MODULATION OF AIRWAY REACTIVITY WITH CHRONIC MECHANICAL STRAIN

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1.0 Background

Asthma is characterized by chronic airway inflammation, repeated episodes of reversible airway obstruction and airway hyper-reactivity. Airway inflammation and clinical symptoms of asthma are most often initiated by IgE-mediated responses following exposure to environmental allergens. Inflammatory cell recruitment into the lungs and airway hyper responsiveness are key components of the allergen-induced inflammatory response, which results from the interaction of resident airway cells and inflammatory cells that release local mediators. Patients with severe, recurrent asthma also have remodeling of the airway wall with increased airway smooth muscle, increased inflammatory cells and collagen deposition.

For many decades, corticosteroids have been used to control airway inflammation, while β-adrenergic bronchodilators have been the primary treatment for bronchoconstriction. Although these therapies are effective for the vast majority of asthmatics, they are limited by high cost, poor adherence, and increasing concern about long-term adverse effects. The FDA has issued a black-box warning linking long-acting β- adrenergic bronchodilators to asthma deaths, and other studies have linked corticosteriods to fracture risk and growth retardation[10-12]. Recently introduced therapies that focus on inhibiting a single component of the allergic inflammatory response, such as anti-IL-5 or anti-IL-13 antibodies, have had limited efficacy[13]. Thus, there is a compelling need for new, safe and effective approaches to asthma treatment, particularly in children with severe asthma, where there is generally a lifelong burden of disease, use of medications, and accounts for the majority of health care expenditures among all children with asthma. **The development of a therapeutic approach that could reduce both airway responsiveness and airway inflammation would be an important advance in the treatment of asthma.**

During the previous funding period of this project, our laboratory demonstrated that chronic mechanical strain imposed on the airways *in vivo* using continuous positive airway pressure (CPAP) results in a dramatic reduction in airway reactivity *in vivo* in mice, ferrets and rabbits[1-3]. Lungs, airways and airway smooth muscle (ASM) tissues isolated from CPAP-treated animals studied *in vitro* exhibited lower responsiveness to bronchoconstrictors[1-3]. We also observed this suppression of airway responsiveness by chronic mechanical strain in a rabbit model of allergic asthma[5]. These animal studies led to a small clinical trial in which adults with asthma were treated with nocturnal CPAP for 1 week. CPAP caused a significant reduction in airway reactivity in these patients[6]. This novel approach for treating airway hyper-reactivity is currently being evaluated in a NIH multi-center Phase II clinical trial of adults with mild to moderate asthma (U01 HL108730).

We now propose to determine the efficacy of chronic mechanical strain as an inhibitor of airway inflammation. Our preliminary studies have demonstrated that chronic mechanical strain can suppress the responses of airway tissues to allergic inflammatory stimuli and suppress the activation of signaling molecules involved in these responses[3,14,15]. Based on these studies, ***we hypothesize that chronic mechanical strain will inhibit signaling processes in airway tissues that lead to airway inflammation, hyper-reactivity and remodeling.*** We propose a novel molecular mechanism for the inhibitory effects of chronic mechanical strain on the responses of ASM to inflammatory stimuli. Anti-inflammatory agents such as corticosteroids remain the primary treatment for severe chronic asthma. The consequences of long term steroid therapy can be extremely deleterious, particularly in children, for whom steroids can have a significant adverse impact on growth and development. Our proposed study will provide the basis for a new non-pharmacologic therapy for the control of asthma that may be particularly important for the treatment of children with severe steroid-dependent disease. We will determine whether mechanical strain, as delivered using CPAP, is an effective therapy for suppressing airway hyper-reactivity and inflammation in pediatric subjects with severe asthma. ***The concept that a non-pharmacologic therapy may play such a pivotal role in suppressing ASM contraction, inflammation and tissue remodeling is novel and exciting.*** Furthermore, elucidation of the molecular mechanisms by which strain suppresses inflammatory signaling pathways could also provide a basis for the development of new pharmacologic targets for asthma therapy.

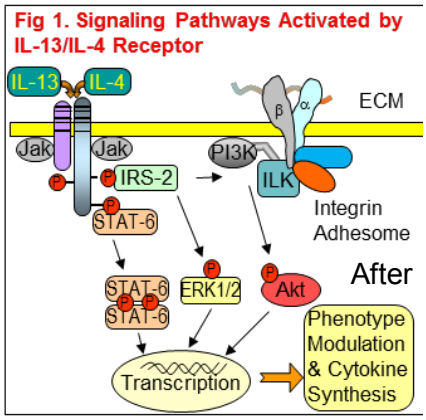
Pathophysiology of airway inflammation in asthma. Th2 cytokines derived from CD4+ T lymphocytes play a pivotal role in the airway pathology associated with asthma[16-19]. The structurally related Th2 cell derived cytokines interleukin (IL)-4 and IL-13 stimulate allergic and eosinophilic inflammation as well as

epithelial and smooth muscle changes, and they are widely recognized as mediators of allergen-induced airway hyperresponsiveness and airway remodeling[8,18-21]. Th2 cytokines can induce airway hyper-reactivity without inflammatory cell recruitment which indicates that many of the pathophysiologic effects of allergic inflammation in asthma result from the direct actions of IL-4 or IL-13 on airway cells[9,19,20,22-24]. The incubation of ASM with IL-
13 or IL-4 *in vitro* causes ASM hypercontractility, inhibits relaxation to isoproterenol and stimulates release of the eosinophil chemo-attractant eotaxin and IL-5[7,9,25-28]. The cytokines secreted by ASM and other airway cells have paracrine effects on inflammatory cells and thus contribute to airway inflammation[29-31].

Phenotypic alterations in airway cells induced by the direct actions of cytokines may be a primary mechanism for airway remodeling in asthma. Changes in gene expression induced by IL-13 or IL-4 have been demonstrated in airway cells including ASM, fibroblasts and airway epithelial cells[15,32,33]**.** Both IL-4 and IL-13 activate signaling pathways that regulate cell growth and proliferation as well as the expression of responsive genes[34]. The direct stimulation of resident airway cells with inflammatory cytokines *in vitro* stimulates cell growth and proliferation and the secretion of matrix proteins[13,35,36]. *In our previous animal studies, , we evaluated the effects of chronic mechanical strain on the physiologic responses of ASM tissues to inflammatory mediators in vitro, and on airway inflammation and remodeling in vivo using murine models of asthma.*

Molecular mechanisms for the direct effects of inflammatory cytokines on ASM cells.

IL-4 and IL-13 both signal through a heterodimeric receptor complex that consists of an IL-4Rα chain and an IL-13Rα chain (Fig.1). In response to stimulation, the IL-4/IL-13 receptor subunits associate with Janus kinases (Jak) that phosphorylate the IL4Rα and IL13 receptor side chains, activating two distinct signaling proteins that bind to the phosphorylated IL-4Rα receptor side chain: insulin receptor-2 (IRS-2) and "signal transducer and activator of transcription 6"(STAT6)[34]. IRS2 activates the PI3 kinase-dependent protein kinase Akt, a regulator of cell growth and hypertrophy, as well as ERK1/2 MAP kinase and other MAP kinases. phosphorylation by Jak, activated STAT6 dimerizes and migrates to the nucleus where it initiates the transcription of IL-4/IL-13 responsive genes. Stimulation of ASM cells or tissues by IL-4/IL-13 *in vitro* results in the phosphorylation of Akt, ERK1/2 and STAT6, the synthesis and secretion of eotaxin and IL-5, and suppresses the expression of smooth muscle phenotype-specific proteins[7,8,15,26,37-40] (Figs.1,4-6). Our previous studies and preliminary data have shown that chronic mechanical strain suppresses the activation of Akt and ERK, and that strain inhibits eotaxin secretion and IL-13-mediated gene transcription (Figs. 5,6,9).



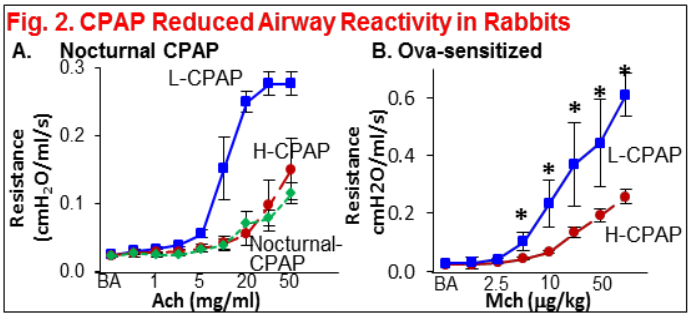
Chronic mechanical strain suppresses airway responsiveness and ASM contractility.

Mechanical strain of the lungs during breathing has an important role in reducing airway responsiveness[41]. Deep inspirations and tidal breathing decrease airway responsiveness acutely in healthy adults and animals and their absence increases airway responsiveness[42-47]. Similar effects of mechanical strain have been observed in isolated airway segments and trachealis smooth muscle strips studied *in vitro*, demonstrating that the effects of mechanical strain on airway reactivity reflect its direct effects on ASM[45,48-52]. Although the effects of deep inspiration on airway reactivity are short-lived, these observations suggest that mechanical strain could be a useful approach for suppressing airway hyperresponsiveness[46,47,53,54].

We initially explored the effects of imposing mechanical strain on the airways for prolonged time periods to determine whether it could cause a longer term reduction of airway responsiveness. Chronic mechanical strain for 1-2 days applied to bronchial segments or tracheal muscle strips *in vitro* resulted in a decrease in their contractile responsiveness to ACh as assessed subsequently under unstrained conditions[4]. These results suggest that chronic strain can induce persistent alterations in the functional properties of the ASM tissues.

We subsequently tested the effects of chronic strain *in vivo* by applying CPAP to rabbits and ferrets for periods of 4 days to 2 weeks and found that CPAP suppressed airway reactivity to ACh *in* vivo[1,2]. Lobes,

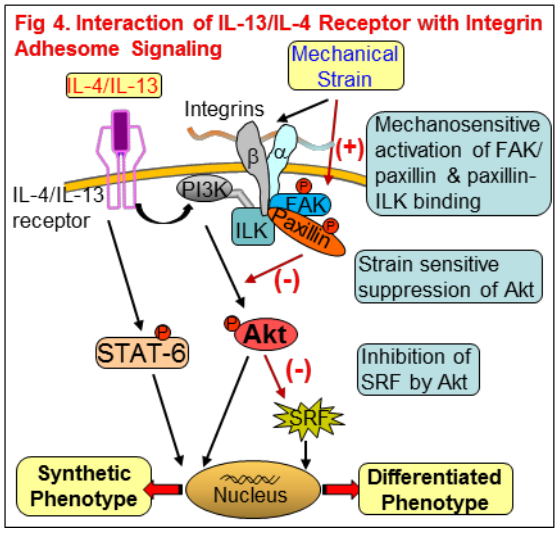
airway segments and tracheal smooth muscle tissues excised from the lungs of these CPAP treated animals also exhibited reduced contractility *in vitro*. The effects of chronic mechanical strain on airway reactivity were persistent and could be detected for at least 24 hours after CPAP[5]. In addition, the continuous use of CPAP was not necessary for it to be effective: limiting CPAP to nocturnal periods for 4 days in rabbits was similarly effective in reducing airway responsiveness (Fig. 2A). We also tested the efficacy of chronic mechanical strain at reducing airway hyperresponsiveness in the presence of allergic airway inflammation. In Ova-sensitized and challenged rabbits continuous CPAP administered for 4 days was effective at suppressing airway reactivity[5] (Fig 2B). We recently extended this study to human asthmatics: Adults with stable asthma and normal spirometry used nocturnal CPAP for 7 days[6]. CPAP resulted in a significant decrease in airway reactivity while a Sham-treated group of asthmatics had no significant change in airway reactivity.



Effects of chronic mechanical strain on ASM phenotype and its responses to inflammatory mediators.

ASM cells in culture modulate their properties between a synthetic and differentiated phenotype[84-86]. Integrin proteins are widely recognized to be important regulators of cell phenotype, and can mediate transitions in the phenotypic status of cells in response to changes in the composition or stiffness of the extracellular matrix. Changes in ECM have been shown to modulate the secretion of eotaxin by ASM cells in culture in response to stimulation with IL-13[87].

Our data indicate that mechanical strain imposed on ASM tissues is a potent stimulus for regulating the phenotype and synthetic status ASM tissues[14,15]. When we imposed chronic mechanical strain on ASM tissues *in vitro*, the activation of pathways mediated by PI3-kinase dependent Akt were suppressed while the expression of smooth muscle phenotype-specific proteins was potentiated[15] (Fig. 4). We confirmed this effect on the airways of mice subjected to chronic mechanical strain *in vivo*: the activation of Akt was significantly depressed in animals subjected to prolonged high mechanical strain using PEEP as compared with control-treated animals (Fig. 13C)[3]. Because Akt is a critical mediator of pathways activated by inflammatory stimuli such as IL-13, **we hypothesized that mechanical loads would suppress the inflammatory responses of ASM to local mediators and might thereby mitigate airway inflammation (Fig.4).**



We performed studies on ASM tissues *in vitro* to evaluate the effects of mechanical strain on the responses of ASM tissues to stimulation with the inflammatory cytokine IL-13[14,15]. The administration of IL-13 to ASM tissues *in vitro* stimulated the synthesis and expression of the chemokine, eotaxin, and suppressed the expression of smooth muscle phenotype-specific proteins (Figs. 5,6). Subjecting ASM tissues to mechanical strain suppressed IL-13 stimulated eotaxin synthesis and secretion and potentiated the expression of smooth muscle specific proteins, promoting the differentiated phenotype. ***Thus, while IL-13 promotes chemokine synthesis and the synthetic phenotype of ASM, mechanical strain opposes this effect, suppressing IL-13 stimulated eotaxin synthesis and promoting the differentiated phenotype.*** (Fig. 4)

Our studies have also established the role of integrin signaling in the regulation of ASM differentiation and mechano-sensitive signaling to the nucleus. We found that integrin-linked kinase (ILK), a multi-domain β integrin-binding protein kinase that regulates Akt, is a critical upstream regulator of signaling pathways that regulate the synthetic and differentiation functions of ASM tissues[88]. ILK forms a stable heterotrimeric complex with PINCH, an adaptor protein, and α-parvin, which binds to actin filaments[89]. ILK also binds directly to β integrin proteins and to the mechano-sensitive adhesome protein paxillin, and paxillin binding is necessary for the localization of ILK to integrin adhesomes[90]. ILK regulates the PI3 kinase-dependent

activation of Akt[91]. We found that Akt activation inhibits the transcriptional regulator, serum response factor (srf) in ASM, thereby suppressing the expression of smooth muscle phenotypic proteins[88]. Mechanical loads inhibit the ILK- dependent activation of Akt, thus promoting the differentiated phenotype[15](Fig. 4). Our preliminary data also show that the ILK complex also plays a critical role in regulating the mechanosensitivity of eotaxin synthesis in response to the stimulation of ASM with IL-13[14] (Fig. 10).

Summary.

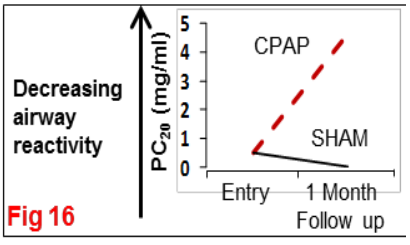
We have shown that chronic mechanical strain suppresses allergen-induced hyper-responsiveness *in vivo* in animal models of asthma and the effectiveness of nocturnal CPAP at reducing airway hyper-reactivity in adults with mild-moderate asthma. Our studies of ASM tissues indicate that mechanical strain is sensed and transduced by integrin adhesomes to signaling pathways that regulate the synthetic and differentiation status of ASM. Our proposed studies will use *in vitro* models to assess the effects of strain on the inflammatory responses of ASM tissues (*SA #1*), will evaluate the suppression of airway inflammation by chronic strain in murine models of asthma (*SA #2*), and will t**est the efficacy of mechanical strain as therapy to suppress airway inflammation and hyper-reactivity in children with severe asthma (*SA #3*).**

SPECIFIC AIM #3: DETERMINE WHETHER CPAP SUPPRESSES AIRWAY HYPER-REACTIVITY AND AIRWAY INFLAMMATION IN CHILDREN WITH SEVERE ASTHMA.

RATIONALE. Whereas some children with asthma have intermittent symptoms that are improved with short-acting bronchodilators, many have persistent symptoms requiring daily treatment with inhaled corticosteroids. Children with severe asthma often have ongoing symptoms, airway inflammation and hyper-reactivity despite treatment with high doses of inhaled corticosteroids. They are at high risk for asthma exacerbation requiring systemic corticosteroids, emergency room visits and hospitalization. Children with severe asthma are often highly atopic with increased peripheral blood eosinophilia, aeroallergen sensitivity, elevated serum IgE concentrations, elevated eNO, and eosinophilia in induced sputum and BALF[120-125]. A novel non-pharmacologic treatment that suppresses airway hyper-reactivity and inflammation in children with severe asthma could provide a significant advance in the therapy for these difficult to treat patients. **We hypothesize that chronic mechanical strain will suppress airway reactivity and inflammation in children with severe asthma.** In *SA #3* we will determine whether the use of nocturnal CPAP for 1 month in children with severe asthma suppresses airway reactivity as assessed by bronchial challenge, and airway inflammation by induced sputum.

PRELIMINARY DATA. Our recent study of adults with mild-moderate clinically stable asthma demonstrated that treatment with nocturnal CPAP for 1 week decreased airway reactivity (increase in PC_{20}) compared to a Sham-treated group[6].These results provided the pilot data for our current NIH phase II multi-center clinical trial designed to assess the effectiveness of using nocturnal CPAP to suppress airway reactivity in subjects with mild-moderate asthma. However, this trial is restricted primarily to adult patients who may not be steroid- dependent and are less likely to have persistent airway inflammation or exhibit airway remodeling. Therefore we plan to evaluate children with severe asthma, as CPAP therapy may be particularly beneficial to this group. Our study will also assess airway inflammation which is not included in previous or ongoing clinical trials.

Fig.16. Nocturnal CPAP suppressed airway hyper-reactivity in an 8 yr old girl.
We assessed the effect of 1 month treatment with nocturnal CPAP (8 cmH_2O) or Sham (0 cm H_2O) in two 8 year old girls who had PC_{20} <1 mg/ml, which is indicative of airway hyper-reactivity. The child treated with CPAP showed a marked decrease in airway reactivity (increased PC20), while the ham-treated child maintained airway. This demonstrates our ability to recruit and evaluate young children, and is consistent with our hypothesis.



2.0 Inclusion/Exclusion Criteria

SUBJECT RECRUITMENT

Inclusion Criteria:

1. Children 8–17 yrs olds with severe asthma (N=120) will be recruited from the Pediatric High Risk Asthma Clinic and Pulmonary Clinics at Riley Hospital for Children at Indiana University Health.
2. Severe asthma will be defined by the need for medication therapies following steps 4-6 according to the National Institutes of Health's Asthma Care Quick Reference, September 2012 or high dose of inhaled corticosteroids.
3. Children on a stable regimen of asthma medications for at least 8 wks prior to enrollment without systemic corticosteroids for ≥4 wks will be eligible.

Exclusion Criteria:

1. Obese (>95% predicted BMI).
2. Congenital heart disease or chronic lung disease.
3. History of pneumothorax.
4. Inability to perform pulmonary function testing.
5. Oxygen saturation <93%.
6. FEV_1 <70% predicted.
7. PC_{20} ≥16 mg/ml of methacholine.

3.0 Enrollment/Randomization

TREATMENT:

1. Nocturnal CPAP (8-12 cmH_2O) or Sham (0 cmH_2O).
2. Subjects will be randomized to use CPAP or Sham CPAP at night for a total of 28 days (+/- 3 days) with a minimum of 4 hrs/night for at least 5 days/wk. If patient is having asthma symptoms at time of final visit, treatment may be extended up to 14 additional days. If patient receives oral steroids at any time during treatment with nocturnal CPAP, the final visit will be extended to at least 21 days (3 weeks) after the last dose of oral steroid was given. Adherence to the use of CPAP will be checked by having the patient mail the SD data card from their machine to the Investigator on day 7 and day 14 of CPAP usage and by downloading the data at the time of the final visit. Adherence and tolerance of CPAP will be evaluated by periodic phone calls and documented daily calendar diary with hours of use.

4.0 Study Procedures:

1. **Spirometry**: Spirometry will be performed after withholding bronchodilators. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) will be measured using ATS guidelines.
2. **Airway Reactivity assessed by MCh Bronchial Challenge:** Testing will use the 5 breath dosimeter protocol recommended by ATS[126]. Following inhalation of saline, MCh will be inhaled in quadrupling concentrations starting with 0.0625 mg/ml and continuing until the MCh concentration required for FEV_1 to decrease by 20% from baseline (PC_{20}) is achieved or a maximum MCh concentration of 16 mg/ml is inhaled.
3. **Airway Inflammation assessed by Exhaled Nitric Oxide (eNO):** eNO will be measured at a constant expiratory flow of 50 ml/s with a chemiluminescence analyzer (NIOX; Aerocrine) using ATS guidelines[127].
4. **Airway Inflammation assessed by Induced Sputum**. Sputum induction will be performed after MCh challenge and treatment with albuterol to return FEV_1 to ≥ 70% predicted. Sputum will be induced by inhaling increasing concentrations (3 to 7%) of saline at 5 min intervals. Oscillating positive expiratory pressure (PEP) therapy may be used if needed to assist with sputum expectoration in conjunction with

saline treatments for up to 20 min or until an adequate sample is produced. Spirometry is performed after each expectoration to check that the FEV_1 has not decreased by >20%, in which case the child would be given bronchodilator and no more hypertonic saline. Sputum will be processed for differential cell counts and supernatants stored at -80ºC for subsequent analysis of eotaxin and eosinophil cationic protein[128].

5. **Asthma Symptom control:** Asthma control will be assessed by using the Asthma Control Test[129].
6. **Berlin score:** Questionnaire will be used to determine high or low risk status for obstructive sleep apnea[130].

5.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse Events
An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the subject internal data collection forms. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study medication, or if unrelated, the cause.

Serious Adverse Experiences (SAE)
An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is considered life threatening (i.e., in the view of the investigator the adverse experience places the subject at immediate risk of death from the reaction, as it occurred; it does not include a reaction that, had it occurred in a more severe form, might have caused death)
- Requires hospital admission or prolongation of an existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event (i.e., when based upon appropriate medical judgment, the adverse experience may jeopardize the subject and may require medical or surgical intervention to prevent one of the above listed outcomes)

Data Safety Monitoring Plan

Data Safety Monitor Gregory Montgomery, MD will monitor this study on an annual basis. Data to be monitored includes: data quality, subject recruitment, accrual and retention and adverse events. The results of related studies that impact subject safety and privacy will also be assessed.

Data monitoring will also include the interpretation of the data and confirming that the data is kept in a secure database. The co-investigators of the study will be doing an interim analysis and interpretation of the data on an annual basis.

Adverse events will be dealt with on a case by case basis and all institutions (IRB/ NIH) involved in the study will be notified by either a formal letter or memo.

The following information will be reported to the IRB on an annual basis with the continuing review:

1. Dates of monitoring
2. Summary of cumulative adverse events

3. Assessment of external factors that impact the safety of subjects
4. Summary of subject privacy and research data confidentiality
5. Any changes in risk-benefit ratio

6.0 Study Withdrawal/Discontinuation

If the parent/guardian chooses to withdraw the subject from the study, then the parent/guardian will inform a member of the research team. At that time, no further testing will be obtained. Samples already obtained from the subject prior to withdrawal will still be included in analysis.

Management of Increased Asthma Symptoms: If a subject's asthma symptoms worsen during the course of the treatment period, they will be directed to their pediatric pulmonologist or the High Risk Asthma clinic for management. Any changes in therapy, including controller medications or courses of systemic steroids will be recorded. As the enrolled subjects have severe asthma, one course of systemic steroids in 4 weeks would not be considered unusual and would not trigger withdrawal from the study. However, an inpatient hospitalization or two courses of systemic steroids in the 4 week treatment period will trigger withdrawal from the study

7.0 Statistical Considerations

ANALYSIS:

Primary Outcome: *The change in airway reactivity (logPC_{20}) measured prior to and after 4 wks of either CPAP or SHAM treatment*. We will compare the change in PC_{20} between two groups using an analysis of covariance (ANCOVA) model, adjusting for baseline PC_{20}. We anticipate that PC_{20} will increase for the CPAP treated group, but not change for the SHAM treated subjects.

Secondary Outcomes: *Airway Inflammation assessed as the change in the percentage eosinophils in the induced sputum measured prior to and after 4 wks CPAP or SHAM treatment*. We will compare the change between two groups using an ANCOVA model adjusting for baseline percentage eosinophils. We anticipate that eosinophilia will decrease for the CPAP treated group, but not in the SHAM treated subjects.

Justification of Sample Size: Change in Airway Responsiveness: We calculated the sample size based on the difference of logPC_{20} changes between groups. Our previous study showed a difference of change in logPC_{20} between CPAP and SHAM groups as 0.41 with a standard deviation of 0.46. With a 1 month intervention period in our study (compared to 1 week in previous study), we expect a larger effect size. Therefore, we conservatively assume a 0.41 difference and a 0.46 standard deviation. With 30 patients in each group, we expect 27 of them will complete the study. We will have more than 85% power to detect such a difference with a 5% Type I error rate using a two-sample t-test. ANCOVA model will be more powerful than the t-test. Change in Airway Inflammation: We calculated the smallest changes in airway inflammation that can be detected with the sample size generated above. With 27 patients completing the study, we have 80% power to detect an effect size as small as 78% (difference of changes is 78% of the standard deviation) using a t-test.

ANTICIPATED RESULTS.

Based upon our previous experience that CPAP suppresses airway reactivity in adults with mild-moderate asthma, our preliminary data in children and our animal models, we anticipate that nocturnal CPAP will suppress airway reactivity in children with severe asthma. These findings would have important clinical implications and justify a multi-center trial for much longer periods of CPAP treatment to evaluate its potential in decreasing asthma symptoms and medication usage in children with severe asthma.

We also expect CPAP to suppress airway inflammation. Although our primary measurement of airway inflammation is induced sputum eosinophilia, we will evaluate additional inflammatory markers. It is possible that our sample size will be too small to obtain statistical significance for these markers. However, if we find that CPAP shows a tendency to decrease airway inflammation, our results could be used to power a larger clinical trial. It is possible that CPAP will increase airway inflammation, but this seems unlikely as

CPAP in adults with obstructive sleep apnea has been found to decrease systemic inflammatory markers[131,132].

8.0 Privacy/Confidentiality Issues

Each study subject will be assigned a subject number. All samples will be identified only by subject number when submitted to the research laboratory for analysis. The enrollment list containing both the subject number and identifiable information will be kept on a password protected server.

Security and confidentiality
To comply with HIPAA guidelines, processes and procedures have been documented and implemented to ensure the security and protection of the study data within the computer operations center, the server, and the database. IU uses a three level security model to secure and protect data collected and stored within REDCap. This model is as follows: 1) an individual must register an account within the Indiana CTSI Hub and either be assigned a username/password if external to IU or use their IU login credentials and authenticate via IU's Central Authentication Service (CAS); 2) once a Hub account has been established, an individual must submit a request for a REDCap account; the request must be approved and then a REDCap account is established; and, 3) an individual must have their REDCap account be granted appropriate access and privileges to the specific project database by the project PI or delegate. In addition, since REDCap is web-based, IU utilizes SSL (https) encryption for secure connectivity.

9.0 Follow-up and Record Retention

Enrollment:
Enrolling sufficient subjects is always challenging; however we were successful at this in our previous CPAP study[6]. Riley Hospital for Children is the primary referral center for all of Indiana and our pediatric pulmonary group manages most of the severe pediatric asthma, so we do not expect problems recruiting subjects for this study. In multiple previous studies we recruited more than 100 subjects[133-137].

CPAP Measures:
In our preliminary studies, the use of CPAP for an average of 4 hrs/night was effective. Our CPAP devices can monitor the number of hours that CPAP is actually being used by measuring respiratory pressure fluctuations. Daily usage statistics can be stored on an SD card that is downloaded after 1 week, 2 weeks and 4 weeks of treatment. To reinforce adherence, we insure mask comfort at the initial visit, use humidification to prevent nasal drying and reinforce adherence with regular telephone calls and documented daily calendar diary with of hours of use.

Data Capture System
The Research Electronic Data CAPture (REDCap) database system will be used to collect data for this study. The REDCap software toolset provides a secure, web-based environment that is flexible enough to be used for a variety of types of research, provides an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. The system offers easy data manipulation with logged auditing, functionality for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages such as: SPSS, SAS, Stata, R/S-Plus. REDCap has been used within Indiana University and affiliates since April 2009 and it currently supports 177 projects and over 380 users within IU, Purdue, Notre Dame, IU Health, VA, Rehabilitation Hospital of Indiana, and Wishard Health. The input data will be stored in secured server for storage and analysis.

Backup
At all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access. At critical junctures of the protocol (e.g.,

production of interim reports and final reports), a permanent archive of the database will be made. Archived versions of the database will be saved for at least three years after the end of the study.

Availability and Retention of Investigational Records:
The Investigator must make study data accessible to authorized representatives of the NIH and IRB/IEC upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the internal data collection forms was derived. All study documents (subject files, signed informed consent forms, copies of internal data collection forms, Study File Notebook, etc.) must be kept secured for a period of three years.

10.0 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).
To maintain confidentiality, all lung function data, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a subject's name to a subject identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

Protocol Amendments
Any amendment to the protocol will be written by the Study Principal Investigators and submitted to the IRB for their approval.

Publications
The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

LITERATURE CITED

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