Alteration In Pulmonary Structure and Function Following *In Utero* and Early Postnatal Arsenic Exposure

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Introduction

- Arsenic is widespread in the environment
  - Inhalation
  - Water
- Epidemiological studies implicate arsenic as a carcinogen
- Lung is a target for both cancer and other chronic diseases

Arsenic exposure is a world-wide problem

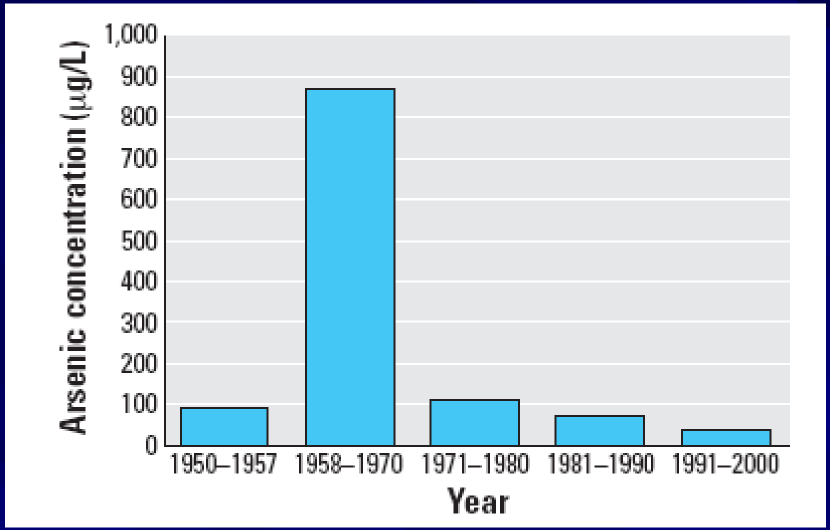
In Bangledesh alone, the British Geological Survey (2001) estimated that over 35 million people were exposed to arsenic levels in drinking water greater than 50 ppb and that over 57 million people were exposed to over 10 ppb arsenic in their water.

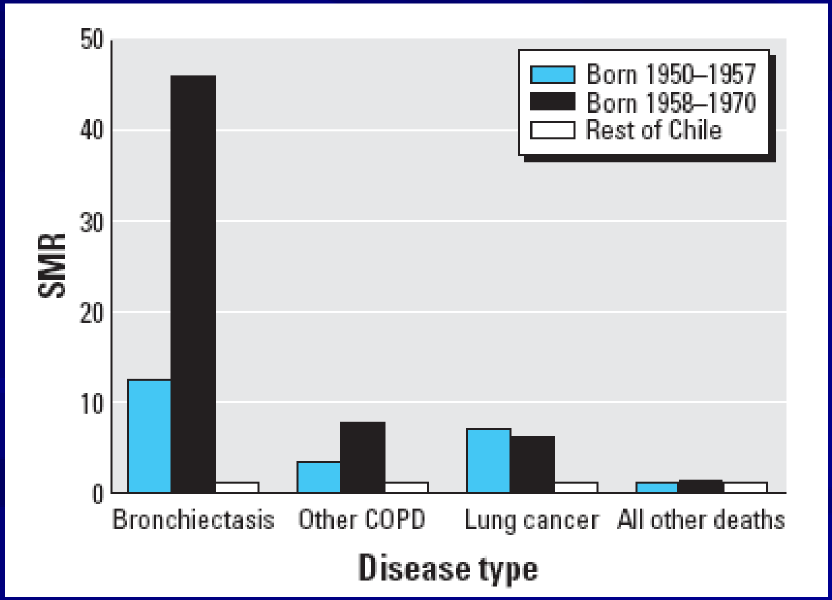
Effect of arsenic exposure in susceptible populations

In utero and early postnatal exposure to toxicants has the potential to affect gene expression, altering organ structure and physiological function which can be manifested as adult disease.

Children may be especially vulnerable to toxicants

- Lung growth and development is still continuing after birth.
- Children are more active and have different metabolic activity than adults.
- The placenta is not a barrier for toxicants.
- High levels of air pollution exposure in children have been associated with increased incidence of asthma and other respiratory disease





- High *in utero* arsenic exposure in mice produces lung tumors in adults (Waalkes)
- Smith results in humans demonstrate the effect of exposures during this developmental time
- Conclusion: Exposure to high levels of arsenic during *in utero* and early postnatal development leads to increased risk of death by chronic lung disease.

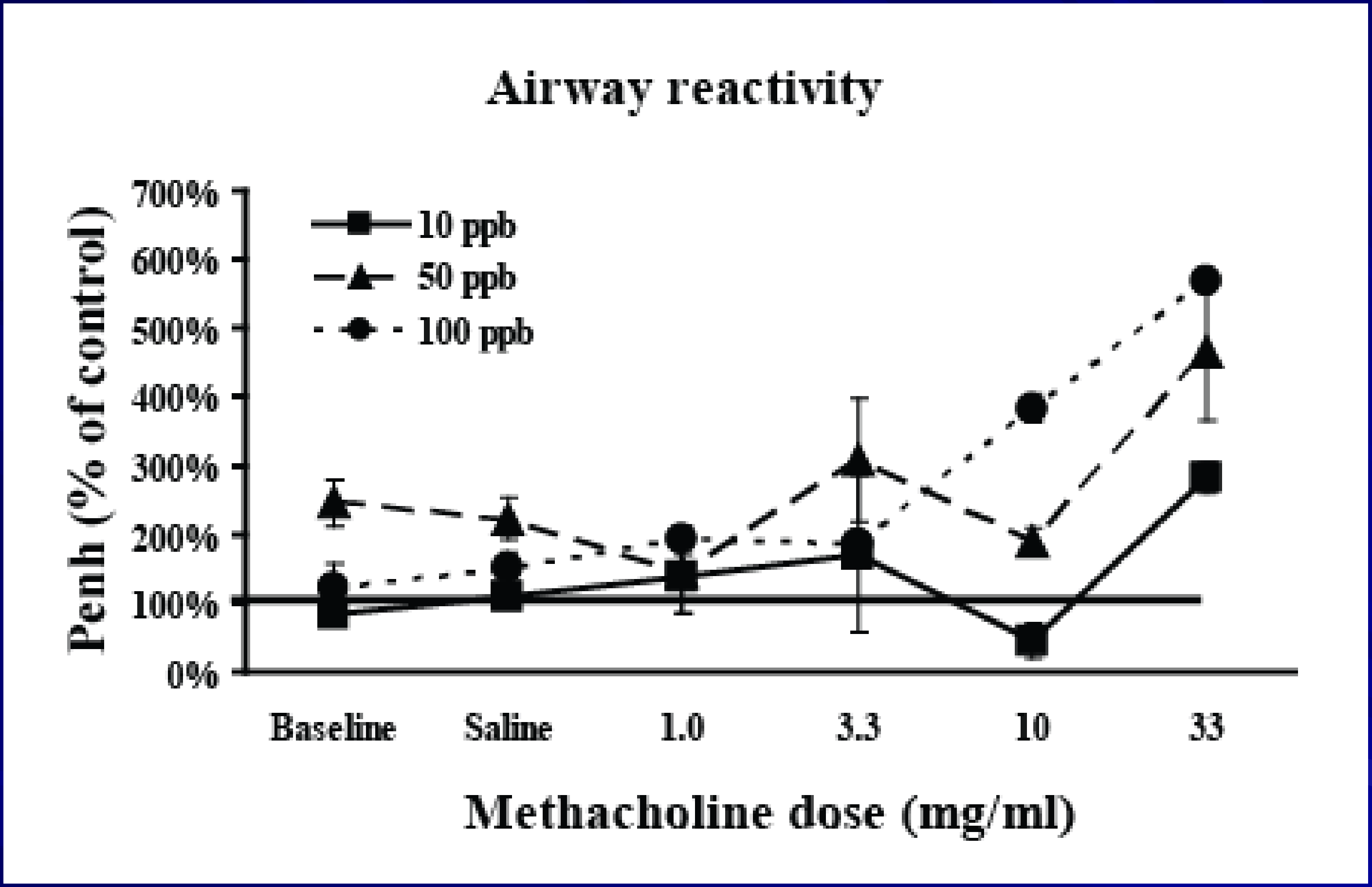
Questions

- Does developmental exposure to environmentally relevant levels of arsenic result in altered lung structure and/or function?

Methods

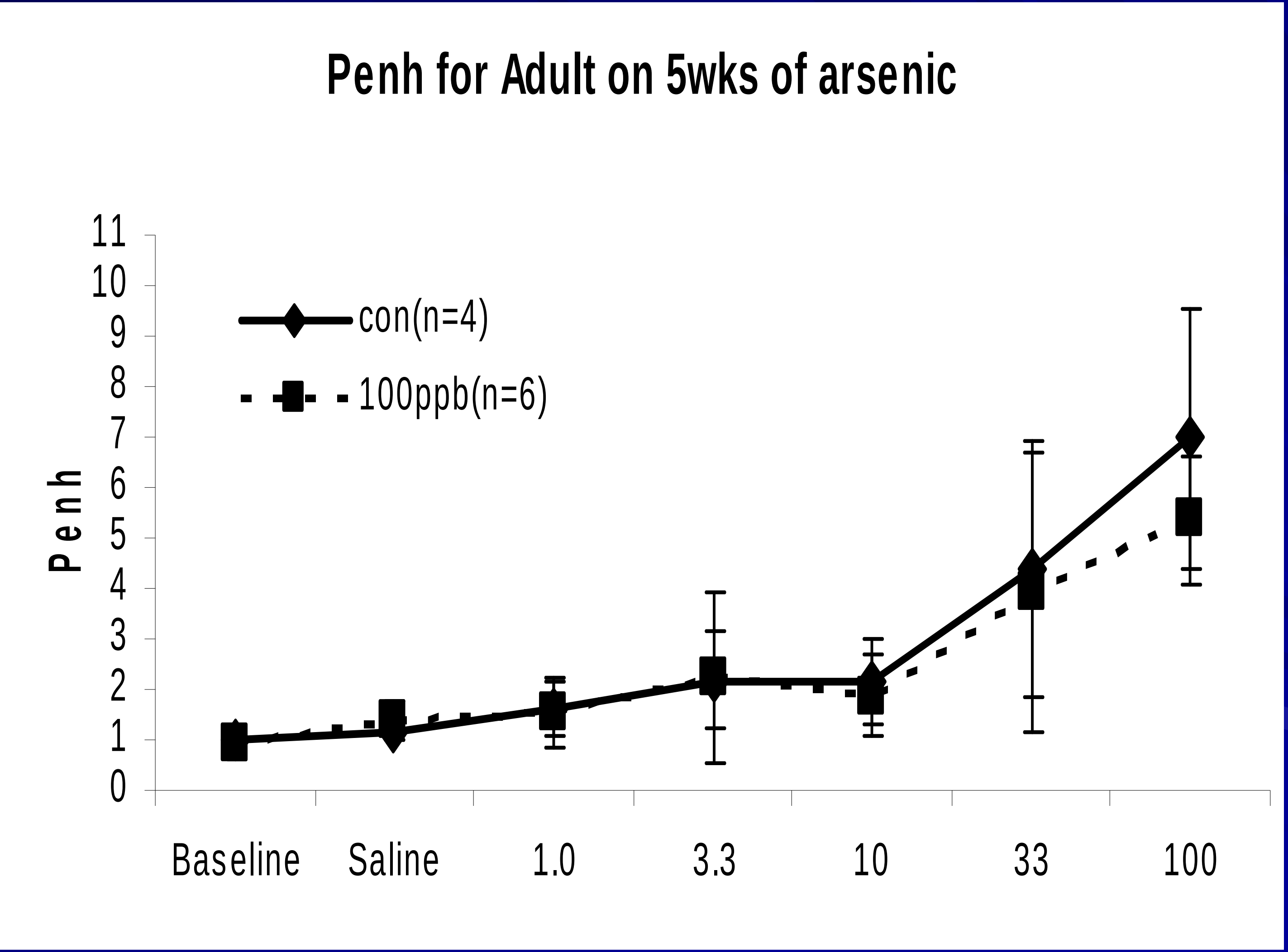
- Established a breeding colony on C57Bl/6 mice
- Mothers are given arsenic in drinking water
  - Sodium arsenite – 5, 10, 50, 100 ppb
  - Exposures continue after birth (either in mothers milk or by direct ingestion)
- Pups are sacrificed on postnatal day 1, 7, 12 and 28
- Determine matrix gene expression
  - Real time PCR and Western blots are used to determine gene and protein expression of collagen 1a1, 1a2, 3a1 and elastin
- Additional lungs are collected for structural and functional analysis

Does *in utero* and early postnatal exposure to arsenic alter lung function?



In utero and early postnatal exposure results in increased airway reactivity.

Is the change in pulmonary function related to developmental exposure?

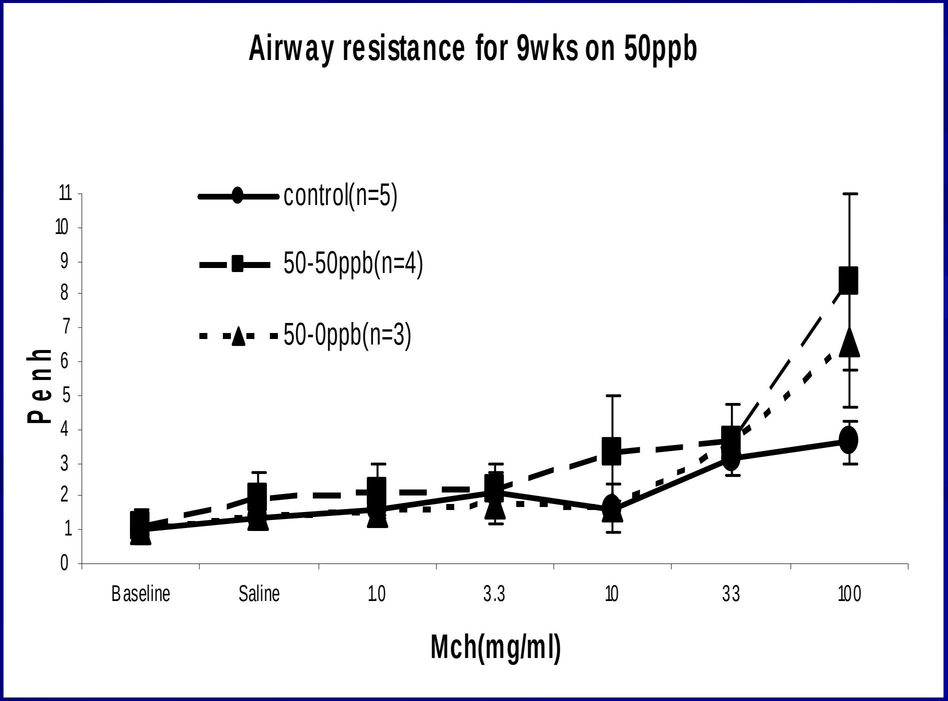


Can the changes be reversed by removal of arsenic exposure?

Animals were exposed *in utero* and up to 28 days after birth.

Animals were then either kept on the arsenic water (50-50) or removed from the arsenic exposure (50-0) for an additional 5 weeks.

Measured pulmonary function



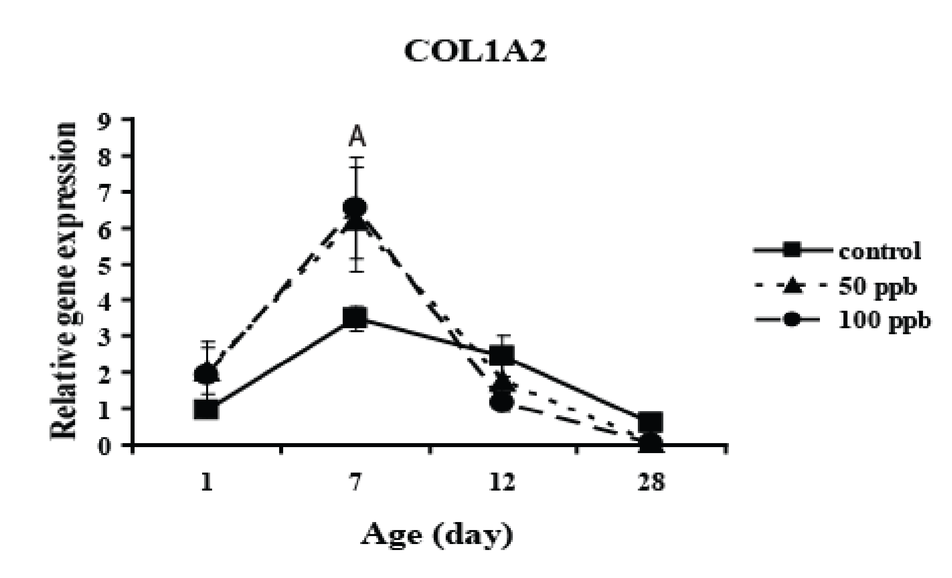
What are the targets that cause the functional change?

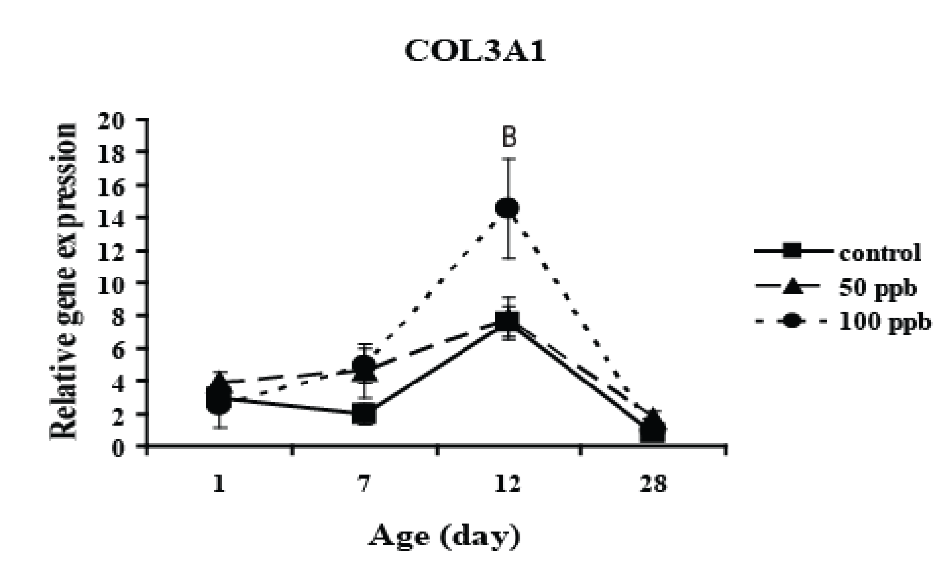
- Anatomical
  - Extracellular matrix genes
  - Airway smooth muscle
- Signaling
  - Growth factors involved

Results

- Real time PCR and Western blot analysis for matrix genes has been performed on pups on postnatal days 1, 7, 12, and 28

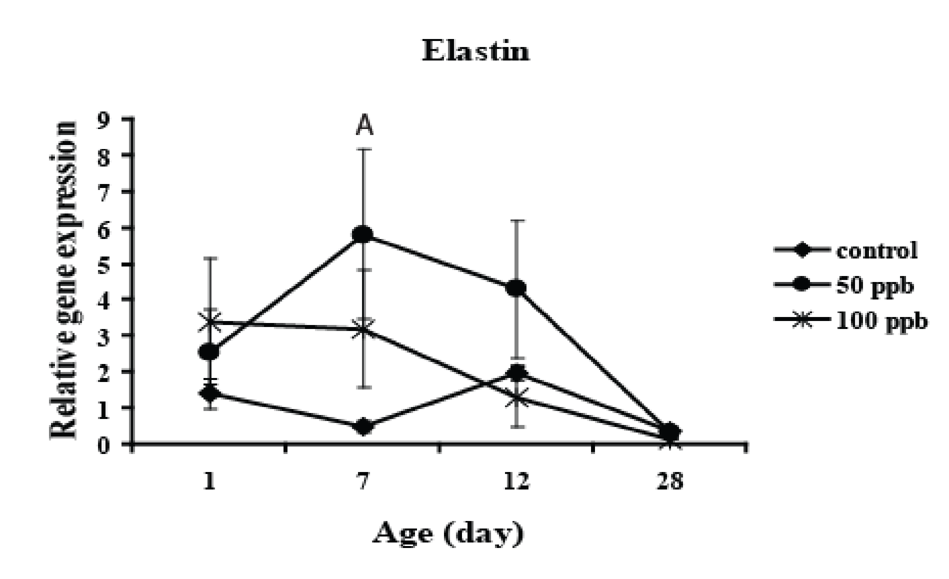
- Used image analysis with specific stains to determine levels of smooth muscle actin and collagen around airways



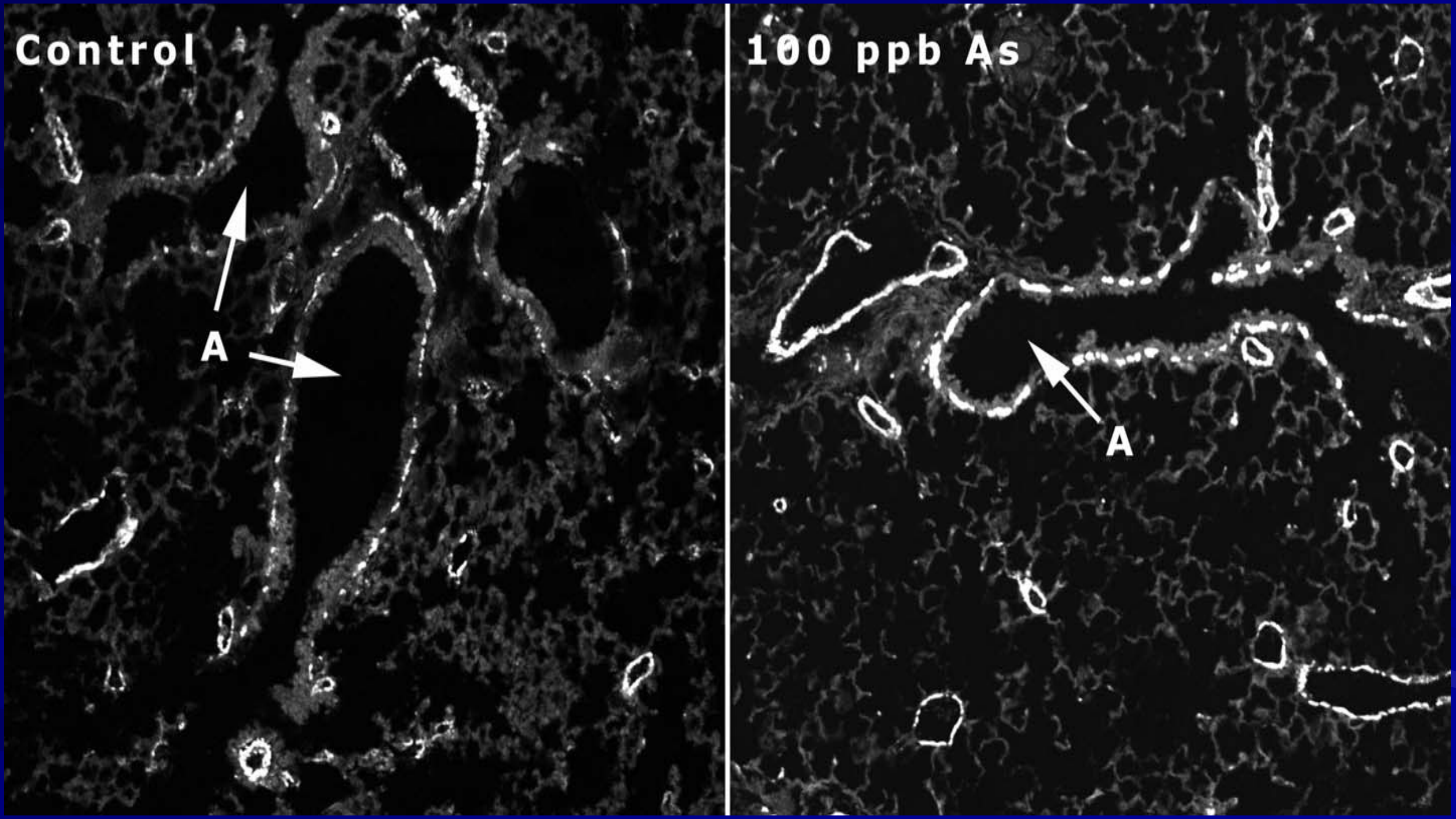


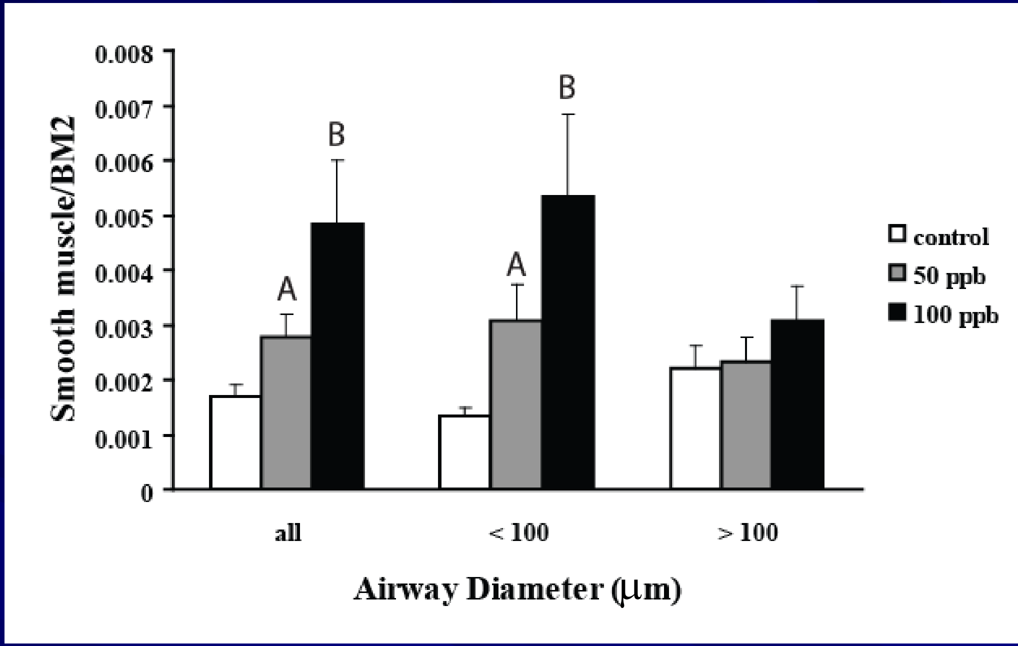
No effect on collagen 1A1

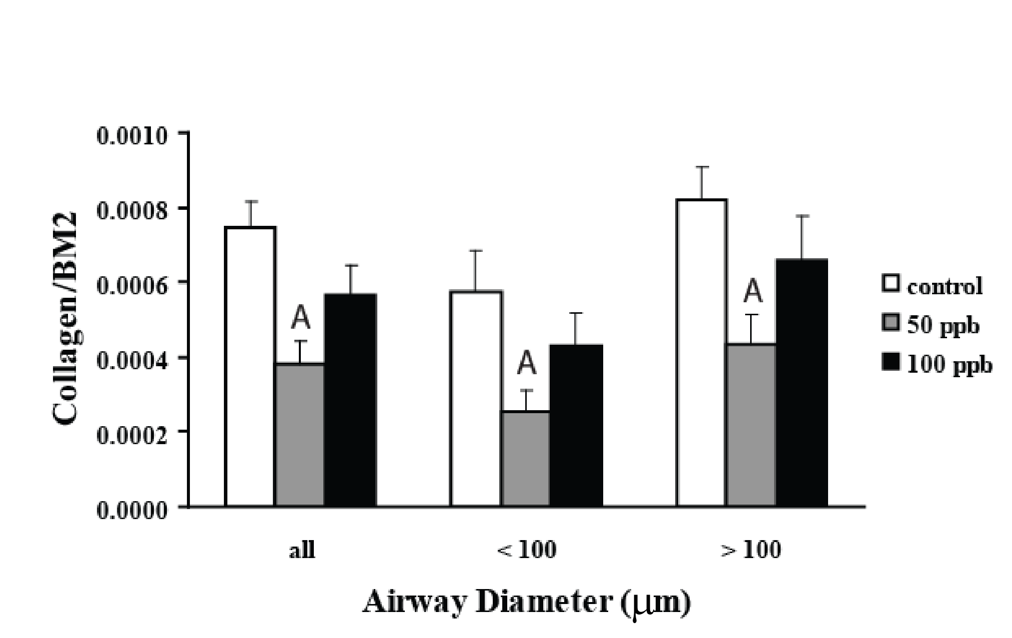
Arsenic increased collagen 1A2 and elastin expression on postnatal day 7 and increased 3A1 expression of day 12



Smooth Muscle Actin Immunostaining







Arsenic exposure resulted in

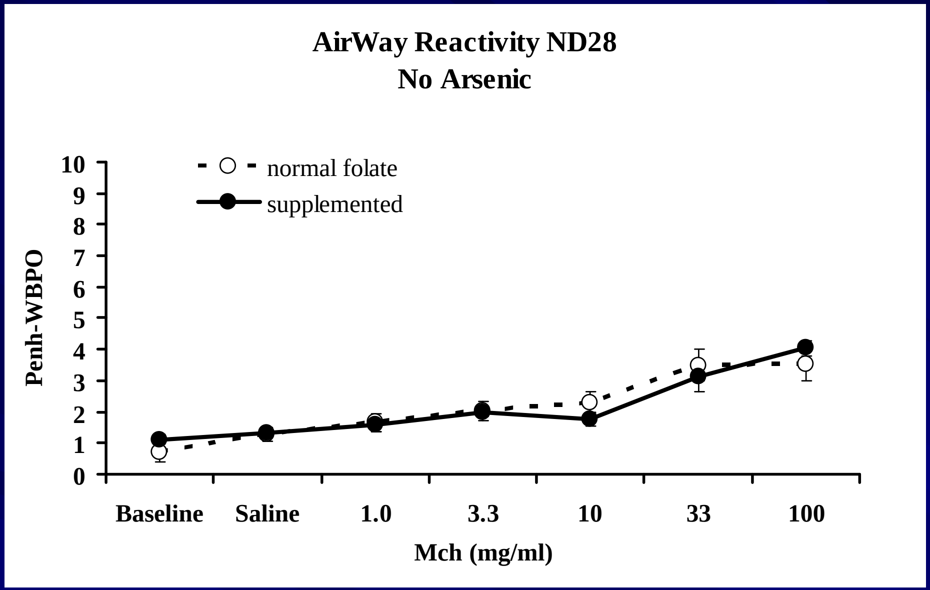
- Dose-dependent increase in smooth muscle
- Changes in smooth muscle were predominantly around small airways
- Decreased levels of collagen around airways with 50 ppb exposure
- Collagen changes were similar between small and large airways.

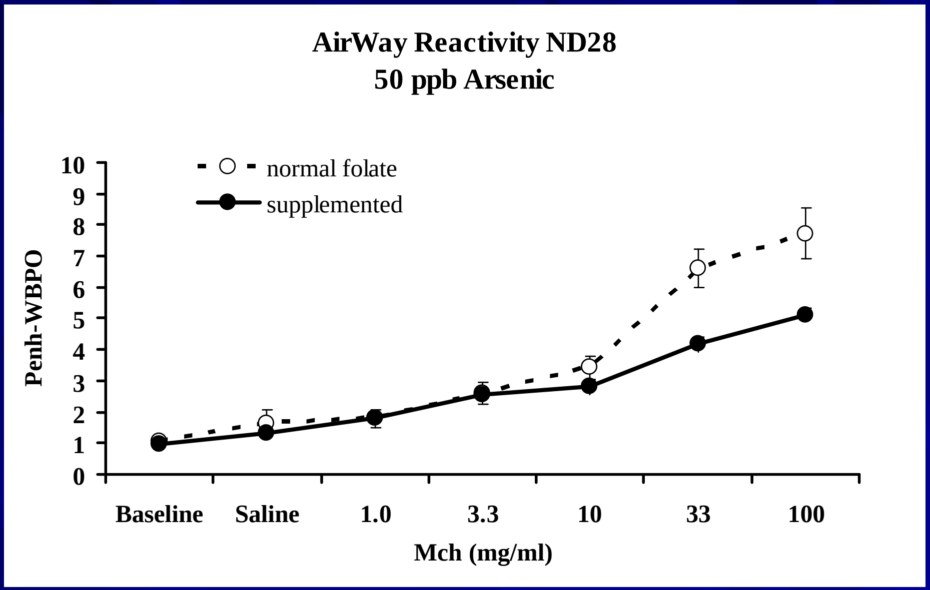
What are the targets that cause the functional change?

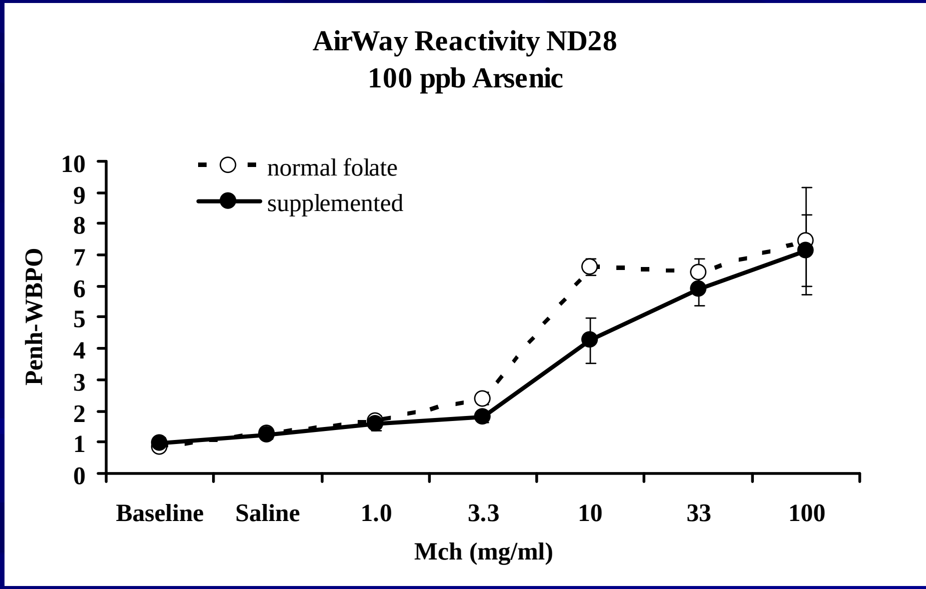
- Anatomical
  - Extracellular matrix genes
  - Airway smooth muscle
- Signaling
  - Growth factors involved
- Does methylation play a role?
  - CpG island methylation status
  - Folate

Role of methylation

- Arsenic effect on CpG island methylation was examined in the matrix genes
  - Arsenic did not affect the methylation status of the regions that were examined
- Evaluated folate supplemented diets as an intervention strategy



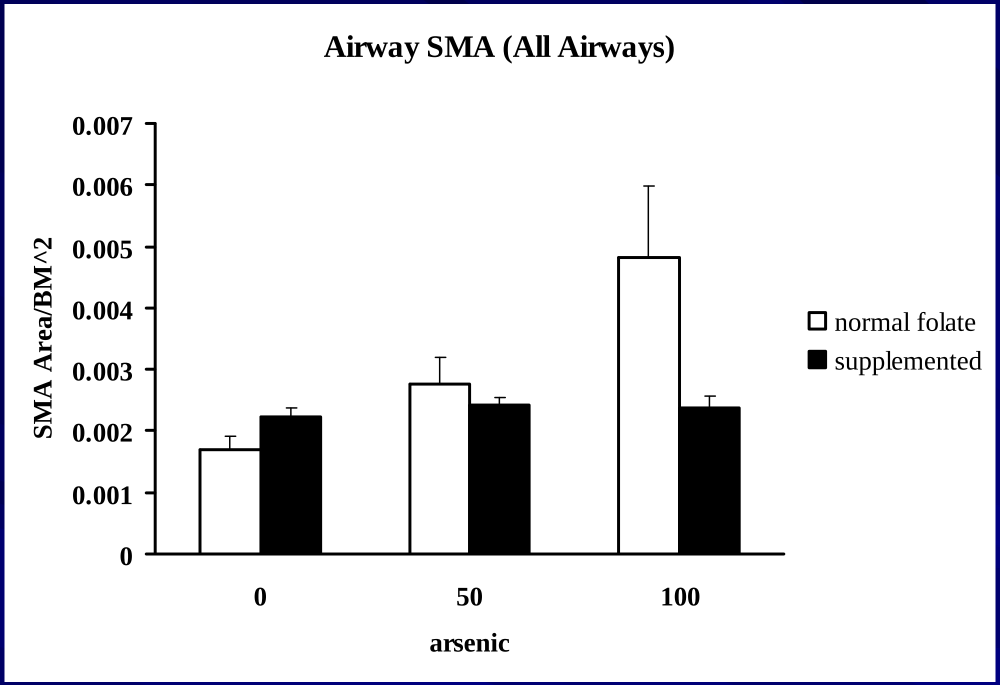




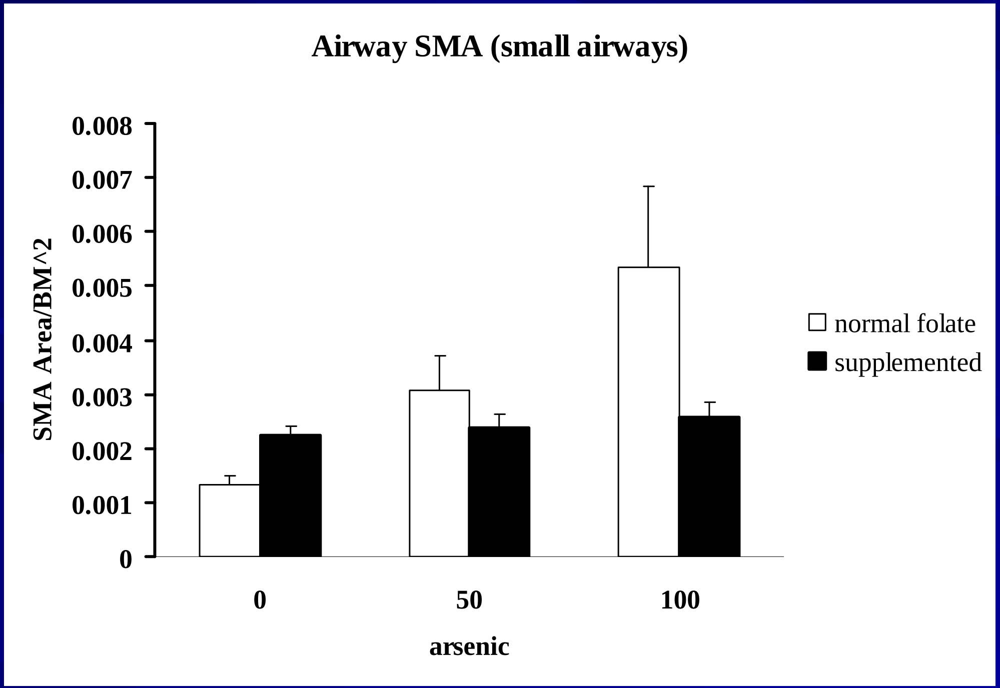
No differences in the control responses

Reactivity of 50 ppb animals is reduced by folate supplementation

No differences in 100 ppb exposed animals



Folate supplemented diets eliminated the arsenic-induced increase in smooth muscle actin around the airways.



Folate supplemented diets reduced the arsenic-induced increased expression of matrix genes

Conclusions

- Arsenic exposure during *in utero* and early postnatal development alter
  - Lung pulmonary function
  - Airway smooth muscle
  - Airway collagen
  - TGF-β1 expression
- Folate supplementation
  - Reduces airway reactivity (50 ppb)
  - Reduces arsenic-induced increases in TGF-β1 on day 12 (50 ppb)
  - Eliminates arsenic-induced increases in airway smooth muscle
  - Reduces arsenic-induced alterations in matrix gene expression

Future Directions

- Future projects
  - Determine developmental exposure time that is important for arsenic induced alterations
  - Human populations (children)
    - Incidence of lower respiratory infections
    - Alterations in pulmonary function
  - Examine arsenic interaction with other developmental regulated genes
  - Examine effects of inhalation of arsenic during early postnatal development

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