is considered a sensitive and specific marker of lung injury in adults.^{1,4,9}

Recent epidemiological and experimental evidence in humans and animals indicates that the lung can be detrimentally affected by an adverse intrauterine environment, resulting in persistent alterations in lung structure and impaired respiratory function during postnatal life.¹³ In this respect, intrauterine growth restriction (IUGR) has been associated with respiratory compromise and higher incidence of lower respiratory tract infections and chronic obstructive airway disease in later life.¹⁴⁻¹⁶ However, data

assessing the impact of IUGR on fetal lung development are limited and predominantly derive from animal experimentation.¹⁷⁻¹⁹

This study was based on the hypothesis that circulating CC16 concentrations may differ between IUGR and appropriate for gestational age (AGA) fetuses and neonates, since the former may present with structural lung immaturity, impaired immunocompetence and increased risk for developing respiratory infections and chronic obstructive lung disease in later life.¹⁴⁻²⁰ Therefore, we aimed to investigate, for the first time to our knowledge, serum CC16 concentrations in IUGR and AGA pregnancies at crucial perinatal time-points and correlate them with several maternal and fetal anthropometric/clinical variables.

Materials and Methods

The Ethics Committee of our teaching hospital approved the study protocol. All included mothers provided signed informed consent before recruitment. Forty parturients giving consecutively birth, either to 20 AGA or 20 asymmetric IUGR full-term singleton infants (birth weight $\leq 5^{\text{th}}$ customized centile) were included in the study. The Gestation Related Optimal Weight computer-generated program²¹ was used to calculate the customized centile for each pregnancy, taking into consideration significant determinants of birth weight, such as maternal height and booking weight, ethnic group, parity, gestational age and gender.²¹ Gestational age was estimated using the date of the last menstrual period and early antenatal ultrasound. Birth weight was measured with an electronic scale.

Ten of the 20 mothers with IUGR offspring were smoking >10 cigarettes/day during the whole duration of pregnancy. The remaining 10 mothers presented with preeclampsia²² (6 cases), hypothyroidism (2 cases), iron-deficient anemia (1 case), and gestational diabetes mellitus (1 case).

Doppler studies were performed in the IUGR group every 10-15 days, starting from the 32nd gestational week. During each Doppler velocimetry evaluation, three consecutive measurements of the pulsatility index (PI) of the studied vessel (uterine, umbilical, cerebral arteries) were done and the mean value was recorded. Concerning uterine and umbilical arteries,^{23,24} mean PI values were progressively found to be in the upper physiological limits for the corresponding gestational age in 10 cases (ranging between the 90th and the 95th percentile), while in the remaining 10 cases PI values showed increased impedance to flow, being above the 95th percentile for gestational age. Regarding middle cerebral arteries,²⁵ Doppler studies showed resistance to be in the lower physiological limits for gestational age, indicating the

initiation of blood flow redistribution process, in order to spare vital organs (brain, heart and adrenals). Nevertheless, amniotic fluid was diminished in all IUGR cases. For the evaluation of the amniotic fluid, the largest fluid column on the vertical plane was assessed and was defined as diminished, if <2 cm. Placental weights were reduced²⁶ ranging from 240 to 450g.

In the AGA group, mothers were healthy and were either non-smokers or abstained from smoking during pregnancy. Placentas were normal in appearance and weight.²⁶ Tests for congenital infections were negative in all women of both groups, and their offspring had no symptoms of intrauterine infection or signs of genetic syndromes. One- and five-minute Apgar scores were in all cases and controls ≥ 8 . All neonates were breastfed. Demographic data of participating subjects are listed in Table 1.

Blood was collected in pyrogen-free tubes from: i) the mothers during the first stage of labor, or before receiving anesthesia in cases of elective caesarean section ii) the umbilical cords after double clamping, reflecting fetal state, and iii) the neonates before feeding on postpartum day 1 (N1) and 4 (N4), characterizing transition and stabilization to extrauterine life, respectively. Serum was separated by centrifugation after clotting and was kept frozen at -80°C until assay.

The determination of serum CC16 concentrations was performed by enzyme immunoassay (EIA) (Biovendor, GmbH, 69120 Heiderberg, Germany). The minimum detectable concentration, intra- and interassay coefficients of variation were 20 pg/mL, 5.7% and 4.1%, respectively.

Statistical analysis

CC16 data were normally distributed (Kolmogorov- Smirnov test); thus, parametric tests (Anova for repeated measures, paired samples t-test with Bonferroni correction for multiple comparisons) were applied in the analysis. Spearman’s or Pearson’s

correlation coefficient, where appropriate, were used to detect any positive or negative correlations. A $P < 0.05$ was considered statistically significant.

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Results

Determined circulating CC16 values are shown in Table 2 (mean±SD), as well as in Figure 1 (mean, [95% confidence intervals]). No significant differences in CC16 concentrations were observed between IUGR cases and AGA controls.

In the AGA group, maternal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ and $P=0.004$, respectively). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P=0.001$ in each case), while N1 CC16 concentrations were significantly higher compared to N4 ones ($P=0.008$). Fetal CC16 concentrations positively correlated with N1 and N4 ones ($r=0.619$, $P=0.004$, $r=0.680$, $P=0.001$, respectively). N1 CC16 concentrations positively correlated with N4 ones ($r=0.549$, $P=0.012$).

In the IUGR group, maternal CC16 concentrations were significantly lower compared to N1 and N4 ones ($P<0.001$ and $P=0.03$, respectively). Fetal CC16 concentrations were significantly lower compared to N1 and N4 ($P<0.001$ and $P=0.005$, respectively) and positively correlated with N1 and N4 ones ($r=0.592$, $P=0.006$ and $r=0.481$, $P=0.032$, respectively). N1 CC16 concentrations positively correlated with N4 CC16 ones and gestational age ($r=0.585$, $P=0.007$ and $r=0.510$, $P=0.022$, respectively).

In a combined group, N1 CC16 concentrations positively correlated with gestational age ($r=0.364$, $P=0.021$, respectively). Finally, the effect of infants' customized centiles, gender, parity, maternal age, as well as smoking on CC16 concentrations was not significant in both groups.

Discussion

The results of this study indicate a lack of significant differences in serum CC16 concentrations between IUGR cases and AGA controls. Serum CC16 concentrations can be affected by lung CC16 production, as well as by CC16 effusion from the lungs across the alveolo-capillary membrane.¹ In human adults, BALF CC16 levels correlate well with serum concentrations, and, therefore, the latter have been suggested as a reliable indicator of the total amount of protein synthesized in the respiratory tract.²⁷

CC16 production seems to be vital in postnatal pulmonary adaptation. However, in animal models, there is no evidence to suggest that the postnatal absorption of lung fluid is dependent on pulmonary perfusion, indicating that lung liquid absorption is dependent on the pulmonary epithelium and not on the pulmonary vasculature.²⁸

Furthermore, CC16 plays an important role in the development of lung injury associated with lung maturity.^{2,11} Thus, low cord blood CC16 concentrations in preterm infants¹², due to reduced production, consequent to Clara cells immaturity,²⁹ independently predict the development of bronchopulmonary dysplasia,¹² while intra-tracheal administration of recombinant human CC16 in preterm infants with respiratory distress syndrome reduces the neutrophil counts, which is indicative of CC16 anti-inflammatory properties.³⁰ Furthermore, the importance of CC16 as an anti-inflammatory pneumoprotein places neonates with CC16 deficiency at possible risk of postnatal respiratory injury.¹¹

IUGR has been proposed to induce permanent changes in lung structure and pulmonary defense mechanisms.¹³ In this respect, increased incidence of respiratory distress and lower respiratory tract infections has been documented in low birth weight, even full-term, infants.^{14,31} On the other hand, it is well established that stress

can lead to maturation of the fetal lung, mediated, at least in part, by an increase in fetal cortisol.³² Thus, IUGR-characterized by hypercortisolaemia³³ - has been previously suggested to be associated with enhanced fetal lung maturation.^{33,34} However, this association remains controversial on the basis of human and animal observations, as well as histological data.^{13,34}

In this respect, increased amniotic fluid indices of lung maturation have been documented in cases with presumed placental insufficiency.³⁴ However, prolonged intrauterine exposure to glucocorticoids in IUGR could have an inhibitory effect on alveolar formation, resulting in an emphysematous state of the lung.³⁵ Moreover, in a series of studies, the lungs of IUGR fetuses appeared to be structurally impaired¹³ with reduced weight, DNA and protein content,³⁶ and immature alveolar wall and air-blood barrier.³⁷ Nevertheless, in our study, the lack of differences in CC16 concentrations between IUGR cases and AGA controls possibly assumes that the lung immaturity and later respiratory diseases associated with the former may not be related to early CC16 deficiency.

This study confirms the presence of a gestational influence on CC16 production, which has also been suggested by previous reports concerning amniotic fluid,^{2,38} BALF of mechanically ventilated preterm neonates³⁹ and umbilical cord blood of both preterm and term infants.¹¹ CC16 is detectable in amniotic fluid from the 15th week of gestation² and increases with advancing gestational age, possibly due to continuous fetal CC16 production, secondary to fetal airway growth and/or lung maturation.^{2,38}

Furthermore, in accordance with a previous report,¹¹ our results indicate that serum CC16 concentrations peak on the first day of life. This production surge at birth has been demonstrated to be more pronounced in term compared to preterm infants, conferring the former with superior extrauterine pulmonary protection.¹¹ Thus, term

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3 infants who are better equipped for extrauterine life had highest serum and lung fluid
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5 CC16 on day 1, indicating that CC16 production at birth may be an important part of
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7 postnatal adaptation.¹¹
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10 Additionally, our data suggest that neonatal CC16 concentrations are higher than
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12 maternal ones. Similarly, a recent study demonstrated increased CC16 concentrations
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14 in newborns compared to healthy adults.¹¹ Such concentrations may be necessary for
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16 postnatal lung adaptation.
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19 Furthermore, we were not able to confirm the reported circadian alterations of serum
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21 CC16, with on average a 20% decrease at the end of the day.⁴⁰
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24 Finally, the absence of a gender effect in circulating CC16 concentrations is in line
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26 with previous reports in healthy adults.^{41,42}
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29 In conclusion, circulating CC16 concentrations are similar between IUGR and AGA
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31 groups, possibly suggesting that the lung immaturity and later respiratory diseases
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33 associated with the former may not be related to early CC16 deficiency. Moreover,
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35 serum CC16 concentrations increase with increasing gestational age and peak on the
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37 first day of life, possibly indicating a vital role of this protein in fetal lung maturation
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39 and extrauterine pulmonary adaptation, respectively. Future research is necessary to
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41 elucidate the role of CC16 in fetal lung development and postnatal respiratory
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43 adaptation in both normal and complicated pregnancies.
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Acknowledgments

No conflict of interest is declared.

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Table 1. Demographic data for AGA and IUGR neonates and their mothers

	AGA	IUGR	<i>P</i>
	Mean (SD)	Mean (SD)	
Birthweight (g)	3280 (333)	2511 (261)	<0.001
Birthweight centile	46 (24.7)	2.95 (1.9)	<0.001
Gestational age (weeks)	39.2 (0.83)	38.5 (1.2)	NS
Gender			NS
Male	10 (50%)	7 (35%)	
Female	10 (50%)	13 (65%)	
Maternal age (years)	30 (4.8)	32.7 (4.3)	NS
Parity			NS
Primigravida	14 (70%)	11 (55%)	
Other	6 (30%)	9 (45%)	
Mode of delivery			NS
Vaginal	15 (75%)	10 (50%)	
Cesarean section	5 (25%)	10 (50%)	

NS: non significant

Table 2. Mean±standard deviation (SD) values (ng/ml) of CC16 in maternal (MS), fetal (UC), postnatal day 1 (N1) and 4 (N4) serum in appropriate for gestational age (AGA) and intrauterine growth restricted (IUGR) groups.

GROUP		MS	UC	N1	N4
AGA	Mean±SD	2.48±0.53	2.67±0.58	8.76±5.98	4.43±1.97
IUGR	Mean±SD	2.54±0.68	2.94±0.69	7.39±3.70	5.52±3.15

Figure legend

Fig. 1. Mean (95% CI) serum CC16 concentrations in mothers (MS), fetuses (UC) and neonates on day 1 (N1) and 4 (N4) postpartum in appropriate for gestational age (AGA) and intrauterine growth restricted (IUGR) groups.

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