

2006

8-isoprostane levels in exhaled breath condensate of pregnant women compared to non-pregnant women; is there a baseline difference?

Rosemary Szollas
University of South Florida

Follow this and additional works at: <http://scholarcommons.usf.edu/etd>

 Part of the [American Studies Commons](#)

Scholar Commons Citation

Szollas, Rosemary, "8-isoprostane levels in exhaled breath condensate of pregnant women compared to non-pregnant women; is there a baseline difference?" (2006). *Graduate Theses and Dissertations*.
<http://scholarcommons.usf.edu/etd/2719>

This Thesis is brought to you for free and open access by the Graduate School at Scholar Commons. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Scholar Commons. For more information, please contact scholarcommons@usf.edu.

8-Isoprostane Levels in Exhaled Breath Condensate of Pregnant Women Compared to
Non-Pregnant Women; Is There a Baseline Difference?

by

Rosemary Szollas, M.D.

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in Public Health
Department of Occupational Medicine
College of Public Health
University of South Florida

Major Professor: Stuart M. Brooks, M.D.
Robert Haight, M.D.
Thomas Truncale, M.D.

Date of Approval:
April 5, 2006

Keywords: inflammatory markers, asthma, pregnancy, preeclampsia, oxidative stress

© Copyright 2006, Rosemary Szollas, M.D.

Dedication

I would like to thank Dr. Stuart Brooks for his guidance through the residency program and through this research project. I would also like to thank Karen Olson for all of her help and support. Throughout my time in the residency with her I have made a wonderful friend. I would like to thank Robert Haight for his help with the statistical calculations, and advice on paper thickness. Last but not least I would like to thank Melinda Tyler for her kindness and patience. It has been a pleasure for me to work with these individuals throughout my time in the occupational medicine residency program. I could not have met my academic and professional goals without their help.

Table of Contents

List of Tables	iii
List of Figures	iv
Abstract	v
Introduction	1
Isoprostanes	1
Pregnancy	3
Exhaled Breath Condensate	6
Research Question	8
Hypothesis	8
Goals and Objectives	9
Utility and Significance	9
Materials and Methods	10
Study Design	10
Facilities and Equipment	10
Participant Recruitment	11
Study Subjects and Restrictions	12
Study Questionnaire and Eligibility	12
Physical Examination	13
Exhaled Breath Condensate Collection and Storage	13

Data Collection and 8-isoprostane Analysis	13
Acquisition of Spirometry	17
Results	20
Study Population Characteristics	20
8-Isoprostane Measurements	21
Biostatistical Data for 8-Isoprostane Measurements	26
Conclusions	29
References	31
Appendices	33
Appendix A: Questionnaire Form	35
Appendix B: Adult University of South Florida Informed Consent Document	37

List of Tables

Table 1. Effects of isoprostanes (8-iso-prostaglandin (PG) _{2_{ex}}) in asthma	5
Table 2. Advantages and limitations of collection and analyzing exhaled breath condensates	8
Table 3. Demographic characteristics of subjects both pregnant and non-pregnant	21
Table 4. 8-isoprostane concentrations in pregnant and non-pregnant females	22
Table 5. Standard concentrations (known) and actual absorbance concentrations Detected	24
Table 6. Standard concentrations (known) and actual absorbance concentrations detected using standard curve without known concentrations 500pg/ml, 250 pg/ml and 125 pg/ml	25
Table 7. Spirometric data for all subjects tested including pregnant and non-pregnant females	25
Table 8. Statistical analysis of mean 8-Isoprostane values of 20 subjects tested	26
Table 9. T-tests used to analyze difference between means of the two groups	27
Table 10. Variables involved in power calculations	27

List of Figures

Figure 1.	Lipid Peroxidation Pathways	2
Figure 2.	Schematic representation of an EBC collection apparatus	6
Figure 3.	Cayman Chemical 8-isoprostane kit precision curve	16
Figure 4.	8-isoprostane Concentrations Taken from Samples in Pregnant Females	23
Figure 5.	8-Isoprostane Concentrations in Samples Taken from Non-pregnant	24

8-Isoprostane Levels in Exhaled Breath Condensate of Pregnant Women Compared to Non-Pregnant Women; Is There a Baseline Difference?

Rosemary Szollas

ABSTRACT

This study investigated whether or not there was an overall difference in the pulmonary oxidative marker, 8-isoprostane (PGF₂- α), found in exhaled breath condensate, during pregnancy versus the non-pregnant state. The utility of this information was important secondary to the effect of maternal asthma on pregnancy and outcome, as it has been demonstrated in past studies that overall pregnant females with asthma have been shown to have an increased risk of exacerbation requiring medical intervention.

The primary goal of this study sought to determine if there is a difference in PGF₂ α levels in the exhaled breath condensate of pregnant versus non-pregnant females. In order to achieve this goal a cross-sectional study was performed consisting of two groups and were compared to one another.

A group of 16 healthy, non-pregnant females aged 18-35 years old was compared to a group of 6 healthy, pregnant females in their third trimester of pregnancy. Both groups exhaled breath condensate was collected and 8-isoprostane levels determined and compared to each other. Both groups compared did not report a history of environmental allergies, asthma, and smoking. The non-pregnant group showed a mean 8-isoprostane

level of 11.513pg/ml (C.I. 8.7633- 14.263). The pregnant group showed a mean 8-isoprostane level of 17.34 pg/ml (C.I. -4.209-38.889).

Although a crude observable difference between the means of the two groups was determined, this pilot study did not show a statistically significant difference between the means of the pregnant versus non-pregnant group when they were statistically compared. This finding is primarily due to the small sample size of both groups. A power calculation determined that each group would require 25 participants in order to establish a statistically significant difference in the 8-isoprostane levels in exhaled breath condensate.

The implication is that a larger scale study is needed in order to conclusively determine if there is a statistically significant difference between the exhaled breath condensate 8-isoprostane levels in pregnant versus non-pregnant females.

Introduction

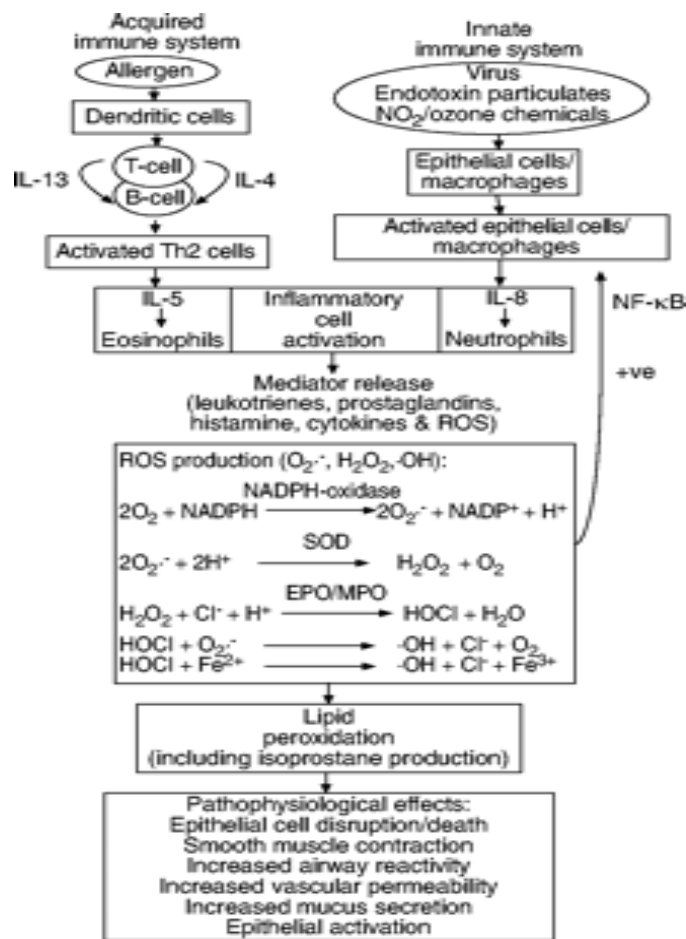
Isoprostanes

Isoprostanes are derived from membrane lipids via peroxidation with compounds such as free radicals and reactive oxygen species¹. This non-enzymatic production of isoprostanes catalyzed by free radicals was first described by Morrow and colleagues in 1990. It is distinctly different from the classical enzymatically produced isoprostanes via cyclooxygenase (COX) enzymatic pathways (Figure 1). Since their discovery, the usefulness of these compounds as bio-markers in various disease states has been studied. In particular, 8-isoprostane or PGF2 α , as a marker of oxidative stress in patients with asthma has been studied by Montushi and colleagues in 1999.² Measurement of PGF2 α has been done by collection of exhaled breath condensates (EBC). This method of collection and analyzing oxidative products in the lung allows for a simple non-invasive mean of examining the human lower respiratory tracts³.

Elevated levels of 8-isoprostanes have shown to correlate with level of disease severity in various disease states. Just why this occurs is not fully understood. 8-isoprostane produces a number of biological responses, such as smooth muscle contraction, vasoconstriction, and platelet activation¹. These responses are thought to be a result of an isoprostane receptor binding and subsequent signaling pathway involving phospholipase and kinase enzymatic activity resulting in Ca ion channel activation¹. Various other markers have been found in the breath and typically vary by

disease state. In particular, PGF2 α in the exhaled breath condensate, plasma, and serum has been found to be elevated in individuals with asthma. Two separate studies have shown PGF2 α to be elevated in a group of asthmatic children compared to a group of non-pregnant children.^{4,5}

Figure 1. Mechanisms leading to lipid peroxidation in asthma.⁶



Other studies have shown 8-isoprostane levels to be elevated in severe respiratory failure in infants.⁷ Therefore various disease states exist where exhaled breath condensates have been showed to be elevated.

Pregnancy

Not surprisingly, given its biological responses, 8-isoprostane has also been studied in its relationship to a healthy state and various disease states during pregnancy. 8-isoprostane, as previously discussed, produces smooth muscle contraction, vasoconstriction, and platelet activation¹. Preeclampsia is defined as a pregnancy-dependent syndrome diagnosed on the basis of increased blood pressure and proteinuria. Endothelial dysfunction is hallmark of preeclampsia and is central in explaining the main features of preeclampsia: hypertension, edema, proteinuria, and activated homeostasis⁸. For example, plasma concentrations of free 8-isoprostane has been found to be significantly greater in a study analyzing levels before delivery in patients with preeclampsia than in control subjects.⁸ It is thought that oxidative lipid derivatives are uteroplacental compounds that may cause dysfunctional maternal endothelium.⁸ Recently, a study reported an increased content of lipid peroxides in preeclamptic decidual placental tissue in preeclamptic patients at delivery compared with controls⁸. In addition, another study by Moretti and colleagues demonstrated increased markers of oxidative stress in preeclamptic women versus uncomplicated pregnancy and non-pregnant controls by measuring various volatile organic compounds in the exhaled breath condensate of these women⁹.

Although levels of 8-isoprostanes have been shown to be elevated in the exhaled breath condensate of individuals with asthma, no study to date has measured the levels of 8-isoprostane in the exhaled breath condensate of pregnant females. Asthmatic females who become pregnant have been demonstrated to be more at risk of low birth weight neonates, pre-term delivery, and complications such as preeclampsia, especially in the absence of actively managed asthma treated with inhaled corticosteroids.¹⁰ These mechanisms responsible for changes in asthma with pregnancy, or alterations in pregnancy outcomes due to asthma have not been thoroughly explored.

Asthma is a chronic inflammatory disorder of the airways involving variable airflow obstruction and increased airway responsiveness to a variety of stimuli. The airway mucosal inflammatory response in asthma is characterized by increased vascular permeability with edema of airway walls, mucus hypersecretion with small airway plugging and infiltration with inflammatory cells, typically eosinophils.⁶ The role of oxidant and antioxidant in asthmatic airway inflammation is ill-defined. Isoprostanes are one of the groups of lipid peroxidation products that contribute to the pathophysiological changes seen in asthma.⁶ 8-isoprostane, in particular, has been demonstrated to be elevated in certain individuals with various disease states, including asthma. These responses may be partially the result of elevated levels of 8-isoprostane.² As previously stated, 8-isoprostane is a known smooth muscle constrictor, as well as vascular constrictor, it has also been demonstrated to cause airway hyperresponsiveness and airway obstruction, as well as plasma exudation.¹¹ effects of 8-isoprostane in asthma can be listed in Table 1 below.

Table 1. Effects of isoprostanes (8-iso-prostaglandin (PG)F_{2α}) in asthma⁶

<i>In vitro</i> /animal models:	Human studies:
↑Smooth muscle constriction 22, 23	↑8-iso-PGF _{2α} in asthma (plasma 28; breath condensate 29)
↑Airway hyperreponsiveness 24	
↑Airway obstruction 25	↑8-iso-PGF _{2α} with asthma severity (plasma 28; breath condensate 29)
↑Plasma exudation 25	
↑Vascular constriction 26, 27	↑8-iso-PGF _{2α} with allergen challenge (urine and bronchoalveolar lavage 30)

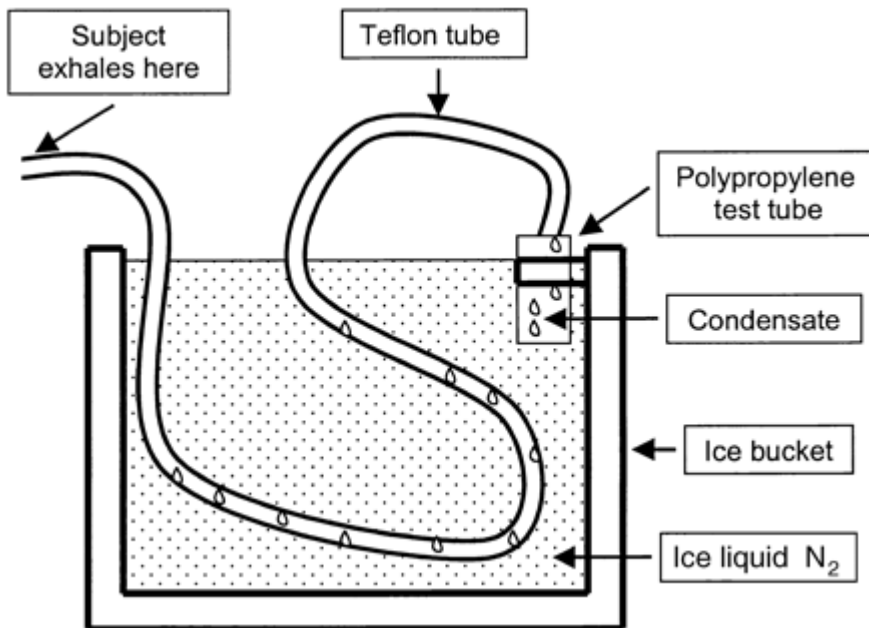
Given that mechanisms responsible for changes in asthma with pregnancy, or alterations in pregnancy outcomes due to asthma have not been thoroughly explored, it seems prudent that more research in this area be done as to why this occurs. It is well known that pregnancy alone produces pulmonary changes including increased tidal volume leading to increased minute ventilation leading to an increased PAO₂ and decreased PACO₂ and PaCO₂. However, whether or not an increase in oxidative stress in the lungs of the pregnant female occurs, also remains unknown. The effect of pregnancy on asthma consensus has remained the same for many years that one-third worsens, one-third remain the same and one-third improve. Regardless, those that worsen are at an increased risk of delivering low birth weight neonates, pre-term delivery and complications including preeclampsia. In addition to the increased risk of poor outcomes, pregnant females with asthma have an increased risk of an exacerbation requiring medical attention which may also increase the risk of a poor outcome.¹⁰

Since reducing asthmatic inflammation and preventing exacerbations contributes to improved outcomes in outcomes for mother and fetus it becomes apparent that studies in this area should be focused on understanding changes in airway inflammation in pregnant females with asthma, which may lead to more effective treatment and target management of pregnant asthmatic females.¹⁰

Exhaled Breath Condensate

Exhaled breath condensate collection (EBC) is a noninvasive tool to collect breath in order to analyze its contents. The method involves an individual breathing into a cooled chamber for approximately 15 minutes. The condensed breath is then analyzed for various substances.¹²

Figure 2. Schematic representation of a EBC collection apparatus¹³



As previously discussed 8-isoprostane measurements in the exhaled breath condensate of pregnant females have not been performed to date. This method of

collection and analyzing oxidative products in the lung allows for a simple non-invasive mean of examining the human lower respiratory tracts³.

The appeal of EBC lies in its ability to non-invasively collect a wide range of nonvolatile molecules from the respiratory tract.¹² However despite its utility with respect to the noninvasive nature EBC portrays, there are drawbacks to analyzing breath in this manner. One of the major problems lies in that of dilution which probably affects the concentration of most biomarkers analyzed with in EBC. In addition, ambient air pollutants may influence biomarkers. Naturally EBC contains mostly water vapor, approximately >99.99%.¹² Previous studies done in attempts to produce repeatability in measuring substances were unsuccessful and showed to be variable between subjects and within repeated samples of the same subject.¹²

The collection chamber of the device and container that collects, cools, and stores the breath is composed either of glass, polystyrene or polypropylene. The composition the EBC may be altered by the adhesive properties of the material from which the container is made. This can be minimized by using a polypropylene container, as less adsorption occurs as compared to polystyrene.¹³

In addition, collection technique to minimize saliva contamination is one of the most important concerns regarding the collection procedure. Certain substances such as nitrite and hydrogen peroxide present in high quantities can contaminate sample data interpretation. This can be minimized by keeping the mouth dry during collection.¹³

Table 2 given below illustrates the various advantages and limitations of collection and analysis of exhaled breath condensate collection.

Table 2. Advantages and limitations of collections and analysis of exhaled breath condensate¹³

Advantages

1. Simple, point-of-care intervention
2. Inclusive rather than intrusive (e.g., healthy children, mechanically ventilated neonates)
3. Domiciliary
4. Longitudinal sampling
5. Nonvolatile compounds associated with pulmonary pathophysiology
6. Amplified DNA and RNA from prokaryotic and eukaryotic cells
7. Pharmacokinetics/pharmacodynamics of drugs
8. Solute clearance

Limitations

1. Lack of standard breath-sampling method
2. Not anatomic site specific
3. Lack of evidence for the origin of the aerosol particles (bronchi versus terminal airways)
4. Concentration artifact (due to evaporation of samples)
5. Feasibility and utility of biomarkers unrelated to oxidative stress not tested
6. Little information on biomarkers of interstitial lung disease

Research Question

Is there a baseline difference between 8-isoprostane levels in the exhaled breath condensate of pregnant women versus those levels in non-pregnant females?

Hypothesis

The null hypothesis states that there was no difference in the exhaled breath condensate oxidative marker PGF2 α in pregnant females in their third trimester of pregnancy versus non-pregnant females. The alternative hypothesis states that there was a difference between the exhaled breath condensate oxidative marker PGF2 α in pregnant females in their third trimester of pregnancy versus non-pregnant females. The utility of the study may prove to provide a better understanding of oxidative stress in the pregnant

state and may prove to provide a baseline measurement of PGF2 α levels in the monitoring of airway, and other disease states in pregnancy.

Goals and Objectives

The primary objective of this study serves to determine if there is a difference in PGF2 α levels in the exhaled breath condensate of pregnant versus non-pregnant females. In addition, the following goals will be were 1) To assess the applicability of measuring baseline PGF2 α levels in pregnant females versus non-pregnant females as a measure of oxidative stress 2) To begin to provide an examination of the generalizability of measurements of exhaled breath markers to pregnant populations, which may serve as the basis for a larger and more in-depth study 3) To examine whether baseline 8-isoprostane levels are appropriately representative of healthy pregnant females.

Utility and Significance

This study was unique in that it examined exhaled breath condensate levels in the breath of pregnant females which has not been previously performed. The utility of the study proved to provide a better understanding of oxidative stress in the pregnant state and proved to provide a baseline measurement of PGF2 α levels in the monitoring of airway and other disease states in pregnancy.

Materials and Methods

Study Design

The study design was a cross-sectional design comparing two groups: a group of pregnant women ages 18 to 35 years in their third trimester and a group of non-pregnant women in the same age group. Exposure consisted of pregnancy and non-exposure consisted of non-pregnant. The outcome consisted of levels of PGF2 α oxidative marker in exhaled breath condensate. Both groups were screened to exclude those with a recent history of upper respiratory infections or smoking and any history of environmental allergies or lung disease. In addition any individual in the pregnant group was excluded for any complication of pregnancy, including but not limited to gestational diabetes, hyperemesis gravidarum, preeclampsia, hypertension, or any other complication of pregnancy.

Inclusion criteria include female sex, within the age range of 18-35 years, non-pregnant state (including individuals that have previously been pregnant), and pregnant state – specifically within the third trimester of pregnancy. Informed consent was obtained before any testing began.

Facilities and Equipment

The participants were seen at the College of Public Health at the University of South Florida in the Breath Laboratory (MHH Room 323). The records were maintained in a secured cabinet in this room. The key to access the laboratory was distributed by the University of South Florida only to authorized personnel (obtained through the College of Public Health).

Exhaled breath condensate samples were collected using Jaeger air condensing device (Jaeger, Wuerzburg, Germany 1999). The Jaeger instrument was located in the Breath Lab – MHH room 323. Samples were stored at approximately -70 degrees Celsius in The Breath Lab freezer. Once samples were ready to be analyzed they were removed from the freezer and de-thawed and analyzed using a commercially available enzyme linked immunoassay (EIA) kit, ordered from Caymen Chemical. The EIA kits were processed on a 96 well plate and analyzed using a spectrophotometer (μ Quant Universal Microplate spectrophotometer,2004), which is also available within the Breath Lab (MHH Room 323). All samples were used in their entirety.

Spirometry was performed using the KoKo spirometry software and devices also located within the Breath Lab – Room MHH 323. Mouthpieces needed for performing the testing were disposable and located with in the Breath Lab.

Participant Recruitment

Subjects were recruited to volunteer to participate in the study through a one time visit to The Breath Lab (Room MHH 323) over a range of months spanning from November 2005 to March 2006. Volunteers were recruited through primarily three mechanisms: 1) Advertisements in the form of a poster for non-pregnant and pregnant females were displayed in various areas throughout the University of South Florida Tampa campus and the Tampa General Hospital Genesis OBGYN clinic (Appendix A,B) 2) A mass email targeting female students and staff in the University of South Florida health science centers was sent out twice 3) Researchers personally manned a booth located in the Tampa General Hospital Genesis OBGYN clinic to recruit in person.

Researchers did not approach volunteers; rather potential subjects voluntarily approached the booth.

Study Subjects and Restrictions

Healthy pregnant and non-pregnant female subjects were recruited who denied any history of environmental allergies, asthma, or other lung diseases. Also, they must not have suffered from any recent upper or lower respiratory tract infections. Subjects must also have no significant smoking history as defined by less than one-half pack-year history of smoking, no smoking within the past 2 years, and no significant second-hand smoke exposure. Subjects must refrain from strenuous exercise, food, or drink for one hour prior to the test.

Pregnant subjects were restricted to the third trimester of pregnancy. Previous history of pregnancy or delivery did not exclude subjects. Gestational dates were taken in the medical history portion of the questionnaire form (Appendix A).

Study Questionnaire and Eligibility

In order to determine eligibility for potential healthy subjects, subjects were initially asked a series of questions. The questions were asked by telephone when the subject called in response to a recruitment flyer or email. If the potential subject denied any previous history of environmental allergy, denied any recent URI infections within the past two weeks, denied any chronic diseases and met the age criteria range the participant was scheduled for an appointment for testing with in The Breath Lab.

Once the subject was met in the Breath Lab the Health Questionnaire was given to each subject. Responses were reviewed by researcher and subject in a private cubicle.

Any medications and health problems were reviewed by the researcher. If subjects met inclusion criteria they were invited to then move on to the informed consent documentation in order to participate in the study.

Physical Examination

All subjects were visually inspected for any cyanosis and clubbing. Also, auscultation of the heart and lungs was performed prior to any breathing tests and at the conclusion of each visit. In auscultation, a stethoscope is used to listen to both heart sounds and breath sounds. Auscultation is routinely and correctly done while disrobed, however, for our purposes assessment was done over outer garments. Pregnant females were examined identically to their non-pregnant counterparts; of note, gestational age was not assessed on physical examination of pregnant females.

Exhaled Breath Condensate Collection and Storage

Data Collection and 8-isoprostane Analysis

Exhaled breath condensate samples were collected using Jaeger air condensing device (Jaeger, Wuerzburg, Germany 1999). The Jaeger instrument was located in the Breath Lab – MHH room 323. The Jaeger instrumentation was turned on at least 15 minutes prior to EBC collection in order for adequate cooling time of the collection chamber to approximately -30 degrees Celsius. All valves and collection tubing was cleaned with cavicide solution and thoroughly dried prior to use. A disposable mouth piece and nose clip was given to each subject for collection. Apparatus was assembled and subjects were asked to sit in a chair during the fifteen minute collection time. Individual were asked to breathe tidally through their mouths, after a nose clip was

applied, into the two-way non-re-breathable mouth piece connected to the collection chamber portion for a total of fifteen minutes. Subjects were asked to discard any excessive saliva produced into a tissue periodically. Reading material was provided to each subject and timer was set to 15 minutes with alarms at 10 and 5 minutes remaining. Subjects were notified that removal of their mouth from the tubing in order to cough, sneeze, etc was permitted. However, subjects were instructed that refraining from removing their mouth from the tubing was ideal in order to optimize sample collection. After fifteen minutes, collection chamber cups were unscrewed from the chamber and breath condensate was immediately transferred to cryo safe storage tubes in a split sample using an analytic pipette. On average two ml of condensate was obtained and 1 ml stored in each tube. Samples were then capped and placed in a -70 degree Celsius controlled freezer. Tubes were pre-labeled with subject identification numbers that identified samples with individual subjects. Key sample ID sheets were stored in a laboratory workbook in Room MHH 323. Samples were collected in this manner from November 2005 to March 2006.

8-isoprostane concentrations were measured in pregnant and non-pregnant subject samples using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical Company, 8-isoprostane EIA kit model # 516351, Ann Arbor, MI). The 8-isoprostane detection limit for the kit is 5 pg/ml. Normal *Plasma* 8-isoprostane levels for healthy subjects: 40-100 pg/ml (Cayman Chemical), Normal *Urine* 8-isoprostane levels for healthy subjects: 10-50 pg/ml (Cayman Chemical), exhaled breath condensate measurements were not provided by Cayman Chemical. *Exhaled Breath Condensate* 8-

isoprostane levels (two different studies) demonstrated 8-isoprostane levels in a sample of 10 children (male) asthmatics - levels were 9.4-29.5 pg/ml, same study sampled 10 healthy children (male) – levels were 2.1-3.0 , (Baraldi, et.al., *Thorax*2003).

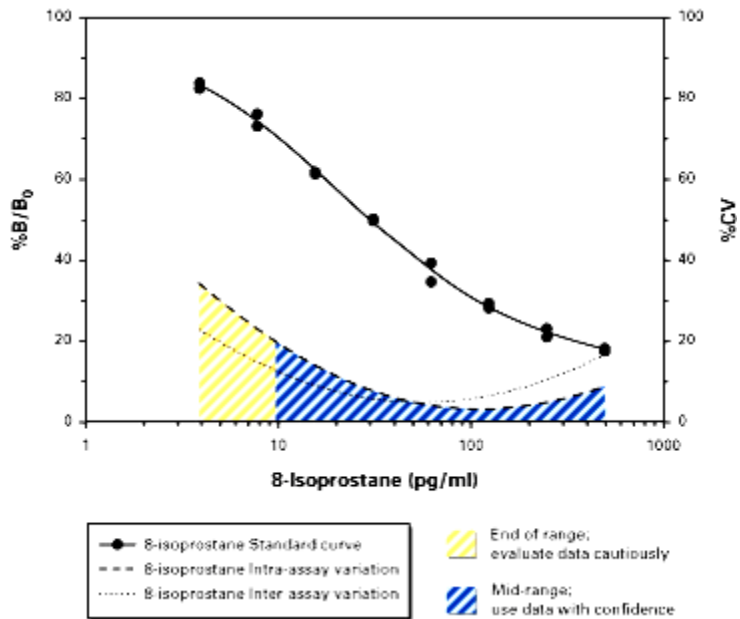
8-isoprostane levels were demonstrated in a sample of 12 healthy children – avg. of 34.2+/-4.5 pg/ml, compared to a group of 12 asthmatic steroid naïve children – avg of 56.4+/-7.7. (Baraldi, et.al., *Chest* 2003).

Analyzing the 8-isoprostane kit was done via spectrophotometry (μ Quant Universal Micro plate spectrophotometer,2004). Absorbance readings of the EIA kit plates were taken according to the manufacturer’s recommendation at 420 nm, 977 nm and 900 nm (the higher wavelengths were taken to negate background measurements).

The EIA 8-isoprostane EIA kit detects 8-isoprostane starting from a concentration of 5 pg/ml. The intra and interassay precision for the kit is depicted below (Figure 3).

Precision which is the component of accuracy that is concerned with the consistency of the stability of results is demonstrated in Figure 3 as the degree to which the kit provides consistent results from one application to the next.

Figure 3. Cayman chemical 8-isoprostane kit precision curve (Cayman Chemical, kit number 516351)



The EIA 8-isoprostane laboratory analysis kit works via an Enzyme Linked Immunoassay Analysis (ELISA) method. It is based on the competition between 8-isoprostane and 8-isoprostane-acetylcholinesterase (AChE) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. So, the concentration of AChE is known, and the concentration of 8-isoprostane is unknown. The reaction between bound AChE and the antiserum binding sites produces a yellow color which is absorbed at 412 nm of light via the spectrophotometer. Therefore the amount of 8-isoprostane in each

sample is inversely proportional to the amount of bound 8-isoprostane AChE, which is in turn directly proportional to absorbance. (See below).

Absorbance α {Bound 8-Isoprostane Tracer} α 1/{8-Isoprostane}

In order to calculate concentrations from the absorbance values the following manner:

1. We average the absorbance readings from the NSB wells. (NSB well = non-specific binding that occurs even in the absence of antiserum – this artificially elevates total antiserum bound).
2. We average the absorbance reading from the B0 wells (B0 is the max tracer the antiserum can bind in the absence of free analyte).
3. We subtract NSB average from the B0 avg. This is the corrected B0 or the actual real maximum amount bound.
4. We calculate % B/B0 (%sample or standard bound /maximum bound) for all our sample wells. Once this is done we can use the standard curve to locate the %B/B0 and find the corresponding concentration on the x- axis.

The standard curve was produced via a set of known concentrations of 8-isoprostane provided in the EIA kit.

Acquisition of Spirometry

Spirometry, which is a common clinical method employed in assessing lung function, was performed on each subject after collecting exhaled breath condensate. The Koko spirometer was used and calibrated using a standard 3-liter syringe to ambient temperature, humidity, and barometric pressure at least once a day on days when participants were examined. The spirometer was additionally re-calibrated when at least

six hours had elapsed since the prior calibration or at the discretion of the examiner. The raw FEV1 and FVC measurements obtained for each participant were automatically compared to their predicted normal values based on age, ethnicity, weight, height, and non-smoking status in determining their percent of predicted values using parameters as set forth by Crapo et. al.

The spirometry parameters examined on each study participant included FEV1, FVC, FEV1/FVC ratio, and the flow-volume loop. At least three spirometric measurements were obtained on each subject, with at least one flow-volume loop showing good effort. Proper technique was ensured by evaluating the flow-volume curve and continuance of the expiratory maneuver for at least six seconds according to ATS criteria.

A nose clip was placed on the subjects' noses during the study to prevent nasal breathing in order to obtain more accurate spirometry results. Subjects were asked to forcibly exhale for at least six seconds through a disposable single-use filter after maximal inspiration, followed by another maximal inhalation. The maneuver was demonstrated to them to help achieve consistency.

Any subject with abnormal spirometry measurements was notified of this information and advised to consult a healthcare provider and disqualified from the study. All subjects with an FEV1/FVC ratio greater than 70% of predicted, FEV1 greater than 80% of predicted, FVC greater than 70% of predicted, and a normal appearing flow-volume loop were eligible for the study by spirometric criteria.

Adverse effects were unlikely to occur during this phase of the study because subjects identified themselves as being in good health, the room was temperature controlled, and this phase of the study was brief in duration. All subjects were seated during the spirometry. In the event that adequate trials could not be obtained in the seated position as demonstrated by their flow-volume loops, subjects were asked to stand and instructed on safety precautions in the event of symptoms.

Nonetheless, there was the slight possibility of unusual symptoms such as lightheadedness, dizziness, chest pains, palpitations, or shortness of breath during the spirometry from overexertion in breathing. As a precaution, subjects were instructed to stop, be seated, and notify the physician examiner immediately if they experienced any symptoms at any time.

Results

Study Population Characteristics

The study population consisted of 22 subjects total; a total of 17 non-pregnant females and 5 pregnant females. Pregnancy dates in terms of weeks ranged from 25-32 weeks. The age of the population ranged from eighteen to thirty five years with a median age of twenty-three years. Fourteen of the twenty-two subjects' occupations were students, one a teacher, two waitresses, one coordinator, one scientist, and one lab tech. None of the subjects were smokers, and one reported a past smoking history of 2 pack-year history. Sixteen of the twenty-two subjects reported no past medical history problems. Nine of the twenty-two subjects reported no use of over the counter medications, vitamins or supplements.

Date of onset of last menstrual cycle was asked and recorded for non-pregnant subjects and ranged from 1-28 days with a mean of 14. Symptoms of cough, phlegm production, itchy or runny nose, throat irritation, and chest tightness or pain was asked on a four point scale zero = none, one = a little, two = moderate, three = very much. One subject reported throat irritation and rated it as a little. Two subjects reported cough and both rated it as a little. Five subjects reported phlegm production one reported it as moderate, four reported it as a little. Four subjects reported an itchy or runny nose and three reported it as a little, one reported it as moderate. One subject reported chest

tightness or pain and rated it as a little. Table 3 lists population characteristics, as well as Appendix I.

Table 3. Demographic characteristics of subjects both pregnant and non pregnant

Subject	Occupation	Pregnant	Weeks	Menses	Smoking	PMH	Meds	Age
A1	Student	No		14	0	Hernia		30
A2	Student	No		7	0	Insomnia	OCP/Ben /Flex	22
A3	Student	No		21	0		OCP/MV I	23
A4	Student	No		21	0			25
A5		No		14	0			20
A6	Student	No		21	0		OCP	23
A7	Teacher	No		28	0			21
A8	Student	No		5	0	Food allergy		20
A9	Student	No		10	0			24
A10	Sales	No		7	0	Childhd asthma	OCP/Mot rin	20
A11	Student	No		21	0			29
A12	Student	No		14	0			28
A13	Student	No		21	0		OCP/Mot rin	20
A14	Waitress	No		7	0		Celebrex	18
A15	Coordinator	No		14	0		Motrin	28
A16	Student	No		1	0	Migraine h/a	Axert	25
A17	Student	No		14	0		OCP	24
B18	Lab Tech	Yes	30	0	0			35
B19	Scientist	Yes	31	0	2		Effexor/ MVI	33
B20	Waitress	Yes	32	0	0	Gestati on. DM	Glyb/MV I	26
B21	Student	Yes	25	0	0		MVI/ Tylen	20
B22	Student	Yes	25	0	0		MVI	28

*OCP = oral contraceptive pill, Ben = Benadryl, Flex = Flexeril, MVI = multivitamin

8-Isoprostane Measurements

The data obtained from analyzing exhaled breath condensate samples from study subjects using the 8-isoprostane EIA kit (Cayman Chemical) are given below in graph and tabular forms. The standard curve used to calculate 8-isoprostane concentrations is given in Appendix K and L. Appendix K standard curve shows values prior to removing concentrations 500 pg/ml and 250 pg/. Removal of these concentrations as shown in standard curve showed no significant change in concentration calculations for study samples. 8-isoprostane concentrations obtained from standard curve were used for biostatistical manipulations.

Table 4. 8-isoprostane concentrations in pregnant and non-pregnant females.

Sample ID	#1 – CONC (PG/ML)	#2- CONC (PG/ML)	#3 – CONC (PG/ML)	AVG	SD
A1	4.182	7.298	16.689	9.389	6.511
A2	16.925	14.207	16.498	15.877	1.462
A3	8.803	4.374	7.555	6.911	2.284
A4	18.469	8.700	13.759	13.642	4.886
A5	-	-	-	-	-
A6	5.372	10.199	7.538	7.703	2.417
A7	7.311	8.553	5.979	7.281	1.288
A8	8.048	10.690	11.368	10.035	1.754
A9	24.335	16.402	15.625	18.788	4.820
A10	24.421	11.708	13.945	16.692	6.787
A11	8.048	11.368	10.621	10.012	1.742
A12	9.837	6.188	12.744	9.590	3.285
A13	16.901	18.010	11.116	15.342	3.702
A14	11.533	5.770	9.616	8.973	2.935
A15	11.389	8.120	7.967	9.159	1.933
A16	38.277	14.207	13.684	22.056	14.050
B1	12.971	10.655	5.562	9.730	3.790
B2	13.502	21.182	10.881	15.188	5.354
B3	10.805	4.642	6.313	7.253	3.187
B4	52.832	42.412	48.122	47.789	5.218
B5	8.349	-	4.954	6.651	2.401

A = NON-PREGNANT, Mean = 11.51333, B = PREGNANT, Mean = 16.64

Concentrations were calculated as a function of the light absorbance of the 8-isoprostane AChE tracer which is indirectly proportional to the concentration of 8-isoprostane.

Given below in figures 4 and 5 are pregnant and non-pregnant concentrations obtain from subject exhaled breath condensate samples.

Figure 4. 8-isoprostane concentrations taken from samples in pregnant females

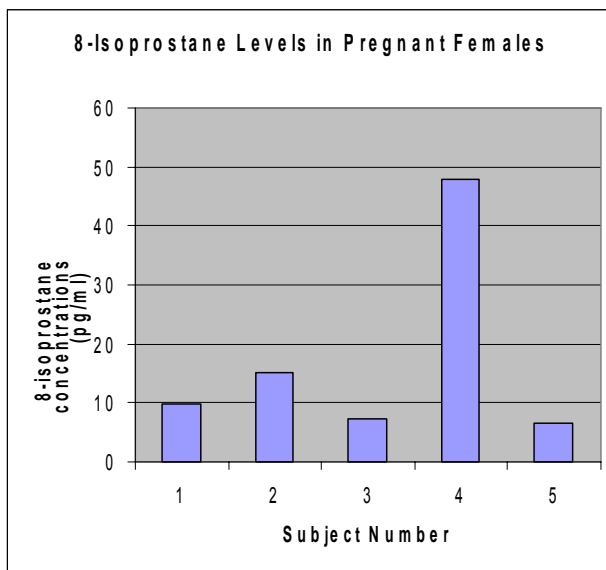


Figure 5. 8-isoprostane concentrations in samples taken from non-pregnant females

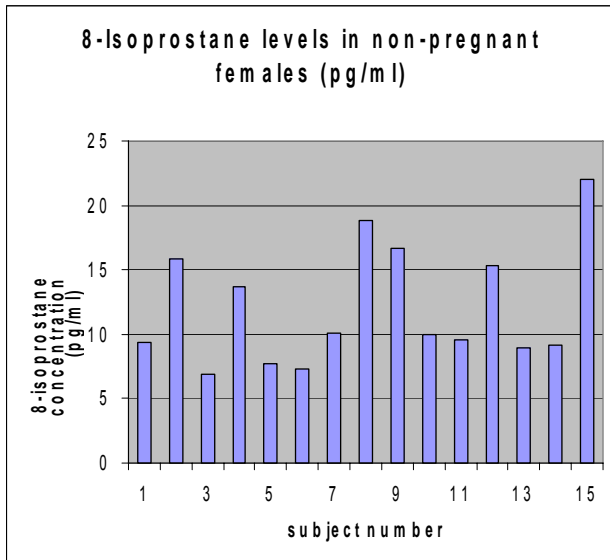


Table 5 depicts standard curve values used for concentration calculations. These values were obtained by using known 8-isoprostane concentration standards given in the kit.

Table 5. Standard concentrations (known) and actual absorbance concentrations detected

STD #	Known Conc (pg/ml)	Calc conc #1 (pg/ml)	Calc Conc #2 (pg/ml)	Calc conc#3 (pg/ml)	AVg	SD
1	500.000	-	-	-	-	-
2	250.000	207.790	164.255	166.470	179.505	24.521
3	125.000	94.526	86.222	141.009	107.253	29.528
4	62.5000	70.312	82.303	90.090	80.902	9.963
5	31.300	26.648	28.106	34.964	29.906	4.441
6	15.600	17.383	11.143	20.294	16.273	4.675
7	7.800	9.304	6.860	4.870	7.011	2.221
8	3.900	4.516	5.228	-	4.872	.504

Table 6. Standard concentrations (known) and actual absorbance concentrations detected using standard curve without known concentrations 500pg/ml, 250 pg/ml and 125 pg/ml

STD #	Known Conc (pg/ml)	Calc conc #1 (pg/ml)	Calc Conc #2 (pg/ml)	Calc conc#3 (pg/ml)	AVg	SD
1	500.000	-	-	-	-	-
2	250.000					
3	125.000					
4	62.5000			57.886	57.886	
5	31.300	27.485	28.807	34.683	30.325	3.832
6	15.600	18.450	11.753	21.408	17.204	4.947
7	7.800	9.700	6.940	4.693	7.111	2.508
8	3.900	4.296	5.096	-	4.969	.565

Utilizing the standard curve data shown in table 6 above to obtain sample data concentrations for subjects did not statistically alter calculated sample concentrations. The statistical calculations for 8-isoprostane concentrations of non-pregnant and pregnant subjects are given below in Table 8.

Table 7. Spirometric data for all subjects tested including pregnant and non-pregnant females

Subject Number	FEV1/FVC
A1	
A2	93%
A3	
A4	111%
A5	90%
A6	98%
A7	77%
A8	84%
A9	91%
A10	88%
A11	
A12	94%
A13	97%
A14	87%
A15	96%
A16	99%
A17	
B1	
B2	91%
B3	88%
B4	94%
B5	92%

No subjects were excluded based on spirometric measurements. The above values do not classify any of the subjects as having an obstructive respiratory component.

Biostatistical Data for 8-Isoprostane Measurements

Table 8. Statistical analysis of mean 8-isoprostane values of 20 subjects tested

Statistical Measure	Non-Pregnant	Pregnant
N	15	5
Mean	11.513	17.34
Median	9.6	9.7
Mode	10.0	
Standard Deviation	4.966	17.355
Skewness	0.841	2.039
Coeff Variation	43.131	100.08
Sum Weights	15	5
Sum Observations	172.7	86.7
Variance	24.659	301.193
Kurtosis	-.2966	4.225
Std Error Mean	1.282	7.761
Lower CL of the mean	8.7633	-4.209
Upper CL of the mean	14.263	38.889
Lower CL of Std Dev	3.6356	10.398
Upper CL of Std Dev	7.8317	49.87

The data given above show a difference between the means of the two groups. The standard deviation of each group is large and demonstrates a largely skewed set of data in each of the two sets. In addition the Lower CL of the means and upper CL for the means of each group overlap.

Performing a T-test on the data in order to determine if the difference between the means of the two groups was significant the following data was obtained as shown in Table 9 below.

Table 9. T-tests used to analyze difference between means of the two groups

Variable	Method	Variances	DF	T value	Pr > t
8-iso	Pooled	Equal	18	-1.22	0.2397
8-iso	Satterthwaite	Unequal	4.22	-0.74	0.4980

The statistical analysis (Satterthwaite Test for normally distributed data with different variances) failed to show a difference between the two means of the data groups as the p-value was greater than 0.05.

The power of the study was calculated and shown to be .518.

Table 10. Variables involved in power calculations

M_1	μ_2	σ	σ	A	Critical Value	Power
$N_1=15$ 11.513	$N_2=5$ 17.34	4.9659	17.355	0.05	4.84434	.518

Because $\beta = 1 - \text{Power}$, beta (or the probability of making a type II error) was calculated to be equal to 48.2%.

Conclusions

To reiterate, the question of interest has been whether a difference exists in the average 8-isoprostane levels between two groups: Pregnant Females and Non-Pregnant Females aged 18-35 years.

When analyzing the demographic characteristics of the two groups it became evident that two subjects in the non-pregnant group could be excluded. One subject reported a history of childhood asthma; another reported a history of food allergies. Both of these subjects were excluded from data calculations. Subject A10 self reported a history of childhood asthma; her 8-isoprostane concentration was 16.692 pg/ml. The subject A8 self reported a history of food allergy; her 8-isoprostane concentration was 10.035. All other data collected from subjects was used for calculations.

The means of the non-pregnant and pregnant groups was 11.513 and 17.34 respectively. There is an obvious difference between these two means, but is this difference statistically significant? According to the t-test performed on the two means there was not a statistical difference found. The null hypothesis stated that there was no difference in the exhaled breath condensate oxidative marker $\text{PGF2}\alpha$ in pregnant females in their third trimester of pregnancy versus non-pregnant females. The alternative hypothesis stated that there was a difference between the exhaled breath condensate oxidative marker $\text{PGF2}\alpha$ in pregnant females in their third trimester of pregnancy versus non-pregnant females. Based on our t-test we must fail to reject the null hypothesis.

As to why a difference between the two groups was not found could primarily be due to the sample size of the study. Power is largely affected by sample size, that is, if

the sample study size is small the study will have insufficient power to detect real associations. Since Power = $1 - \beta$ the smaller the power the larger the beta (probability of not finding an association in a sample when one exists in the population).

The standard deviations of both the pregnant group and non-pregnant group were large; 4.966 and 17.355 respectively. This could be in part due to the distribution of 8-isoprostanes in females, both pregnant and non-pregnant is large in nature. However, we cannot statistically confirm this as our sample size is not large enough for us to conclude that we have statistically found a difference between the two means.

As previously discussed, Staff, et.al. reported an increased content of lipid peroxides in preeclamptic decidual placental tissue in preeclamptic patients at delivery compared with controls.⁸ Another study by Moretti and colleagues demonstrated increased markers of oxidative stress in preeclamptic women versus uncomplicated pregnancy and non-pregnant controls by measuring various volatile organic compounds in the exhaled breath condensate of these women⁹ This study analyzed non-pregnant, healthy pregnant and preeclamptic pregnant females. We sought out to determine if a difference exists between baseline levels of 8-isoprostanes between a group of healthy pregnant females and non-pregnant females. This difference between the means of the two data sets was shown and is an observed difference, however is not a statistically significant difference.

The implication that the crude means of the two groups indicated an observable difference but not a statistically significant difference provides a basis for the need of future studies. Future studies would need to address the necessity of larger sample sizes

in each group. This serves as a challenge as participant recruitment of pregnant females in their 3rd trimester was low in this study.

References

1. Janssen LJ. Isoprostanes: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol Lung Cell Mol Physiol* 2001;280(6):L1067-82.
2. Montuschi P, Corradi M, Ciabattini G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-Isoprostane, a Marker of Oxidative Stress, in Exhaled Condensate of Asthma Patients. *Am J Respir Crit Care Med* 1999;160(1):216-20.
3. Van Hoydonck PGA, Wuyts WA, Vanaudenaerde BM, Schouten EG, Dupont LJ, Temme EHM. Quantitative analysis of 8-isoprostane and hydrogen peroxide in exhaled breath condensate. *Eur Respir J* 2004;23(2):189-92.
4. Baraldi E, Carraro S, Alinovi R, et al. Cysteinyl leukotrienes and 8-isoprostane in exhaled breath condensate of children with asthma exacerbations. *Thorax* 2003;58(6):505-9.
5. Baraldi E, Ghiro L, Piovan V, et al. Increased Exhaled 8-Isoprostane in Childhood Asthma. *Chest* 2003;124(1):25-31.
6. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur Respir J* 2003;21(1):177-86.
7. Goil S, Truog WE, Barnes C, Norberg M, Rezaiekhaligh M, Thibeault D. Eight-epi-PGF₂[α]: A possible marker of lipid peroxidation in term infants with severe pulmonary disease. *The Journal of Pediatrics* 1998;132(2):349-51.
8. Staff AC, Halvorsen B, Ranheim T, Henriksen T. Elevated level of free 8-isoprostaglandin F₂[α] in the decidua basalis of women with preeclampsia. *American Journal of Obstetrics and Gynecology* 1999;181(5):1211-5.
9. Moretti M, Phillips M, Abouzeid A, Cataneo RN, Greenberg J. Increased breath markers of oxidative stress in normal pregnancy and in preeclampsia. *American Journal of Obstetrics and Gynecology* 2004;190(5):1184-90.
10. Murphy VE, Gibson PG, Smith R, Clifton VL. Asthma during pregnancy: mechanisms and treatment implications. *Eur Respir J* 2005;25(4):731-50.
11. Held H-D, Uhlig S. Mechanisms of Endotoxin-Induced Airway and Pulmonary Vascular Hyperreactivity in Mice. *Am J Respir Crit Care Med* 2000;162(4):1547-52.

12. Holz O. Catching breath: monitoring airway inflammation using exhaled breath condensate. *Eur Respir J* 2005;26(3):371-2.
13. Mutlu GM, Garey KW, Robbins RA, Danziger LH, Rubinstein I. Collection and Analysis of Exhaled Breath Condensate in Humans. *Am J Respir Crit Care Med* 2001;164(5):731-7.

Appendices

Appendix A: Questionnaire Form

Health Questionnaire

What is your date of birth? _____

Your current age? _____

What is your current
occupation? _____

Have you ever been pregnant? YES NO

G__P__A__

Are you currently pregnant? YES NO

EDC _____

When was your last menstrual
period? _____

Do you have any medical complications related to your pregnancy such as diabetes,
hypertension, morning sickness, etc? YES NO

If yes,
what conditions? _____

Do you smoke? YES NO

Are you exposed to second hand smoke? YES NO

Have you ever smoked? YES NO

How many packs per day did you smoke? _____

For how many years did you smoke? _____

Appendix A (Continued)

When did you quit smoking? _____

Do you have any medical problems? YES NO Please list them here.

- _____
- _____
- _____

Have you ever had any of the following conditions?

- Chest pain, palpitations, irregular heart beat, or heart disease? YES NO
- High blood pressure? YES NO
- Asthma, bronchitis, emphysema, or other lung disorder? YES NO

Do you see your health care provider on a routine basis for any medical condition?

YES NO

If so, what? _____

Do you take any prescription or over-the-counter medications? YES NO If so, what?

- _____
- _____
- _____

When is the last time you remember being ill? _____

What illness did you have? _____

To what degree do you have the following symptoms at this time?

Cough: NONE A LITTLE MODERATE
VERY MUCH

Appendix A (Continued)

Phlegm production:	NONE VERY MUCH	A LITTLE	MODERATE
Itchy or runny nose:	NONE VERY MUCH	A LITTLE	MODERATE
Throat irritation:	NONE VERY MUCH	A LITTLE	MODERATE
Chest tightness or pain:	NONE VERY MUCH	A LITTLE	MODERATE

Have you been exposed to any gases, dusts, or fumes at home or on the job? YES

NO

If so, please explain:

Post Study Questionnaire

Did you have any concerns about the way the informed consent process was handled?

YES NO

Did you have any problems with the Health Questionnaire? YES NO

Did you have any discomfort or concerns after the physical exam? YES NO

Did you have any discomfort or concerns after the nitric oxide test? YES NO

Did you have any discomfort or concerns after completing the spirometry? YES NO

Please elaborate. Use the back of this page is necessary.

Informed Consent for an Adult

University of South Florida

Information for People Being Asked to Take Part in Research Studies

IRB Study #103718c

Doctors and researchers at University of South Florida (USF) study diseases and other health problems people have. We try to find better ways to help treat these health problems. To do this, we need the help of people who agree to take part in a research study.

Title of Research Study: 8-isoprostane levels in exhaled breath condensate of pregnant women compared to non-pregnant women, is there a baseline difference?

Person in Charge of Study: Rosemary Szollas, M.D. and Stuart Brooks, M.D.; Karen Olson, M.D.

Where the study will be done: University of South Florida, College of Public Health, MHH Room 323

Who is paying for the study: Sunshine Education Resource Center

Should you take part in this study?

This form tells you about this research study. You can decide if you want to take part in it. You do not have to take part. Reading this form should help you decide if you want to take part in the study. If, at any time, you have any questions feel free to ask the person explaining this study to you.

Before you decide:

- Read this form.
- Talk about this study with the study doctor or the person explaining the study. You can have someone with you when you talk about the study.
- Find out what the study is about.

This form explains:

- The purpose of this research study.
- What will happen during this study and what you will need to do.
- The potential benefits of being in this study, if any.
- The risks of having problems because you are in this study.
- The answers to any questions you might have.

You can ask questions:

- You may have questions this form does not answer. If you do, ask the study doctor as you go along.

Appendix B (Continued)

- You don't have to guess at things you don't understand. Ask the people doing the study to explain things in a way you can understand.

After you read this form, you can:

- Take your time to think about the information that has been provided to you.
- Have a friend or family member read the form.
- Talk it over with your regular doctor.

It's up to you. If you choose to be in the study, then you can sign the form. If you **do not** want to take part in this study, you **do not** sign the form.

Why is this research being done?

The purpose of this study is to find out if:

- The purpose of this study is to find out if there is an increased level of 8-isoprostane in the exhaled breath condensate of pregnant women. 8-isoprostane is a chemical made in the body naturally. The amount of 8-isoprostane also increases in the lungs in some diseases of the lung. This study will help find out if pregnancy causes an increase in the production of 8-isoprostane in the lungs.
- This study will help find out if 8-isoprostane levels increase during pregnancy and help in understanding of how pregnancy affects asthma.
- To measure 8-isoprostane in the breath we need to collect your exhaled breath. Breathing normally into a mouth size tube will do this. The tube is cooled to a very low temperature thereby changing the gas you exhale into a liquid form. The liquid is a suitable way to collect your breath and store it for analyzing.

Why are you being asked to take part?

We are asking you to take part in this study because you are a woman of child bearing age who is pregnant or who is not pregnant. We need to compare these two groups.

In order to participate in this study, you need to be between 18 – 35 years of age, a non-smoker with no history of asthma, environmental allergies, chronic illness, or recent respiratory infection (in the past month). If you are pregnant, you need to be at 28 weeks gestation or more (as determined by your personal health care provider) when you come to the Breath Lab for the study.

How long will you be asked to stay in the study?

You will be asked to spend about three hours in this study.

The sample collected from your breath will be stored for a maximum of three months, the

Appendix B (Continued)

analyzing of your sample will take about two hours, you will not be required to be present for this portion of the study.

One hour includes the time it takes to complete the informed consent process and fill out a health questionnaire. You may choose to take more time to complete the informed consent.

A brief physical examination, collection of exhaled breath condensate and spirometry will take about an hour.

How often will you need to come for study visits?

A study visit is one you have with the study doctor. This visit is different than the visits you make with your regular doctor. You will need to come for one study visits in all.

How many other people will take part?

About fifty people will take part in this study at USF.

Will the medical treatment you get from your regular doctor change if you take part in this study?

The kind of treatment you now get from your regular doctor will not change because you take part in this study. The only way that it could potentially change is if we discover a medical condition which was not previously diagnosed and your doctor chooses to treat you for it.

You will keep seeing your regular doctor. Your regular doctor will give you the same kind of treatment you would get anyway, whether you take part in the study or not.

This study includes only healthy women whose only condition requiring treatment should be pregnancy. If you require any other treatment, please do not hesitate to get it. We need you to inform us of any treatment in the month before testing or in the week following testing, other than routine prenatal care, because it may affect or ability to use your results in this study.

What other choices do you have if you decide not to take part in this study?

If you decide you do not want to take part in this study, that is okay. We are performing some simple diagnostic tests. These are probably not necessary for your medical care if you are being included in this study.

If you wanted or needed them done, your personal health care provider could perform a physical examination and order spirometry. If you wanted to know the level of 8-

isoprostane in your breath, you would only be able to have it measured in a study such as this one. It is an experimental study which is not generally available.

How do you get started?

If you decide to take part in this study, you will need to sign this consent form. Then, we will do some screening tests. Screening tests are tests done to see if you are able to be in the study. Screening tests are different from the actual study procedures. The definition of screening is the application of a test to detect a potential disease or condition in a person who has no known signs of that disease or condition. The screening tests are done, therefore, prior to the actual study procedures to make sure the researchers identify the correct population of individuals to include into the study.

We will do these screening tests:

1. The health questionnaire will be used to confirm whether you meet the criteria for being involved in this study. In order to be included you need to

- be a non-smoker
- be 18 – 35 years of age
- have no environmental allergies such as hay fever
- have no chronic illnesses which require routine monitoring or treatment, especially asthma or other pulmonary diseases
- be twenty-eight or more weeks pregnant when you are scheduled to be tested

OR

- be non-pregnant, having had a normal menstrual period in the month prior to testing

2. The brief physical examination will include listening to your heart and lungs. In order to be

included you need to have

Appendix B (Continued)

- clear lung sounds without wheezing, rales, or rhonchi (abnormal lung sounds)
- a regular heart beat without any murmurs, rubs, or gallops

There is not a separate visit to complete the screening tests. The results will be available once the collected samples are analyzed. Then you and the research team will decide whether or not you continue in the study.

What will you need to do to get ready for this study?

For one hour prior to your scheduled appointment at the Breath Lab, you will need to refrain from eating, drinking, or performing any strenuous exercise such as running, weight lifting, or heavy physical labor.

What will happen during this study?

You will need to complete five things to complete this study:

1. This informed consent document
2. A health questionnaire
3. A brief physical exam where we listen to your heart and lungs
4. Spirometry
5. The collection of exhaled breath condensate

If you have not completed the informed consent before you come to the Breath Lab, we will complete it at the beginning of your visit. If you agree to participate in the study and sign the informed consent, we will proceed.

The health questionnaire is a short assessment of your health history and any current symptoms you may be experiencing. It will be used to collect demographic (a group of characteristics defining human populations) information about you such as your age and medical information about your health history and recent symptoms.

After you complete the health questionnaire, the principal investigator (PI) will listen to your heart and lungs. She needs to see if you have wheezing or rhonchi (abnormal breath sounds) or an irregular heart beat or murmur. Any of these findings will probably result in your withdrawal from the study.

Next, we will measure how fast you can blow the air out of your lungs and how much air you can blow out in a test called spirometry. This test is used routinely to monitor people of all ages who have lung disease. We are using it in our study to be certain that you do not have any type of undiagnosed lung disease which would affect our results.

Spirometry takes about as much effort as blowing up a big balloon and is the most difficult test to perform in this study. Once again, we need to have three acceptable

Appendix B (Continued)

measurements to complete this test. We will not repeat this test more than eight times even if we do not get three acceptable tracings. Most people are able to complete this test in five attempts or less.

You can take your time; you can take a break; or you can stop at any time.

Finally, your exhaled breath will be collected and cooled in order to condense (turn from a gas to a liquid form) your breath into a liquid for collection and storage. In order to collect your breath condensate we will ask you to breathe as you normally would through a disposable tube. We will ask you to do this for a maximum of fifteen minutes, in order to collect an adequate sample amount. If you need to break to cough, sneeze, or blow your nose, or for any other reason you will be asked to remove your mouth from the tube and resume breathing through the tube when comfortable once again. We can collect at your pace if necessary. The time limit is not concrete as it is a recommended time period for collection by the manufacturer of the collection device, however if adequate sample size is obtained prior to fifteen minutes you may stop if you would like to do so.

After collection of exhaled breath condensate, we will ask you if you have any questions about the tests, any discomfort at all, or any other concerns. All of these responses will be recorded on your health questionnaire.

Will you be paid for taking part in this study?

Subjects will be offered twenty dollars upon completion of exhaled breath condensate collection and for their time spent participating in the study.

If for any reason a subject is unable to complete the study, the following payment schedule will be used:

1. Completion of exhaled breath condensate collection - \$20

We are not able to reimburse you for additional costs you incur such as parking fees, bus or taxi fare, childcare costs, or time away from work.

What will it cost you to take part in this study?

It will not cost you anything to be part of the study.

You will have to pay for your regular care or any other costs. Your insurance plan should cover your regular costs. Your insurance plan will not have to pay for any study costs.

What are the potential benefits if you take part in this study?

We don't anticipate that you will get any health benefits from taking part in this study. We are performing diagnostic (used to identify disease or conditions) tests only; not providing any treatment.

Appendix B (Continued)

You may potentially benefit by learning more about your health as a result of your physical examination and testing during this study.

You may enjoy being part of a study and seeing how it is conducted.

No matter what, we will learn more about changes in the lungs during pregnancy. What we learn may guide further research about asthma and pregnancy.

What are the risks if you take part in this study?

There is very little risk that you incur by being part of this study.

You may experience some discomfort during the collection of the exhaled breath condensate or during the spirometry. Spirometry, in particular, requires you to blow out as hard and fast as you can. This is something like blowing up a big, stiff balloon.

You could potentially get an infection in the Breath Lab. This is probably less likely than getting an infection at the grocery store. Currently, all subjects being tested are healthy subjects. Everyone gets a new, disposable mouthpiece to use. Anything that gets contaminated is cleaned according to lab protocol.

Should you experience any adverse symptoms or event, you will be under the direct supervision of a physician at all times during the study. We have emergency equipment available and will call for emergency personnel (911) if needed.

Is there any risk to your unborn children if you take part in this study?

This study does not involve any treatment which could affect you unborn child.

Two of the diagnostic studies which are being performed, physical examination and spirometry, are routinely performed in pregnant women who have asthma without any adverse outcome.

The experimental diagnostic study, collection and analyzing levels of exhaled breath 8-isoprostane, does not expose you to any physical, chemical, or biological hazard which could potentially harm you or your unborn child. It is simply a measurement of a common constituent in exhaled breath.

What if you get sick or hurt while you are in the study?

If you need emergency care:

- **Go to your nearest hospital or emergency room right away. Call 911 for help.** You should know that USF does not provide emergency care.
- Call the study doctors as soon as you can. They will need to know that you are hurt or ill. Call Rosemary Szollas, M.D., or Stuart Brooks, M.D. at (813) 974-

Appendix B (Continued)

7545.

- **If it is NOT an emergency, and you get hurt or sick while you are taking part in this study:**
- Go to your regular doctor.
- Please inform the study doctors. They will need to know if you are hurt or ill. Call Rosemary Szollas, M.D. or Stuart Brooks, M.D. at (813) 974-7545.
- The USF Medical Clinics may not be able to give the kind of help you need. You may need to get help somewhere else.

If you are harmed while taking part in the study:

The state of Florida enjoys what is called "sovereign immunity." This means that you usually cannot sue the state of Florida. However, the state has waived sovereign immunity (agreed to be sued) in certain situations. One of those situations is if a state employee, such as your study doctor or other USF employee, is negligent in doing his or her job in a way that harms you during the study. The money that you might recover from the state of Florida is limited in amount.

You can also call the USF Self Insurance Programs (SIP) at 1-813-974-8008 if you think:

- You were harmed because you took part in this study.
- Someone from the study did something wrong that caused you harm, or didn't do something they should have done.
- Ask the SIP to look into what happened.

Recruitment for this project has been conducted at a Tampa General clinical facility therefore participation in this research project will make you subject to the following:

ADULT TAMPA GENERAL INJURY STATEMENT

In the event you suffer an injury or illness as a result of participating in this research study, please be aware that immediate, short-term medical treatment for the injuries or illness will be available to you from Tampa General Hospital. The cost of the medical treatment will be billed to you to the extent not covered by your insurance company or government program or study sponsor. No other compensation will be offered. You are not giving up any legal rights by signing this form. If you believe you have experienced a reaction to the study medication or have been injured as a result of research procedures performed at Tampa General Hospital, please contact the Department of Risk Management at (813) 844-7666.

What will we do to keep your study records private?

Federal law says we must keep your study records private. We have made prior arrangements to do this.

To maintain subject confidentiality, each subject will be given a random three digit subject number which will be recorded on the informed consent document and compiled in a master list of all subjects. This number will be the only identifier listed on the health questionnaire, the data collection form, the appointment list, spirometry report, and sample storage tubes. Without the informed consent documents or the master list, it will not be possible to link any confidential information to the subject. The informed consent document and a master list of subjects will be kept in a locked file cabinet in the Breath Lab or in the Occupational Medicine Residency Office. Access to this material will only be available the investigators in the study.

Questionnaires and recorded data from the study will be kept in a locked file cabinet in the Breath Lab or in the Occupational Medicine Residency office separate from the informed consent documents and the master list whenever they are not in direct possession of the investigators. These documents will not contain any information which could, by itself, link them to you.

However, certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:

The medical staff who are taking care of you.

Certain government and university people who need to know more about the study. For example, individuals who provide oversight on this study may need to look at your records. These include the University of South Florida Institutional Review Board (IRB) and the staff that work for the IRB. Other individuals who work for USF that provide other kinds of oversight may also need to look at your records. Other individuals who may look at your records include: the Florida Department of Health, people from the Food and Drug Administration (FDA) and the Department of Health and Human Services (DHHS) (This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.)

We may publish what we find out from this study. If we do, we will not let anyone know your name. We will not publish anything else that would let people know who you are.

What happens if you decide not to take part in this study?

You should only take part in this study if you want to take part.

If you decide not to take part:

- You will not be in trouble or lose any rights you normally have.
- You will still have the same health care benefits.

Appendix B (Continued)

- You can still get your regular treatments from your regular doctor.

What if you join the study and decide you want to stop later on?

You can decide after signing this informed consent document that you no longer want to take part in this study. If you decide you want to stop taking part in the study, tell the study staff as soon as you can.

- You can stop at any time without any adverse outcome.
- If you decide to stop, you can continue getting care from your regular doctor.

Are there reasons we might take you out of the study later on?

Even if you want to stay in the study, there may be reasons we will need to take you out of it. You may be taken out of this study if:

- We find out it is not safe for you to stay in the study.
- You experience a change in your health which is inconsistent with the study criteria.
- You miss your appointment at the Breath Lab twice without calling ahead of time to reschedule.

You can get the answers to your questions.

If you have any questions about this study, call Rosemary Szollas, M.D. at (813) 974-7545.

If you have questions about your rights as a person who is taking part in a study, call the Division of Research Compliance of the University of South Florida at (813) 974-9343.

Appendix B (Continued)

Signatures for Consent to Take Part in this Research Study

It is up to you to decide whether you want to take part in this study. If you want to take part, please read the statements below and sign the form if the statements are true.

I freely give my consent to take part in this study. I understand that this I am agreeing to take part in research. I have received a copy of this consent form to take with me.

Signature of Person Taking Part in Study

Date

Printed Name of Person Taking Part in Study

Optional - Witness

Signature of Witness

Date

Printed Name of Witness

Statement of Person Obtaining Informed Consent

I have carefully explained to the person taking part in the study what he or she can expect.

I hereby certify that when this person signs this form, to the best of my knowledge, he or she understands:

What the study is about.

What needs to be done.

What the potential benefits might be.

What the known risks might be.

I also certify that he or she does not have any problems that could make it hard to understand what it means to take part in this study. This person speaks the language that was used to explain this study.

This person reads well enough to understand this form or, if not, this person is able to

Appendix B (Continued)

hear and understand when the form is read to him or her.

This person does not have a medical problem that makes it hard to understand what is being explained and can, therefore, give informed consent.

This person is not taking drugs that make it hard to understand what is being explained and can, therefore, give informed consent.

Signature of Person Obtaining Informed Consent

Date

Printed Name of Person Obtaining Informed Consent

Optional - Witness

Signature of Witness

Date

Printed Name of Witness

**Addendum to the Consent
Consent to take and store additional samples.**

We are asking you to allow us to take and store additional samples of your exhaled breath for use in the future. These samples may be used for a variety of purposes.

This means we will take some of your exhaled breath and cool it in order to collect the liquid form of your breath. We will then freeze this small amount of liquid (about half of a teaspoon) at below freezing temperatures in order to stabilize the compounds within your breath. Once we are capable of analyzing your sample within the breath lab we will de-thaw the sample and utilize it in a chemical assay kit that determines the concentration of 8-isoprostane within your breath. Your entire sample collected will be used for this technique and we will not discard any of the sample.

When you sign this below, you are agreeing to let us store and use your exhaled breath condensate for future research studies.

We may use these samples to help us:

- Determine if 8-isoprostane is elevated in the exhaled breath condensate of pregnant women.
- Help us with understanding how pregnancy affects asthma.

____ I give my consent to provide my exhaled breath condensate for that purpose.

Appendix B (Continued)

____ I do not give my consent to my exhaled breath condensate for that purpose.

Signature of Person Taking Part in Study Date

Printed Name of Person Taking Part in Study

Optional - Witness

Signature of Witness

Date

Printed Name of Witness