Prenatal iodine deficiency results in structurally and functionally immature lungs in neonatal rats

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Godbole MM, Rao G, Paul BN, Mohan V, Singh P, Khare D, Babu S, Nath A, Singh PK, Tiwari S. Prenatal iodine deficiency results in structurally and functionally immature lungs in neonatal rats. Am J Physiol Lung Cell Mol Physiol 302: L1037–L1043, 2012. First published March 16, 2012; doi:10.1152/ajplung.00191.2011.—Maternal hypothyroidism affects postnatal lung structure. High prevalence of hypothyroxinemia (low T4, normal T3) in iodine-deficient pregnant women and associated risk for neuropsychological development along with high infant/neonatal mortality ascribed to respiratory distress prompted us to study the effects of maternal hypothyroxinemia on postnatal lung development. Female Sprague Dawley rats were given a low-iodine diet (LID) with 1% KClO4 in drinking water for 10 days, to minimize thyroid hormone differences. Half of these rats were continued on iodine-deficient diet; ID (LID with 0.005% KClO4) for 3 mo, whereas the rest were switched to an iodine-sufficient diet; IS [LID + potassium iodide (10 µg iodine/20 g of diet + normal drinking water)]. Pups born to ID mothers were compared with age-matched pups from IS mothers at postnatal days 8 (P8) and 16 (P16) (n = 6–8/group). ID pups had normal circulating T3 but significantly low T4 levels (P < 0.05) and concomitantly approximately sixfold higher thyroid hormone receptor-β mRNA in alveolar epithelium. Lung histology revealed larger and irregularly shaped alveoli in ID pups relative to controls. Lung function was assessed at P16 using a double-chambered plethysmograph and observed reduced tidal volume, peak inspiratory and expiratory flow, and dynamic lung compliance in ID pups compared with IS pups. Significant lowering of surfactant protein (SP)-B and SP-C mRNA and protein found in ID pups at P16. ID pups had 16-fold lower matrix metalloproteinase-9 mRNA levels in their alveolar epithelium. In addition, mRNA levels of thyroid transcription factor-1 and SP-D were significantly higher in ID pups at P16. ID pups had 16-fold lower matrix metalloproteinase-9 mRNA levels in their alveolar epithelium. In addition, mRNA levels of thyroid transcription factor-1 and SP-D were significantly higher (3-fold) compared with IS pups. At P16, significantly lower levels of surfactant protein (SP)-B and SP-C found in ID pups may be responsible for immature lung development and reduced lung compliance. Our data suggest that maternal hypothyroxinemia may result in the development of immature lungs that, through respiratory distress, could contribute to the observed high infant mortality in ID neonates.

hypothyroxinemia; surfactant; plethysmography

ALVEOLAR STAGE OF LUNG DEVELOPMENT corresponds to week 36 until ~1.5 yr in humans and postnatal days 1 to 28 (P1–P28) in rodents and is an important step required for transition from structurally immature lungs to mature lung. The postnatal increase in thyroid hormone (TH) availability coincides with the alveolar stage of lung development and has been indicated to accelerate alveolar septation, suggesting the importance of TH in lung maturation. Moreover, TH has been shown to accelerate lung maturation (21). Various transgenic mouse models have been prepared to study the effects of TH deficiency on lung maturation. Tuyl and colleges (35) demonstrated that maternal hypothyroidism alters postnatal alveolar structure even before the functioning of fetal thyroid. They suggested that prenatal exposure to TH is necessary for normal development of lungs. Furthermore, iodine deficiency has been implicated in high incidence of neonatal and infant mortality (10, 11, 13, 31, 37), asphyxia, respiratory distress, and deviation of immune system (25). Thus TH deficiency poses a risk for impaired lung development.

More than nine million neonatal deaths occur each year, 98% of these occurring in developing countries. Neonatal deaths account for two-thirds of deaths in infancy and 40% of deaths before the age of 5 yr. The major causes of neonatal death are infections, preterm delivery, and asphyxia (11). The Indian National Health and Family Survey 3 (NHFS-3) has recorded the highest infant mortality of 72/1,000 in Uttar Pradesh, the largest state in India, with the considerable problem of iodine deficiency, the leading causes of death being diarrhea and respiratory failure (NIH Survey 2008).

Normally TH deficiency, or hypothyroidism, is established when the levels of TH hormones T4 and T3 are below normal range. Maternal thyroid deficiency during pregnancy has been reported as a major risk factor for impaired neuropsychological development of the child (16, 18). Furthermore, in an elegant report by Morreale de Escobar and colleagues (22), it was concluded/stated that “the screening of pregnant women for thyroid disorders should include the determination of free T4 as soon as possible during the first trimester as a major test, because hypothyroxinemia has been related to poor developmental outcome.” The frequency with which this may occur is probably 150 times or more that of congenital hypothyroidism, for which successful screening programs have been instituted in many countries. In light of this report, it becomes almost imperative to determine the effect of maternal hypothyroxinemia on lung development and function of their progeny (22).

In view of the above reports and the observed neonatal mortality attributable to respiratory failure in iodine-deficient (ID) areas, we tested the hypothesis that iodine deficiency may compromise the alveolar cell maturation and function during lung development in rats. To address this hypothesis, a lung structure and function of ID pups born to hypothyroxinemic Sprague Dawley rats were examined at P8 (early alveolar stage of the lung development) and P16 (toward the completion of alveolar stage and transition into mature lung). The alveolar stage development coincides with the increase in postnatal TH availability, suggesting significance of TH at this stage. There-
fore, in this study, we have selected P8 and P16 (flanking alveolar stage of development in rats) to study the effects of maternal hypothyroxinemia on lung development.

MATERIALS AND METHODS

Animals and treatments. Seven-week-old female Sprague Dawley rats weighing 120–150 g were used for experimentation with approval from the Institutional Animal Ethics Committee. Animals were housed under humane conditions, with 12-h:12-h light/dark cycles at 22–25°C, under Veterinary Control Guidelines. They were fed a stock pelleted diet for rats and water ad libitum.

ID model. On day 60, animals were switched to a low-iodine diet (LID) and drinking water containing 1% KCIO4 for 10 days to minimize thyroid store and individual variations among test animals. KCIO4 blocks uptake of circulating iodide and accelerates depletion of thyroid store. On day 60, animals were randomly divided into two groups: 1) iodine-sufficient groups (IS) and [LID + potassium iodide (10 μg iodine/20 g of diet + normal drinking water)], and 2) ID group. IS group received 10 μg iodine per 20 g diet + normal drinking water, and ID group continued to receive LID + 0.005% KCIO4 for 3 mo. LID and the supplements were prepared as described by us in our recent publication (3).

After 3 mo, blood (~0.5 ml) was obtained under slight ether anesthesia from the jugular vein to determine T4 and T3. The plasma was separated by centrifugation at 3,000 revolution/min for 5 min and kept at ~20°C. The rats were mated with normal males. Vaginal smears and microscopic visualization of spermatozoa confirmed the date of mating (EO). The date of birth was marked as P0. Pups were killed at P8 (n = 15) and P16 (n = 12). Lungs were dissected out at different developmental stages from three different litters and used for real-time qPCR, immunohistochemistry, and fluorescent staining. Eight pups from each group were used for the lung function study by plethysmography. Total T4 (TT4) and total T3 (TT3) were measured in the serum of the rat pups by radioimmunoassay using DPC kits (DPC, New York, NY).

Hematoxylin and eosin staining. Lung sections from pups at P8 and P16 (n = 6) for each group were stained with Harris’s hematoxylin and washed in running tap water after acid alcohol wash for 5–10 s. Sections were counterstained in eosin and washed in tap water for 15–30 s. The sections were then dehydrated through gradient alcohol series, cleared in xylene, and mounted in DPX.

Measurement of alveolar average diameter. For measuring the alveolar diameters, we used diaminobenzidine staining images (data not shown) for surfactant protein (SP)-B at P16 for both the ID and IS group. Maximum and minimum diameters were measured for 10 alveoli per section in three random fields by using the Image J software (NIH, Bethesda, MD); for calculating the average diameter, the mean of these maximum and minimum diameters were taken. These means were compared by Student’s t-test for unpaired differences between ID and IS.

Immunofluorescence. The animals were killed and were perfusion fixed; lower right lobes of their lungs were then harvested. Paraformaldehyde (4%)-fixed paraffin-embedded (5 μm) lung sections were used. After deparaffinization and rehydration steps, the sections were boiled in a microwave oven using 10 mM citrate buffer (pH 6.0) for antigen retrieval. Sections were blocked with 10% normal sheep serum for 20 min and were stained with polyclonal antibodies (1:200) against SP-C and SP-B (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. The next day after washing with 1× PBS, these sections were stained with Alexa Fluor 488 secondary antibody (Invitrogen, Carlsbad, CA) for 1 h at room temperature. The sections were counterstained with Hoechst 33258 (Invitrogen) nuclear stain and mounted in antifade mountant. The stained sections were observed, and images were collected with a Nikon 80i fluorescence microscope. Fluorescence intensities were measured from Image J software.

Western blot analysis. Western blot analysis was performed on tissue homogenates as described previously (3). Briefly, proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. The blots were then incubated for 2 h or overnight with primary antibodies SP-C (1:1,000, Santa Cruz Biotechnology) or SP-B (1:1,000, Santa Cruz Biotechnology) in the antibody buffer. The blots were developed for visualization using an enhanced chemiluminescence detection kit (Amersham Biosciences, Little Chalfont, UK). Relative expression of each protein was determined by densitometric analysis using LabWorks 4.0 software (Ultra-Violet Products, Cambridge, UK).

RNA extraction and real-time PCR. Total RNA was isolated from the lung tissues by single-step RNA isolation method using TRI reagent (MRC, Cincinnati, OH). Total RNA (2 μg) was reverse transcribed to cDNA using random hexamer primers (ABI) following manufacturer’s instructions. Real-time analysis for proteins [SP-A, SP-B, SP-C, SP-D, matrix metalloproteinase (MMP)-9, thyroid transcription factor (TTF)-1, and TH receptor (TR)-β] with normalizing gene, GAPDH, was performed using specific SyberGreen PCR MASTER mix assays per the manufacturer’s instructions (Applied Biosystems). Foster City, CA) on ABI Prism 7500 Sequence Detection System, and fold changes in gene expression was calculated using 2-ΔΔCT method as described previously (32, 33).

Lung function assessment. Lung function was assessed using a double-chambered whole body plethysmograph (Buxco Electronics, Wilmington, NC) with slight modification. In this system, rat pups were restrained in mouse plethysmograph using the biggest collar plate, and different lung function parameters were assessed. There were eight rats tested for both ID and IS groups. Tidal volume (TV), peak expiratory volume in 1 s (Pef), peak inspiratory volume (Pif), and specific airway resistance (sRaw) are derived in the presence of nebulized normal saline. These parameters were also assessed at 0, 3.12, 12.5, and 50 μg/ml concentration of nebulized methacholine that was administered through the inhalation route. From the box flow signal, we derived the following: TV, Pif, Pef, relaxation time (RT), and sRaw. Empirical assessment of dynamic lung compliance (cDyne) was made using the following formula (23): cDyne = TV/PIP-PEP, where PIP is peak inspiratory pressure and PEP is the peak end expiratory pressure. With the knowledge that pressure = resistance × flow rate, we derived the pressure values and used the same for empirical calculation of cDyne and expressed the value as ml/cmH2O.

Statistical analysis. All the experiments were repeated with three different litters. Statistical analysis was performed by using unpaired Student’s t-test. The data are presented as means ± SE. A P value <0.05 was considered statistically significant.

RESULTS

Hypothyroxinemia in pups born to ID mothers. A circulating T3 level was similar in ID and control pups at P8 and P16 stages, whereas circulating T4 levels were significantly lower in ID pups, confirming hypothyroxinemia in these pups; T4 levels in ID pups were ~26% and ~21% lower relative to IS pups at P8 and P16, respectively (Table 1). The relative lung
and body weight of the ID pups (both male and female) was found to be similar to IS pups (Table 2). In addition, ID pups had significantly higher mRNA levels of TR-β in their lung tissues; sevenfold at P8 and fivefold at P16 relative to IS pups (Fig. 1A).

Reduced lung capacity in pups born to ID mothers. To study the effect of maternal iodine deficiency on lung function, small-animal plethysmography was performed on pups at P16. ID pups have significantly reduced lung volume as evident from the exhaled or inhaled air volume derived from (Pef and Pit) relative to IS pups ($P < 0.05$) (Table 3). Similarly TV (ml) in ID pups is significantly low ($P < 0.05$) relative to IS pups, suggestive of poor lung function (Table 3). Lung compliance data revealed that iodine deficiency decreases lung compliance in the pups, reflecting the role of the lung surfactant. The unaltered sRaw under basal condition rules out any kind of airway obstruction (Table 3). Although sRaw was similar in both groups in response to saline on administration of increasing dose of methacholine, there was an increase in sRaw in the IS group, but the increase was significantly less in ID relative to controls at 50.0 mg/ml (Fig. 1B), indicating low air reactivity in ID pups. This is in consonance with histochemical demonstration of larger and irregularly shaped alveoli seen in the lower right lobe in ID pups (Fig. 1C and Table 3). The numbers of alveoli per field are also significantly less in ID compared with IS group (IS, 29 ± 5 vs. ID, 15 ± 3; $P < 0.05$). It is interesting to note that alveoli from ID pups not only have significantly larger average diameter but significantly larger differences observed in maximum and minimum diameter, indicating irregular shape of alveoli under ID (Table 3). In absence of airway defect, lung function and structural abnormalities seen in ID pups suggest surfactant alteration.

Lower SP-B and SP-C levels in lung tissue from ID pups. The mRNA levels of all four types of SPs were analyzed in lung tissue using real-time PCR technique. We observed a significant decrease in SP-B and decreasing trend in SP-C mRNA levels, but significantly higher SP-A and unaltered SP-D mRNA levels were observed in ID at P16 relative to IS of the same age (Fig. 2, A–D). To check whether alterations in mRNA levels of SP-B and SP-C seen in ID pups at P16 also reflect the changes in protein levels of these surfactants, we performed Western blot and immunofluorescence to quantify the protein levels of these surfactants. SP-B and SP-C protein expression levels were significantly decreased in ID at P16 (Fig. 2, E–G). The surfactant expression profile changes seen along with structural abnormalities seen in alveoli of ID pups indicate that iodine nutrition may play a major role in lung development and function in early infancy.

Table 2. Body weight and lung/body weight ratios and sexes of pups born to IS and ID rats at P16

<table>
<thead>
<tr>
<th>Sexes</th>
<th>Lung Weight, g</th>
<th>Body Weight, g</th>
<th>Lung/BODY Weight, %</th>
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<tbody>
<tr>
<td>IS Male (n = 10)</td>
<td>0.551 ± 0.07</td>
<td>26.54 ± 2.7</td>
<td>2.10 ± 0.38</td>
</tr>
<tr>
<td>IS Female (n = 10)</td>
<td>0.469 ± 0.06</td>
<td>24.57 ± 1.77</td>
<td>1.92 ± 0.31</td>
</tr>
<tr>
<td>ID Male (n = 10)</td>
<td>0.533 ± 0.05</td>
<td>26.21 ± 2.41</td>
<td>2.04 ± 0.25</td>
</tr>
<tr>
<td>ID Female (n = 10)</td>
<td>0.464 ± 0.07</td>
<td>23.71 ± 2.42</td>
<td>1.96 ± 0.27</td>
</tr>
</tbody>
</table>

Results are shown as mean values ± SE.

Reduced MMP-9 transcript levels in lung tissue from ID pups. MMPs, especially MMP-1 and MMP-9, are expressed strongly in all lung developmental stages (19). They play an important role in the required extracellular matrix remodeling during late lung development to allow the epithelial cytoplastic extensions to reach in close proximity of the interstitial fibroblast (2). Furthermore, MMP-9 has been described as a TH-responsive gene (26). Therefore, we analyzed MMP-9...
mRNA levels in the lung tissue. Real-time PCR data revealed that at P8, the lung tissue of ID pups had significantly lower (16-fold, **P < 0.01) MMP-9 mRNA levels relative to controls. At P16, there appeared to be a compensatory increase in MMP-9 level (5.5-fold, **P < 0.001, Fig. 3A).

**DISCUSSION**

Severe prenatal and postnatal malnutrition can have a significant effect on lung development, decreasing both lung function and lung size (1). In addition to malnutrition, insults such as exposure to hypoxia during the critical period of development have also been shown to cause reduced lung function in rats (27). Ong and colleagues (24) have demonstrated poor lung function in malnourished preschool children from rural India and suggested nutritional deficiency as one of the causes. Furthermore, based on the placebo-controlled, dou-

**TTF-1 expression in ID pups.** TTF-1 is expressed in the epithelium of the lung and is a critical regulator of transcription for SP-A, SP-B, and SP-C and is essential for lung morphogenesis. Therefore, TTF-1 mRNA expression was analyzed in both the groups at different developmental stages. At P8, TTF-1 expression was approximately threefold higher in ID pups relative to controls (Fig. 3A).

**Table 3. Lung function parameters assessed by a double-chambered whole body plethysmograph**

<table>
<thead>
<tr>
<th>Lung Function Parameters, per kg body wt</th>
<th>IS</th>
<th>ID</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV, ml</td>
<td>11.69 ± 0.54</td>
<td>9.65 ± 0.53</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PIF, ml/s</td>
<td>123.4 ± 6.83</td>
<td>93.7 ± 4.29</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>PEF, ml/s</td>
<td>103.9 ± 7.13</td>
<td>86.3 ± 5.63</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SRaw, cmH2O × s</td>
<td>126.08 ± 39.3</td>
<td>94.61 ± 9.66</td>
<td>NS</td>
</tr>
<tr>
<td>Lung compliance, ml/cmH2O</td>
<td>0.153 ± 0.06</td>
<td>0.021 ± 0.004</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>AAD, average alveolar diameter</td>
<td>49.4 ± 5.8</td>
<td>79.33 ± 12</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>AMMD, alveolar max-min diameter</td>
<td>24.33 ± 4.04</td>
<td>40.66 ± 9.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results are shown as mean values ± SE, n = 8/group. Dynamic lung compliance in pups born to IS rats and pups born to ID rats at P16. TV, tidal volume; PIF, peak inspiratory flow; PEF, peak expiratory flow; SRaw, specific airway resistance; AAD, average alveolar diameter; AMMD, alveolar max-min diameter.

**Fig. 2.** Lung tissue obtained from pups born to IS and ID rats at P16. A–D: surfactant protein (SP)-A, SP-B, SP-C, and SP-D mRNA levels using real-time PCR. Bar represents mean ± SE. E: representative Western blot of SP-B, SP-C, and β-actin. F: relative density of SP-B and SP-B in IS and ID groups. Each bar represents the mean of the respective individual levels ± SE (n = 5) fetuses from different pups at each developmental stage. Significant differences compared with age-matched euthyroid counterpart are indicated (**P < 0.005). G: immunofluorescence images of SP-B, SP-C at ×40. Fluorescence intensity of SP-B and SP-C, (n = 8/group/stage). *P < 0.05 by unpaired t-test. Scale bar = 20 μm.
We found that pups born to ID mothers (ID pups) have reduced lung compliance and altered lung function in terms of Pef and Pif values at P16 (Table 3). This may suggest improper lung development in ID pups; however, no obstruction in the airflow was observed in these ID pups as evident by lack of any significant differences in specific airway resistance at baseline level (Table 3). Furthermore, histological analysis of lung tissue revealed larger alveolar space and irregular shape at P16 relative to controls (IS), suggesting impaired alveolarization or improper alveolar formation in ID pups (Table 3). In rats and mice, extensive subdivision of gas exchange units and thinning of the alveolar walls, required for lung maturation, occur around P3 and P4 and are virtually complete by P14 (7, 8). In our study, we demonstrated decreased maximal inspiratory and expiratory flow rates and decreased TVS in ID pups relative to IS pups. This is further supported by lower TV in ID pups.

Taken together, our histological and plethysmograph data suggest improper lung maturation/development in ID pups, resulting in reduced lung volume and impaired lung function. Significantly lower SP-B and SP-C gene expression in the lung tissue from ID pups indicates poor lung maturation and compromised function in these pups relative to controls. Lower SP-B mRNA and protein levels in ID pups could be the cause for reduced lung compliance and lower lung volumes (34). The SP is under multifactorial control and is regulated by a number of hormones and factors, including glucocorticoids, prolactin, insulin, growth factors, estrogens, androgens, THs, and catecholamines acting through β3-adrenergic receptors and cAMP (21). ID pups had a significant increase in TR-β expression in lung tissue in response to iodine deficiency.

Thus reduced levels of SP-B and SP-C levels found in the lung tissue of ID pups could be direct effect of lower T4 levels in these pups. Effect of THs on fetal lung maturation has been demonstrated on the rate of type II alveolar cell differentiation (17). There are some clinical studies in the literature that support the importance of thyroxine administration in reducing the incidence and severity of the respiratory distress syndrome in prematurely born infants. The effect of 1-thyroxine on prenatal lung maturation investigated in pregnant women with high risk for preterm delivery indicated that 1-thyroxine accelerates the maturation of fetal lung (14). Similarly, study performed in fetal sheep of 125 days gestation showed that combined administration of glucocorticoids and TR accelerated lung maturation in the fetus (29). Furthermore, a study by Gross et al. (15), based on randomized clinical trials indicated the importance of antenatal thyrotrophin-releasing hormone in reducing respiratory distress syndrome where surfactant therapy is unavailable, further supports our findings.

Moreover, surfactant dysfunction, reduced SP-B levels and its promoter, TTF-1, are associated with respiratory distress syndrome (6). Lowered SP-B and SP-C mRNA and protein levels in the lungs of ID pups with concomitant lowering in TTF-1 mRNA levels indicate similar conditions in pups born to ID rats. During normal fetal human lung development and in the adult lung, TTF-1 expression remained constant, indicating that TTF-1 levels may not be subject to developmental regulation (5). Reduced TTF-1 expression is associated with respiratory distress syndrome (6). However, limited information is available on the regulation of TTF-1 expression in lung tissue during prenatal and postnatal malnutrition. We found lowered TTF-1 expression in lung tissue from ID pups (Fig. 3); however, it is not known whether reduced T4 has a direct effect on TTF-1 gene expression or whether this effect is secondary to iodine deficiency-induced impaired differentiation of alveolar type II cells. Nevertheless, TTF-1 controls the expression of several important lung-specific genes such as expression of SP-A, SP-B, SP-C, Clara cell secretory protein, and ATP-binding-cassette transporter A3 genes (5). Thus reduced levels of SP-B and SP-C genes in ID pups could be a consequence of TTF-1 deficiency found in their lung tissues (6, 30). However, higher expression of SP-A and SP-D in ID pups support the role of impaired alveolar differentiation on TTF-1, SP-B, and SP-C gene expression. SP-A and SP-D glycoproteins take part in host defense unlike SP-B and SP-C, which are required for alveolar function (12, 36). Taken together, the mechanisms for differential gene expression of SP and TTF-1 in postnatal lung of pups exposed to iodine deficiency need to be explored.
Signals that regulate lung maturation in ID needs to be determined; MMP may be a potential candidate (4, 20). MMPs play a key role in extracellular matrix remodeling during development and pathogenesis. Expression of MMP-1 and MMP-9 has been detected in lung epithelial cells in all developmental stages throughout lung development (19). It plays an important role in the remodeling that occurs in the interstitium and epithelial basement membrane during lung development and in vascular development. MMP-9 has been demonstrated as TH-responsive genes (26). Under normal conditions, expression and activity of MMP-9 increase substantially after the saccal stage of lung development (28). This increase in MMP expression and activity may have an implication in the disruption of basement membrane, which occasionally occurs toward the alveolar stage of lung development to allow the epithelial cytoplasmic extensions to reach in close proximity of the interstitial fibroblast. Thus MMPs have a capacity to remodel the stromal/epithelial interface during lung development. The pups born to ID rats had a 16-fold lower MMP-9 mRNA levels in their alveolar epithelium compared with control at P8. The lowered MMP-9 levels in ID pups could have impaired the process of basement membrane disruption required during lung development.

Data presented here indicate that exposure to iodine deficiency during lung development impairs lung function in newborn pups. Moreover, immature lung formation in ID pups is indicated by lower SP-B, SP-C, and substantially lower MMP-9 mRNA in lung tissues of these rats. The repercussions of regulation of these molecules may have caused impaired lung maturation and function. This could significantly contribute to infant mortality that can be prevented with adequate iodine supplementation to mothers during pregnancy and neonatal life. These findings are noteworthy given the high prevalence of maternal hypothyroxinemia in ID areas, as immature lung, through respiratory distress, could contribute to the observed high infant mortality in neonates. The results of present study are likely to pave the way to future studies on how the weakened lung of infants cope with secondary challenges like bacterial infections highly prevalent in rural areas with poor sanitary conditions.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: M.M.G. and B.N.P. conception and design of research; M.M.G., A.N., P.K.S., and S.T. interpreted results of experiments; M.M.G., P.K.S., and S.T. drafted manuscript; M.M.G. and A.N. edited and revised manuscript; M.M.G. approved final version of manuscript; G.R., V.M., P.S., D.K., and S.B. performed experiments; G.R., B.N.P., V.M., and S.T. analyzed data; V.M. prepared figures.

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