Abstract

Abstract of thesis entitled: Exhaled Nitric Oxide in Chinese Schoolchildren

Submitted by LIU Kin Hang

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Exhaled nitric oxide (eNO) has been found to be increased in patients with inflammatory pulmonary disease such as asthma and it could serve as a noninvasive marker of airway inflammation. Monitoring of the level may help doctors to adjust the dosage of anti-inflammatory therapy. Exhaled NO levels vary greatly depending on the extent of the airway inflammation and the treatment used. Levels are increased in individuals experiencing asthmatic exacerbation and are lower in patients with stable asthma receiving anti-inflammatory treatment. Therefore, the use of standardized methodology of eNO measurement and the establishment of a reference range for eNO level will make both monitoring and treatment of asthma more reliable. Since data on reference normal level of eNO is limited, a study with a large sample of schoolchildren would potentially reveal important insights into the possible influence of gender and anthropometric measurements on the level of eNO.
The levels of exhaled NO were determined in a large number of schoolchildren recruited randomly from secondary schools distributed throughout Hong Kong aiming at determining the eNO levels among normal control children as well as children with other atopic disorders. In our paediatric asthma clinics, a group of asthmatic patients without corticosteroid treatment and recent upper respiratory tract infection were also recruited for comparison. Exhaled NO levels were measured by standardized methodology using a FDA approved instrument, NIOX® (Aerocrine AB, Stockholm, Sweden), according to ATS/ERS guideline.

Among the normal control children, there were no significant correlations between exhaled NO level and anthropometric measurements such as height, weight, body mass index (BMI) and body surface area (BSA).

Exhaled NO can be used in epidemiologic studies as a noninvasive marker of allergic airway inflammation in schoolchildren. The results demonstrated significant difference of the eNO level between boys and girls. In addition to gender, there may be other determinants of eNO levels such as genetic variations. Our data also demonstrated that allergic disorders such as allergic rhinitis and eczema can also result in an elevation of the eNO of an individual.
摘要

研究指出，患有呼吸道發炎的人士，如哮喘病人呼出之一氧化氮含量會較高。此外，呼出一氧化氮能對呼吸道發炎作一種非侵入性指標。透過監察呼出一氧化氮的含量，能幫助醫生調整抗發炎治療藥物的劑量。同時，呼出一氧化氮的含量會隨着氣道發炎的程度和治療方法，而有所改變。當哮喘病發時，呼出一氧化氮含量會較高；相反，接受適當抗發炎治療後，含量則會隨之下降。因此，引進量度呼出一氧化氮含量的標準方法，並設定正常含量範圍，會更可靠地監控和治療哮喘。由於現在正缺乏呼出一氧化氮正常含量之參考數據，大規模的學童研究實在有潛力去顯明一些重要的發現，如性別與人體測量的量度對呼出一氧化氮含量影響之可能性。

我們從全港分佈各區的中學裏，隨機抽樣了一群學童去量度呼出的一氧化氮含量。目的是為了確定健康學童與患有過敏性疾病學童的呼出一氧化氮含量。我們還在兒科哮喘診所選出那些沒有接受類固醇治療和近期沒有患上呼吸道感染的哮喘病人，以作比較。此外，我們使用了一臺已被（美國）食品及藥物管理局檢定的儀器，NIOX® (Aerocrine AB, Stockholm, Sweden)，並按照歐洲呼吸學會及美國胸肺學會所發出的指引，採用標準方法來量度呼出一氧化氮的含量。
在健康的學童中，呼出的一氧化氮含量與身高、體重、身體質量指數、體表面積沒有顯著的關係。

藉着學童流行病學研究，呼出一氧化氮是其中一種可以用來反映過敏性呼吸道發炎的非侵入性指標。研究結果顯示了男孩和女孩的呼出一氧化氮含量確有顯著分別。除了性別引起的差異之外，亦有其他因素決定呼出一氧化氮含量的可能性，例如基因差異。數據顯示，過敏性疾病如過敏性鼻炎和濕疹都有可能引發病人增加呼出一氧化氮之含量。
I wish to express my sincere gratitude to my supervisor, Prof. Gary WK Wong, and co-supervisor, Prof. TF Leung, who have given me a lot of guidance and support throughout my study period.

I would like to thank all the schoolchildren and teachers for their participation and preparation in this health survey. I also thank the field workers, Edmund Yung and Brenda Li, for their efforts.

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Procedure-related Factors

Nasal Nitric Oxide Contamination

Exhalation Procedure — Starting Lung Volumes

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Circadian Rhythm

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Sex

Upper Respiratory Tract Infection

Diet and Exhaled Nitric Oxide

Effect of Spirometry and Exercise

Environmental Factors

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Study Population

The International Study of Asthma and Allergies in Childhood

ISAAC Questionnaires

Standardized Approach for Answering Questions in the Field

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Exhaled Nitric Oxide Measurement

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<td>ATS</td>
<td>American Thoracic Society</td>
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<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
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<td>BHR</td>
<td>bronchial hyperresponsiveness</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>BSA</td>
<td>body surface area</td>
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<tr>
<td>BTS</td>
<td>British Thoracic Society</td>
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<td>EBC</td>
<td>exhaled breath condensate</td>
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<td>ECP</td>
<td>eosinophil cationic protein</td>
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<td>eNO</td>
<td>exhaled nitric oxide</td>
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<td>ERS</td>
<td>European Respiratory Society</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FEV₁</td>
<td>forced expiratory volume in first second</td>
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<td>FRC</td>
<td>functional residual capacity</td>
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<td>GINA</td>
<td>Global Initiative for Asthma</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
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<tr>
<td>Ig</td>
<td>immunoglobulin</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>IQR</td>
<td>inter-quartile range</td>
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<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
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<tr>
<td>MBP</td>
<td>major basic protein</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>NOS</td>
<td>nitric oxide synthase</td>
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<tr>
<td>ppb</td>
<td>parts per billion</td>
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<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<td>SPT</td>
<td>skin prick testing</td>
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<tr>
<td>TLC</td>
<td>total lung capacity</td>
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<tr>
<td>Th</td>
<td>T-helper</td>
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<tr>
<td>URI</td>
<td>upper respiratory tract infection</td>
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Chapter 1: Introduction

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   1.1.3 Exhaled Nitric Oxide as a Diagnostic Marker and Its Correlation with Other Markers of Inflammation
   1.1.4 Normal Reference Studies of Exhaled Nitric Oxide

1.2 Aim of Study

Chapter 2: Plan of Study
1.1 Asthma and Assessment of Airway Inflammation

Asthma is one of the most common chronic disorders in children, and its prevalence varies widely in different regions of the world (ISAAC Steering Committee, 1998). Recent epidemiological studies suggested that the prevalence of asthma and allergic diseases is increasing in many Western and developing countries (Aberg et al., 1995; Venn et al., 1998). The prevalence rate of asthma in children has been reported as high as 20-30 % in Australia and New Zealand.

Asthma is a complex disease which is characterized by the following features — intermittent reversible airway obstruction, airway hyperresponsiveness, and airway inflammation as well as airway remodeling (Haley and Drazen, 1998). The most important pathogenic mechanism causing airway obstruction is airway inflammation. The cornerstone of current treatment of asthma is the use of anti-inflammatory drugs (GINA, 2004). However, there is no firm agreement as to whether therapeutic intervention ought to be aimed at controlling symptoms alone.
optimizing lung function, or minimizing airway inflammation, and hyperresponsiveness (Crimi et al., 1998).

The diagnosis and monitoring of asthma are primarily based on conventional measurements (British Thoracic Society, 2003), such as assessment of symptoms reported by the patient (history of wheeze, shortness of breath, and cough), measurements of airway obstruction by means of lung function study, assessment of bronchodilator response, and occasionally bronchial challenge tests to assess bronchial hyperresponsiveness (BHR). However, self-reporting of symptoms is much dependent on perception of symptoms with the potential for underestimation of the severity by the patients.

International guidelines recommend the use of symptoms assessment and the measurement of serial peak expiratory flows or spirometry to classify the severity of asthma (GINA, 2004). The response to either inhaled bronchodilator or to a trial of corticosteroid is also included as part of current recommendations for the diagnosis of asthma. However, these traditional approaches would lead to some problems. First, lung function testings are primarily based on demonstrating abnormal airway physiology. Particularly in mild asthma, lung function testing is often normal and
the results may not reflect the degree of airway inflammation (Hunter et al., 2002).

1.1.1 Assessment of Airway Inflammation

Asthma is a chronic inflammatory disorder characterized by the presence of inflammatory cells and the release of several inflammatory mediators in the airways. Assessment of airway inflammation may be useful for the diagnosis and monitoring of asthma (Shelhamer et al., 1995). There are different invasive and noninvasive means for assessing airway inflammation. Clinical studies have suggested that monitoring these molecules might help to improve the control of asthma as they provide an objective marker for adjusting asthma medications (Smith et al., 2005). If the markers are to be used for the daily care of asthmatics, they should be relatively easy to obtain in both the primary care or referral settings.

1.1.2 Invasive and Noninvasive Methods

As inflammation is a central feature of asthma, measuring airway inflammation may be useful for the diagnosis and evaluation of asthma (Shelhamer et al., 1995). Until recently, only invasive techniques such as bronchoscopy can directly sample
bronchial tissue and fluids for the quantification of inflammatory cells and measurements of inflammatory mediators (Saetta et al., 1998; Heaney et al., 1996; Stevenson et al., 1997). In the last decade, there has been an increasing interest in the development of noninvasive techniques in assessing the extent of underlying airway inflammation and monitoring the efficacy of treatment (Little et al., 2000).

Noninvasive monitoring of the effect of anti-inflammatory drugs in asthma would potentially be very useful as asthmatic patients may require a long period of anti-inflammatory therapy. One would like to use the lowest possible dose of such therapy (Wilson et al., 2002). Recently, a variety of noninvasive approaches, such as exhaled breath analysis (Kharitonov and Barnes, 2001) for inflammatory markers (exhaled gases and condensate) and sputum induction (Hargreave and Leigh, 1999) have been developed. These approaches allow clinicians to assess the degree of airway inflammation repeatedly. Examination of induced sputum is relatively noninvasive and produces valuable information on airway inflammation. However, induction of sputum can be difficult to use for day to day monitoring, as it provokes transient neutrophilia (Holz et al., 1998) and it may produce temporary decrements in lung function. Furthermore, the processing of sample is time-consuming, expensive, and requires skilled technicians (Magnussen et al., 2000). The exhaled
breath condensate (EBC) approach is only in the early stage of development. The methodology needs to be standardized and validated before they can be used in the clinical setting.

The measurement of exhaled nitric oxide (eNO) has attracted considerable interest, as it is simple, rapid and noninvasive for the assessment of airway inflammation (Lundberg et al., 1996). It can be performed repeatedly without causing undue discomfort for the patients. It can also be used in children, infants (Baraldi et al., 1999b) and in patients with severe airflow obstruction, when other invasive methods are not possible or safe. Over the past decades, there have been many studies suggesting that eNO might be useful in assessing airway inflammation and its level correlated well with other markers of inflammation (Jatakanon et al., 1998b; Mattes et al., 1999; Payne et al., 2001; Warke et al., 2002).

1.1.3 Exhaled Nitric Oxide as a Diagnostic Marker and Its Correlation with Other Markers of Inflammation

Recent evidence suggests that eNO may be an excellent way to confirm or refute a diagnosis of asthma (Smith et al., 2004). Exhaled nitric oxide has been
found to be the first marker to increase during asthma deterioration indicating that it may be used as a loss of control marker in asthma (Kharitonov et al., 1996a).

Exhaled nitric oxide levels were increased prior to any significant changes in lung function, BHR, sputum eosinophilia or asthma symptoms. The deterioration of lung function is related to airway obstruction as a result of airway inflammation. Subclinical inflammation most likely precedes actual functional deterioration so that conventional lung function measures may deteriorate only after a period of ongoing airway inflammation.

Exhaled nitric oxide has been validated against invasive measurements of airway inflammation such as biopsy findings obtained by bronchoscopy and induced sputum in asthma (Jatakanon et al., 1998b; Mattes et al., 1999; Payne et al., 2001; Warke et al., 2002). The inflammatory origin of elevated levels of exhaled NO in asthma (Alving et al., 1993; Kharitonov et al., 1994; Persson et al., 1994), its responsiveness to suppression by corticosteroids (Kharitonov et al., 1996b) and its association with asthma severity may make measurement of exhaled NO an effective and practical marker to monitor the effect of anti-inflammatory treatment in asthma.
1.1.4 Normal Reference Studies of Exhaled Nitric Oxide

Exhaled nitric oxide may be very useful to guide diagnostic and therapeutic decisions in patients. In the past, the lack of standardization of eNO measurement has made it difficult to assess the clinical utility of eNO measurement. Previous studies on reference eNO values show that the range of eNO values varies widely among different studies (Dotsch et al., 1996; Silvestri et al., 1999; Latzin et al., 2002; Jouaville et al., 2003). It may be due to the use of different measurement conditions and techniques in each study (Latzin et al., 2002; Pedroletti et al., 2002). In addition, the sample sizes of these studies were too small to reveal any possible effects of gender and anthropometric measurements on the eNO values (Baraldi et al., 1999a; Franklin et al., 1999).

The normal reference range of exhaled NO levels has been reported to be 10 to 20 ppb in nonatopic control adults (Kharitonov et al., 2003). Levels in patients with steroid-treated asthma are in the range of 30 to 40 ppb, whereas levels are often greater than 80 ppb in steroid-naïve asthmatic patients (Silkoff et al., 2000).

There are no published studies large enough to provide accurate reference
values of eNO in children. The majority of the published studies of exhaled nitric oxide are from Caucasians (Baraldi et al., 1999a; Franklin et al., 1999; Jouaville et al., 2003). There has been a recent study of a small group of Japanese children (n = 278) showing a slightly higher eNO values than in Caucasian children (Saito et al., 2004). There have not been any systematic studies of eNO in Chinese children. Therefore, a reference study of the normal levels of eNO in Chinese is needed. As the production of endogenous NO is dependent on the level of the enzyme NO synthase (NOS) (Yates et al., 1995), it is possible that differences in genetic background may affect the activity of this enzyme resulting in differences in eNO production (Wechsler et al., 2000; van's Gravesande et al., 2003).
1.2 Aim of Study

Traditionally, assessment of asthma is based on symptoms and simple lung function testing. Many of the objective markers of airway inflammation cannot be measured easily in a clinical setting. Exhaled NO potentially provides a very useful noninvasive marker for assessing airway inflammation in asthmatic patients. It is particularly useful in children as the procedure of measurement would not cause any discomfort.

Therefore, the aims of the current study are:

1) To establish the normal reference range of exhaled nitric oxide in the normal Chinese children.

2) To assess the effects of gender and anthropometric measurements on the level of exhaled NO in Chinese schoolchildren.
In order to establish the normal reference range of eNO in Chinese children, recruitment of a representative sample of normal children for the measurement of eNO is necessary. In Hong Kong, there are approximately 7,000,000 people with almost 20% aged under 18 years. In order to establish the reference values of eNO for children, a random sample of normal control children needs to be recruited. There are approximately 400 secondary schools distributed in different districts of Hong Kong. A random sample of secondary schoolchildren can be recruited for participation in this survey. For comparisons, asthmatics identified in the school survey and patients from our paediatric asthma clinic will also be recruited for eNO measurement.

In order to differentiate between normal controls and asthma in a population study, a standardized and validated tool is needed. The International Study of Asthma and Allergies in Childhood (ISAAC) was designed to allow comparisons of asthma prevalence between populations in different countries (ISAAC Steering Committee, 1998).
ISAAC questionnaires consist of a written questionnaire and a video questionnaire. The use of the video one is to overcome the linguistic problems of interpretation of a written questionnaire. The translated ISAAC questionnaires have been validated among Hong Kong schoolchildren in predicting bronchial hyperresponsiveness (BHR) (Lai et al., 1997). Therefore, ISAAC questionnaires can be employed for conducting population-based epidemiological study in order to identify whether the schoolchildren have asthma or not.

Schoolchildren will be recruited for the measurement of eNO. They will complete the ISAAC questionnaires and they will be classified as asthmatic or not according to their responses to the questionnaire. A group of asthma patients followed up at the Prince of Wales Hospital will also be recruited for comparison. For the diagnosis of asthmatic patients from the hospital, the criteria established by the British Thoracic Society (BTS) will be used (British Thoracic Society, 1993). As the use of anti-inflammatory therapy was associated with reduction of eNO level (Baraldi et al., 1997; Kharitonov et al., 1996b), only asthmatics who have not been on steroid for the previous three months will be recruited.
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5.2 Exhaled Nitric Oxide, Induced Sputum Analysis and Sputum Eosinophil Cationic Protein
3.1 Exhaled Nitric Oxide Production in Airway

Nitric oxide (NO) is a simple molecule which unites neuroscience, physiology, and immunology, and revises scientists' understanding of how cells communicate and defend themselves. Nitric oxide has generated much interest in the field of science and it was crowned as “Molecule of the Year” in 1992 (Koshland, 1992). Nitric oxide was first found in the exhaled breath of human by Gustafsson et al. (Gustafsson et al., 1991). Inhibition of the enzyme nitric oxide synthase (NOS) would reduce the level of this gas in the exhaled air in guinea pigs and rabbits. In the respiratory tract, nitric oxide is produced by a wide variety of cell types including epithelial cells, airway nerves, inflammatory cells (macrophages, neutrophils, mast cells), and vascular endothelial cells (Kobzik et al., 1993; Hamid et al., 1993).
3.2 Nitric Oxide Production and Function

Nitric oxide serves as a key signaling molecule in physiological processes such as host defense, neuronal communication, and the regulation of vascular tone (Gross and Wolin, 1995; Liew and Cox, 1991). Although NO is an essential molecule, its production is not always beneficial. Nitric oxide has several key effects on airways. It is important for host defense and has toxic effect on microorganisms (bacteria or viruses). It may increase mucus secretion and act as potent vasodilator. Nitric oxide derived from airway epithelial cells may amplify asthmatic inflammation, by means of inhibition of T-helper 1 (Th1) cells and their production of interferon γ (IFN-γ) (Barnes and Liew, 1995). This would lead to an increase in the number of T-helper 2 (Th2) cells and increase the levels of cytokines such as IL-4 and IL-5.

Once nasal contamination is controlled, several studies have established that NO in exhaled breath originates from the lower airways (Massaro et al., 1996) and is synthesized by NO synthases (NOSs). In normal controls, nitric oxide has been found to have a very high concentration in the paranasal sinuses (Alving et al., 1993; Kharitonov et al., 1996a). Nitric oxide levels are significantly increased in orally exhaled air of patients with inflammatory airways disorders such as asthma,
respiratory tract infection, bronchiectasis, and chronic rejection following lung transplantation.
3.3 Nitric Oxide Synthase Pathway

Nitric oxide is produced by a variety of cells via the nitric oxide synthase (NOS) pathway (Barnes and Belvisi, 1993). Nitric oxide synthase (NOS) is expressed in several cell types in the respiratory tract, including epithelial and vascular endothelial cells, macrophages, eosinophils and neurons (Kobzik et al., 1993; Hamid et al., 1993). Nitric oxide in the exhaled air is produced in the epithelial cells of the bronchial wall. It is generated from the oxidation of guanidine nitrogen of L-arginine catalyzed by the enzyme NOS (Palmer et al., 1988; Moncada and Higgs, 1993; Hemmens and Mayer, 1998). After the hydroxylation of the amino acid L-arginine to N⁵-hydroxy-L-arginine intermediate, nitric oxide is produced by oxidation of N⁵-hydroxy-L-arginine to L-citrulline.

Nitric oxide synthase exists in several distinct isoforms, including neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3) (Nathan and Xie, 1994). NOS1 and NOS3 were named neuronal NOS and endothelial NOS respectively because they were first discovered in the corresponding tissue, the nervous tissue and vascular endothelium (Lamas et al., 1992). Functionally, all of them can be classified into two major classes,
constitutive (cNOS) and inducible (iNOS) isoforms. All three types of NOS have been shown to be expressed in bronchial airways or nasal mucosa. Among them, two of which are constitutive (NOS1 and NOS3) and the other one is inducible (NOS2). Constitutively expressed NOS is present largely in endothelial cells (eNOS) and neuronal tissue (nNOS) while inducible NOS (iNOS) is largely located in airway epithelial cells. In addition to neuronal, epithelial and endothelial cells, constitutively expressed NOS isoforms have been found in platelets (Bates and Silkoff, 2003). This constitutive form enzyme is calcium and calmodulin-dependent and releases picomolar levels of NO within seconds upon receptor stimulation by agonists such as acetylcholine and bradykinin (Berdeaux, 1993). Activation of the enzyme in the neural tissue or endothelium will produce NO for the regulation of neurotransmission and vascular homeostasis.

Inducible NOS (iNOS or NOS2) found in bronchial epithelium can be upregulated many times at sites of inflammation by mediators such as pro-inflammatory cytokines (Morris and Billiar, 1994). Once iNOS is activated, it can produce nanomolar level of NO and the increased production may continue for days (Robbins et al., 1994). This mechanism allows large amounts of NO to be produced at sites of inflammation, thus reflecting the putative role of inducible NOS.
in disease. In addition to bronchial epithelium, inducible NOS are found in several other cell types including hepatocytes and vascular smooth muscle cells.
3.4 Factors Affecting Exhaled Nitric Oxide Level

There are two approaches to measure eNO level in the expired air. They are the online and offline methods (American Thoracic Society, 1999). Online measurement is obtained in a single, flow-controlled exhalation against a resistance which can be easily performed by cooperative children and adults. On the other hand, offline measurement is using similar controlled exhalation during a single exhalation into the suitable collection reservoir which must be sealed for delayed analysis within a short period of time (Baraldi et al., 2002).

Published eNO values for normal controls and asthmatic subjects vary widely among different studies (Baraldi et al., 1999a; Franklin et al., 1999; Jouaville et al., 2003) as the values are highly depending on the measurement techniques (Latzin et al., 2002; Pedroletti et al., 2002). For example, variations in sampling and breath acquisition techniques, mouth pressure and flow of the exhaled air, portion of flow profile taken as the prevailing eNO level (peak versus plateau values) can affect the results. Furthermore, mixing of NO originally produced in the paranasal sinuses and nasal cavity can contaminate the exhaled NO resulting in a much higher level (Lundberg et al., 1994). Differences in measurement techniques make it very
difficult to compare results from different studies (Byrnes et al., 1997; Silkoff et al., 1997).

Therefore, there is a need to standardize the measurement technique if eNO measurements are to be used in the clinical setting and for comparison of results from different laboratories. The factors leading to variation in measured eNO values can be grouped into procedure-related factors, patient factors and environmental factors.

3.4.1 Procedure-related Factors

3.4.1.1 Nasal Nitric Oxide Contamination

It appears that the variation in eNO values has been largely due to nasal NO contamination (Lundberg et al., 1994) and variations in expiratory flows (Latzin et al., 2002). Nasal NO can accumulate to high concentrations relative to the level of NO in the lower respiratory tract. Since nasal NO can enter the posterior nasopharynx and contaminate the exhaled air, it is necessary to close the velopharyngeal aperture in exhalation procedure in order to reduce the leakage of
nasal NO to a minimal level (Baraldi et al., 2002). By maintaining the mouth pressure between 5-20 cm H2O during exhalation, it can ensure the closure of velum effectively thereby preventing nasal contamination.

3.4.1.2 Exhalation Procedure — Starting Lung Volumes

Exhaled NO values can also be influenced by starting lung volumes. Single breath exhalations are frequently performed from total or near-total lung capacity (Silkoff et al., 1997). At the same expiratory flow, the degree of lung expansion affects eNO values. Exhaled NO plateau values obtained with exhalations from the functional residual capacity (FRC) were significantly lower than those from total lung capacity (TLC) by almost 20 % (Baraldi et al., 2002). Therefore, standardization of starting lung volume is important.

3.4.1.3 Exhalation Procedure — Flow

The level of eNO is affected by exhalation flow or sampling flow while breath holding will lead to accumulation of NO in the airways (Silkoff et al., 1997). Expiratory flow has been shown to have great effect on the eNO levels, with an
inverse relationship of eNO and expiratory flow.

The probable reason for the reduction in NO concentration with increasing expiratory flow (Pedroletti et al., 2002) is that the same amount of NO produced by the airway epithelium will be dispersed in a large volume of expired air. Thus, with a higher exhalation rate, NO will be diluted resulting in a lower eNO concentration (Figure 3.1).

![Graph showing Exhaled FeNO at six different flows.](image)

**Figure 3.1:** Exhaled FeNO at six different flows. The difference between groups was significant for FeNO at all flows (Pedroletti et al., 2002).

Studies have shown that children above eight years could achieve good and reproducible results (Silkoff et al., 2004). From a practical standpoint for children,
high exhalation flows cause a rapid decrease in lung volume before the desired pressure is reached (Pedroletti et al., 2002). It will be difficult for young children to maintain exhalation long enough to achieve a plateau. On the other hand, low flows may be impractical, because the exhalation period is too long before NO plateau can be achieved (Pedroletti et al., 2002).

3.4.1.4 Circadian Rhythm

It has been suggested that serial eNO measurement is better performed at the same time of the day as there might be variation of the eNO level in different time of the day. However, several studies have failed to confirm the relationship between the eNO value and the time of measurement recently (Georges et al., 1999; ten Hacken et al., 1998; Latzin et al., 2002; Kharitonov et al., 2003). These studies did not show any significant circadian variation. Therefore, eNO can be measured at any time of the day.
3.4.2 Patient Factors

3.4.2.1 Sex

A number of other factors unrelated to methodology may also affect the eNO levels. Tsang et al. has showed that eNO level is slightly higher in males than in females (Tsang et al., 2001). Other studies of children have been unable to confirm this relation but all these studies were relatively small (Latzin et al., 2002; Silvestri et al., 1999). Further studies with larger sample size are necessary to clarify the possible influence of gender on the eNO levels.

3.4.2.2 Upper Respiratory Tract Infection

The expression of iNOS can also be induced by viral infections in both upper and lower respiratory tract. This phenomenon occurs in normal and asthmatic individuals (Kharitonov et al., 1995; Alving et al., 1993). Therefore, it is important to take this factor into consideration when interpreting the results.
3.4.2.3 Diet and Exhaled Nitric Oxide

There has been a concern that nitrite in the saliva can be converted by nonenzymatic reaction to produce NO which may contribute to eNO level (Zetterquist et al., 1999). There are a variety of foods containing high levels of nitrate. After absorption in the gut, nitrate is actively transported from the blood to the salivary glands and excreted in the saliva (Tannenbaum et al., 1976). Some of the nitrate would undergo reduction to produce nitrite by anaerobic bacteria that colonize in the oral cavity (Zetterquist et al., 1999). This nitrite will then be converted to NO within the oral cavity.

Nitrate is found in large amounts in many green vegetables like spinach and lettuce (Olin et al., 2001). There are not enough data in the literature to make any conclusion concerning how long patients should refrain from eating or drinking before eNO measurement. Elevation of eNO has been found after ingestion of nitrate or nitrate-containing foods (Zetterquist et al., 1999). Further studies are necessary to determine how diet can affect eNO level in normal controls or asthmatic subjects.
3.4.2.4 Effect of Spirometry and Exercise

The effects of prior spirometry and exercise on subsequent eNO measurements have been studied. Tee and Hui investigated 106 asthmatic patients to determine if the eNO levels were different before and after a spirometry maneuver (Tee and Hui, 2005). There was an insignificant decrease of the mean eNO by 2.2 ppb after spirometry. Gabriele et al. performed a study to investigate if exercise would affect the eNO levels. A total of 24 asthmatic children were studied (Gabriele et al., 2005). They performed the eNO measurement before and 5, 15, 30, 45 and 60 minutes after a 6-min walk test. There was a significant drop of mean eNO by 7.1 ppb five minutes after exercise. Subsequently, the eNO levels returned to baseline within 30 minutes after exercise. Therefore, subjects should be resting for at least 30 minutes prior to eNO measurement to avoid this potential confounding factor.

3.4.3 Environmental Factors

As environmental NO can reach high levels relative to those in exhaled breath (Baraldi et al., 1998), standardized techniques must prevent the contamination of biological samples with ambient NO (American Thoracic Society, 1999). The
single-breath NO profile usually consists of an early peak and plateau. The early peak is due to nasal or ambient NO in the airways. Silkoff et al. found that nitric oxide concentration of inhaled gas in the order of 1000 ppb was associated with a huge early peak but had no effect on nitric oxide plateau (Silkoff et al., 1997). Inhaled NO is rapidly taken up by hemoglobin in pulmonary capillaries. Therefore, plateau values appear to be unaffected when the online method of measurement is used. However, if the exhaled gas is collected in a bag for analysis where dead spaces gas is included, exhaled NO values may vary depending on the ambient NO concentrations because of possible contamination.

Adoption of a standardized measurement technique and establishment of a reference range in normal controls are crucial for the use of eNO measurement as a clinical tool. Recently, international investigators in the field of exhaled NO research from the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have jointly developed a guideline for eNO measurement (American Thoracic Society, 2005). Furthermore, the NO monitoring system, NIOX® (Aerocrine AB, Stockholm, Sweden), is the first analyzer which has been approved by the US Food and Drug Administration (FDA) for clinical monitoring of NO in asthmatics (Silkoff et al., 2004). For meaningful comparisons between laboratories,
the use of standardized techniques and validated instrument are important.
Since the discovery of NO in the exhaled breath in 1991 (Gustafsson et al., 1991), several studies have confirmed that exhaled NO is elevated in asthmatic patients when compared with those in normal controls (Alving et al., 1993; Kharitonov et al., 1994; Persson et al., 1994). Furthermore, exhaled NO has been confirmed to decrease with the use of corticosteroids in asthmatic patients (Kharitonov et al., 1996b).

van Rensen et al. studied a group of 25 asthmatic adult patients aged 19-34 years to determine the relationship of eNO and inhaled steroid treatment (van Rensen et al., 1999). The subjects studied were divided into two treatment groups either using inhaled steroids or placebo twice-daily for four weeks. The markers of inflammation were monitored at weeks two and four of treatment, and two weeks after cessation of treatment. The results showed that exhaled NO level would decrease significantly during treatment period in the group taking inhaled
corticosteroid. In addition, the eNO level would increase back to the pretreatment level after washout for two weeks. These findings suggested that monitoring eNO may be an effective way to assess compliance with treatment.

**Figure 4.1:** Inhaled corticosteroids may inhibit transcription of several inflammatory genes in airway epithelial cells.
Chapter 5: Relationship of Exhaled Nitric Oxide with Other Inflammatory Markers

5.1 Correlation of Findings from Biopsy and Bronchoalveolar Lavage with Exhaled Nitric Oxide

In order to characterize and quantify the degree of airway inflammation, several invasive methods have been used and these include bronchoscopy with endobronchial biopsy (Saetta et al., 1998) and bronchoalveolar lavage (BAL) (Heaney et al., 1996; Stevenson et al., 1997). These methods are considered to be the most direct ways to assess the nature and the extent of airway inflammation (Fabbri et al., 1998). However, repeated examination with these invasive methods may not be ethical or acceptable to the patients. In order to evaluate the accuracy of NO measurement as a marker of eosinophilic inflammation indirectly in asthmatic patients, it is important to study the relationship between exhaled nitric oxide level and markers of airway inflammation obtained by these invasive means.
Payne et al. performed a study to investigate the relationship between exhaled NO and the extent of eosinophilic inflammation obtained from biopsy samples of bronchial epithelium of asthmatic children (Payne et al., 2001). In their study, a total of 31 severe asthmatic children were recruited. Biopsy samples were processed with MBP immunostaining and assessed by point counting to determine the “eosinophil score” (Lim et al., 2000). There was a strong correlation ($r = 0.67; P = 0.001$) between the eNO level and eosinophil score of the biopsy specimens.

The relationship between exhaled NO levels of asthmatic subjects and percentage eosinophils in BAL fluid has also been studied recently (Warke et al., 2002). A total of 71 children admitted for elective surgical procedures were recruited for this study. Among them, 29 were asthmatics. BAL fluid obtained during the elective operations were processed for the eosinophil count. Again, a strong correlation between eNO and the percentage of BAL eosinophils was found ($r = 0.78; P < 0.001$).
5.2 Exhaled Nitric Oxide, Induced Sputum Analysis and Sputum Eosinophil Cationic Protein

Eosinophilic inflammation in the airway correlates with the severity of bronchial asthma (Bousquet et al., 1990). The eosinophil counts and the amount of sputum eosinophil cationic protein (ECP) obtained from induced sputum increase in accordance with the severity of airway inflammation (Covar et al., 2004). By inhalation of hypertonic saline through an ultrasonic nebulizer, sputum could be induced successfully by the individuals (Pin et al., 1992). Recently, sputum induction analysis was considered to be a noninvasive method to assess airway inflammation (Spanevello et al., 1995). The relation between exhaled NO and sputum eosinophils has been studied (Jatakanon et al., 1998b). The investigators recruited a total of 35 stable asthmatics for the measurement of eNO and sputum induction by hypertonic saline (3.5 %) inhalation. The results demonstrated a significant correlation ($r = 0.48$) between eNO and sputum eosinophils. Apart from counting the percentage of eosinophils in the induced sputum, sputum ECP can also be analyzed in the supernatants of the sputum plugs using radioimmunoassay (Covar et al., 2004). Exhaled NO has also been found to have a significant correlation with sputum ECP in a group of asthmatic children (Mattes et al., 1999).
In conclusion, there are very strong evidence that eNO is a reliable marker of airway inflammation as shown by its relationship with markers of airway inflammation obtained by either invasive or noninvasive means.
Section III: Original Study

Chapter 6: Methodology

6.1 Study Population
6.2 The International Study of Asthma and Allergies in Childhood
6.3 ISAAC Questionnaires
6.4 Standardized Approach for Answering Questions in the Field
6.5 Anthropometric Measurements
6.6 Exhaled Nitric Oxide Measurement
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Chapter 7: Results

7.1 Subjects and Demography
7.2 Exhaled Nitric Oxide in Chinese Children
7.3 Exhaled Nitric Oxide in Caucasians and Other Ethnic Groups

Chapter 8: Discussion

Chapter 9: Conclusion and Further Studies
6.1 Study Population

Schoolchildren aged 11-18 years were targeted for the study as part of the ISAAC Phase III study (ISAAC Steering Committee, 2000). A complete list of secondary schools was obtained from the Education and Manpower Bureau of the Government of the Hong Kong Special Administrative Region. Each school was individually allocated a number, and by computer randomization, a list of 30 schools was generated. Each school was invited to take part in the study down the order of the school list. Out of the first 15 schools contacted, a total of 12 schools agreed to participate and were able to provide 5,029 schoolchildren as potential subjects. These schools were distributed randomly throughout the three main regions of Hong Kong (Hong Kong Island, Kowloon and New Territories). The Ethics Committee of the Chinese University of Hong Kong approved the study protocol, and the study was performed in the time between February and November 2004. Informed parental consent was distributed to parents or guardians beforehand and 4,320 agreed to enroll their children in the study, representing a response rate of 86%.
Generally, a class sized of 35-40 was assigned as a unit and a double-lesson period was given to each unit for the whole procedures to undertake. Schoolchildren with written agreements of parents or guardians were given a copy of questionnaire to complete. The questionnaire consisted of demographic questions in addition to ISAAC Phase III questionnaires. Since time was not allowed for every participant to perform the following assessments, only one-third of them were randomly recruited. Anthropometric characteristics such as height and weight were assessed for the selected participants followed by exhaled NO measurement. The subjects were asked not to eat for an hour prior to the measurements.
The International Study of Asthma and Allergies in Childhood (ISAAC), was established in the early nineties to study the worldwide variation of asthma and related atopic disorders using standardized methodology (ISAAC Steering Committee, 1998). ISAAC developed from a merging of two multinational collaborative projects each investigating variations in childhood asthma at the population level. These were an initiative from Auckland, New Zealand to conduct an international comparative study of asthma severity, and an initiative from Bochum, Germany for an international study to monitor time trends and determinants of the prevalence of asthma and allergies in children. ISAAC is a unique project which has attracted worldwide interest and unprecedented large scale participation. ISAAC Phase One used simple methods for measuring the prevalence of childhood asthma, allergic rhinitis and atopic eczema for international comparisons.

The ISAAC programme has allowed a worldwide assessment of the prevalence of self-reported symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema in children by standard methods (ISAAC Steering Committee, 1998). For many
collaborating centres, the ISAAC study was the first in their country to assess the epidemiology of these atopic diseases. The core questionnaires of the ISAAC protocol provide means to classify subjects with or without asthma in epidemiology studies.
Each recruited subject was given an ISAAC Phase III written questionnaire to complete, followed by the video questionnaire at the same session in school (Appendix 1). Each subject was required to complete both questionnaires by themselves. The written questionnaire was translated into Chinese following the ISAAC protocol. This involved an independent person who was bilingual to translate the original English questionnaire into Chinese. Another bilingual person then back translated this into English for comparison in accordance with the guidelines laid down by the ISAAC Committee. The translated questionnaire was pilot tested and necessary modifications were made before the survey was carried out.

All students completed the ISAAC questionnaires about wheezing, rhinitis and eczema. In addition, questions about recent health condition (e.g. fever, cold, upper respiratory tract infection) were asked for our reference. During the surveys at schools, written questionnaire was completed before the video questionnaire. The ISAAC written questionnaires asked for information on demography, symptoms of wheeze, asthma, rhinitis and eczema. "Current" symptoms referred to symptoms in
the past 12 months; “asthma ever” was defined as having been diagnosed with asthma by a physician in the subject’s lifetime. “Current asthma” was defined as having symptom of wheeze in the past 12 months in addition to having a diagnostic label of “asthma ever”. “Rhinconconjunctivitis” was defined as sneezing or having a runny nose or blocked nose accompanied by itchy-watery eyes when the subject did not have an upper respiratory tract infection. “Eczema” was defined as an itchy rash which was coming and going for at least six months affecting the folds of elbows, behind the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes. The ISAAC video questionnaire consisted of five scenes displaying young people of different ethnic origins with wheezing at rest, wheeze after exercise, night waking with wheeze, night waking with cough and severe asthma attack. After each sequence, students were asked if their breathing had ever been like that shown in the video and the frequency of such symptoms. There is another set of materials in English prepared for the students who are not familiar with the Chinese language (Appendix 2). The use of the video questionnaire is to overcome the translation problems associated with the written questionnaire as populations with different cultural and language background may have different interpretation of the written questions.
6.4 Standardized Approach for Answering Questions in the Field

Some students would ask questions about their understanding of the questions during the survey conducted at the schools. The teachers were not allowed to explain the study protocol or the method of answering questions. Our research team would be responsible to answer the students’ questions in a standardized way (ISAAC Steering Committee, 1998). The principles are shown as follows:

1. Speak only to the individual who has the problem (identified by a raised hand). The first step is to read the written question out softly, exactly as it is written.

2. *If the student is still unable to answer the question,* the next step is to encourage them to think about the meaning.

3. *If the student is still unable to answer the question,* the next step is to give a little information without explaining the response required.

4. *If the student is still unable to answer the question,* the last step is to state that if the student really does not know how to respond, they should leave the question blank.
6.5 Anthropometric Measurements

Anthropometric measurements are measures to examine subjects' body weight and size in routine clinical practice. It was safe and simple which could be applied easily in our school visits. Standing heights were measured by a portable stadiometer. Weights were measured by Seca digital physician’s scale. During height and weight measurements, subjects should be standing upright, arm resting by their side and without shoes but with socks on. In addition, any personal belongings including wallets, keys and outer clothing should be put down beforehand.

By using height and weight obtained from each subject, body mass index (BMI) (Deurenberg et al., 1991) and body surface area (BSA) (Mosteller, 1987) were calculated according to the following formulas.

$$
\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height (m)}]^2}
$$

$$
\text{BSA} = \sqrt{\frac{\text{Height (cm) x Weight (kg)}}{3600}}
$$
6.6 Exhaled Nitric Oxide Measurement

6.6.1 NIOX® (Aerocrine AB, Stockholm, Sweden)

NIOX® (Aerocrine AB, Stockholm, Sweden) is the first FDA approved Nitric Oxide Monitoring System (Silkoff et al., 2004) for routine clinical use in asthma patients. It measures nitric oxide (NO) in exhaled air according to ATS/ERS standard of eNO measurement (American Thoracic Society, 1999) and defined in parts per billion (ppb). It employs a chemiluminescence gas analyzer and integrated computer software to measure NO molecules at very low concentrations accurately. Based on the principal of chemiluminescence (Archer, 1993), NO in exhaled breath is drawn to a cooled reaction chamber and reacts with ozone as described follows:

\[
\text{NO} + \text{Ozone} \rightarrow \text{Nitrogen dioxide} + \text{Oxygen} + \text{Photon}
\]

Upon the reaction shown above, nitrogen dioxide is formed under a photochemical reaction with the emission of photon which can be detected by a photomultiplier tube. The quantity of photon is proportional to the amount of NO in the exhaled breath sample.
The NIOX® analyzer provides a single-breath online measurement of NO in a single exhalation with a measurement range of 2-200 ppb which is suitable for analysis. Despite the advanced technology employed, the instrument is relatively easy to use and is suitable not only for adults, but also for cooperative children (Baraldi et al., 2002).

6.6.2 Calibration Procedures

A daily calibration of the NIOX® instrument was not absolutely required unless it was moved or shut off. According to NIOX® analyzer manufacturer recommendation, regular calibration was usually performed every 14 days. However, if the environmental conditions were unstable, recalibration command was requested by the NIOX® instrument automatically in order to maintain a constant environment (e.g. stable temperature and humidity). Measurement of exhaled NO should not be continued until recalibration procedure was carried out properly. The NIOX® instrument was calibrated using standard NO calibration gas with known concentration which was manufactured by AGA Linde Healthcare. A two-point calibration was performed at 0 ppb and 200 ppb. After the zeroing procedure, the high calibration point was regulated by using the certified NO gas with guaranteed
concentration. A reference line was constructed in accordance with the calibration result each time.

6.6.3 Exhaled Nitric Oxide Measurement

Subjects first completed the ISAAC questionnaires and have been resting for at least 30 minutes prior to eNO measurement. Since the determination of eNO varies widely depending on the measuring techniques and conditions, exhaled NO was measured online using the NIOX® (Aerocrine AB, Stockholm, Sweden) according to the recommendation of the ATS/ERS guidelines (American Thoracic Society, 1999).

Exhaled NO measurement was obtained with the subjects in the standing position. Subjects did not wear nose clips, and were instructed not to hold their breath before exhalation. Subjects were required to exhale to residual volume. In order to eliminate possible interference from ambient NO levels, subjects inhaled through an ambient air filter to total lung capacity and then exhale immediately for about ten seconds. In order to keep the soft palate closed for preventing nasal NO contamination, the column of collecting device has an internal restrictor that creates a mouth pressure of 10-20 cm H₂O during exhalation. The mouth pressure was
displayed on a screen instantaneously with the help of visual animation for the subjects to maintain a constant flow. At this mouth pressure, the expiratory flow would be kept constant at 50 ml/s. In the current study, NIOX® ambient air filter, which was able to filter against bacteria and virus, was employed. After each individual performance had completed, one’s NIOX® filter should be disposed and subjected to replace with a new one for every new patient. Since environmental NO may sometimes be very high (> 100 ppb), inhalation of NO-free air through the NIOX® filter was preferable. At the same time, ambient air levels of NO were also measured by the NIOX® instrument automatically for each test which should be recorded immediately before assessing each new subject.

The plateau value of exhaled NO was recorded automatically with the manufacturer’s software. Participants repeated the maneuver after a minimum of 30-second resting period until three consecutive acceptable tests with plateau values of exhaled NO were obtained. The ATS/ERS standard requires three eNO values varied less than 10 % (American Thoracic Society, 1999). The mean of these three readings was recorded as the exhaled NO level for a particular subject. Any exhalation which did not meet the standard requirements was not accepted by the manufacturer’s software automatically and the subjects were asked to attempt
continuously until three acceptable readings were obtained. However, care should be taken in order not to make the subjects too exhausting if repeated measurements were required to perform. The maximum allowable number of exhalations per participant is eight according to the ATS/ERS standard (American Thoracic Society, 1999).
6.7 Classification of Subjects

Data from subjects who had symptoms of upper respiratory tract infection (URI) within the past two weeks were excluded as respiratory tract infection was known to affect eNO level (Kharitonov et al., 1995). The subjects were classified into three groups consisting of “normal controls”, “allergic non-asthmatics” and “asthmatics”. Those subjects who were not classified as anyone of the groups of “normal controls”, “allergic non-asthmatic” or “asthmatics” but having symptom of wheezing ever only were grouped as “others”.

For those subjects who had never been diagnosed to have asthma and did not have any symptoms of wheeze, rhinitis or eczema in their lifetime according to the written and video questionnaires were classified as “normal controls”. Subjects who had a physician diagnosis of asthma ever or current wheeze as documented by either written or video questionnaires were considered as “asthmatics”. “Current asthma” was defined as having symptom of wheeze in the past 12 months in addition to having a diagnostic label of “asthma ever”. The remaining subjects who did not have asthma and wheezing symptoms but had symptoms of rhinitis or eczema as documented by the ISAAC questionnaires were categorized as “allergic
non-asthmatics”.

For comparison, another group of subjects with confirmed diagnosis of asthma according to the British Thoracic Society (BTS) criteria (British Thoracic Society, 1993) was recruited for eNO measurement. They were recruited from the paediatric asthma clinic at the Prince of Wales Hospital (Hong Kong). These patients have not been taking anti-inflammatory treatment for three months prior to eNO measurement as the use of anti-inflammatory therapy was associated with reduction of eNO level (Kharitonov et al., 1996b).
6.8 Statistical Analysis

All data were entered into a computer twice by two independent persons. The data were categorized and analyzed using Statistical Package for Social Sciences (SPSS) for Windows Release 10.0 (SPSS Inc., Chicago, IL, USA). Exhaled NO levels in parts per billion (ppb) were presented as mean and median with inter-quartile range (IQR) unless otherwise stated. The eNO data was analyzed and was skewed to the right. The eNO values were therefore logarithmically transformed before analysis. Comparisons between groups were performed using Student’s *t* test or Mann-Whitney *U*-test. The correlation between eNO and anthropometric measurements, such as height, weight, body mass index (BMI), and body surface area (BSA), were determined by Spearman rank correlation test. All comparisons were made two-sided, and *P*-values less than 0.05 were considered significant.

Furthermore, the influence of other atopic diseases such as rhinoconjunctivitis or eczema on eNO levels was also assessed by comparing the eNO level in children with and without these conditions.
Chapter 7: Results

7.1 Subjects and Demography

A total of 4,320 schoolchildren completed the questionnaire survey as part of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase III study. A random subgroup of 1,399 students was recruited for eNO measurement. Data from subjects who had symptoms of upper respiratory tract infection (URI) within the past two weeks were excluded as respiratory tract infection was known to affect eNO level (Kharitonov et al., 1995). Among the participating subjects without recent URI, 1,089 were Chinese and 165 were Caucasians. The other 106 children were of other ethnic backgrounds such as Indian, Japanese, Korean, Malaysian or mixed ethnic background.
Subjects examined (n = 1,399; 738 males)

Caucasians (n = 175; 97 males)
No URI (n = 165; 91 males)

Other ethnic groups (n = 111; 60 males)
No URI (n = 106; 56 males)

Chinese (n = 1,113; 581 males)
Not successful (n = 1; 1 male)
(unable to perform)
Successful (n = 1,112; 580 males)
URI (n = 23; 15 males)
No URI (n = 1,089; 565 males)

“Others” (n = 81; 45 males)

“Normal controls” (n = 752; 368 males)
“Allergic non-asthmatics” (n = 197; 118 males)
“Asthmatics” (n = 59; 34 males)

Figure 7.1: Flow chart of subjects with eNO measurement.
The demographic characteristics of children who participated in the study are summarized in Table 7.1. There were a total of 752 Chinese (368 males) and 57 Caucasian (25 males) controls. In addition, there were 59 Chinese asthmatics (34 males) and 65 Caucasian asthmatics (43 males). There were 20 Chinese children (14 males) with “current asthma”. A total of 106 subjects (56 males) were of the other ethnic backgrounds. The remaining 197 Chinese (118 males) and 23 Caucasians (13 males) did not have asthma and wheezing symptoms but they had symptoms of rhinitis or eczema as documented by the ISAAC questionnaires. These subjects were categorized as “allergic non-asthmatic”. Among 1,089 Chinese without recent upper respiratory tract infection, 81 subjects who were not classified as anyone of the groups of “normal controls”, “allergic non-asthmatic” or “asthmatics” but having symptom of wheezing ever only were grouped as “others”. 
Table 7.1: Demographic and anthropometric data of participants.

<table>
<thead>
<tr>
<th></th>
<th>Chinese Controls</th>
<th>Allergic non-asthmatics</th>
<th>Asthmatics</th>
<th>Caucasian Controls</th>
<th>Other ethnic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>752</td>
<td>197</td>
<td>59</td>
<td>57</td>
<td>106</td>
</tr>
<tr>
<td>Male</td>
<td>368</td>
<td>118</td>
<td>34</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>Female</td>
<td>384</td>
<td>79</td>
<td>25</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
<td>14.6 ± 1.8</td>
<td>14.7 ± 2.0</td>
<td>15.2 ± 2.1</td>
<td>13.1 ± 1.6</td>
<td>13.9 ± 1.9</td>
</tr>
<tr>
<td>(IQR)</td>
<td>(13.3-15.7)</td>
<td>(13.1-16.0)</td>
<td>(13.5-16.9)</td>
<td>(12.0-13.8)</td>
<td>(12.5-15.4)</td>
</tr>
<tr>
<td>Mean height ± SD (cm)</td>
<td>160.0 ± 8.9</td>
<td>160.7 ± 9.1</td>
<td>163.0 ± 9.9</td>
<td>158.0 ± 10.5</td>
<td>159.0 ± 8.7</td>
</tr>
<tr>
<td>Mean weight ± SD (kg)</td>
<td>50.2 ± 10.4</td>
<td>51.7 ± 11.2</td>
<td>53.5 ± 10.3</td>
<td>50.0 ± 18.5</td>
<td>51.4 ± 10.0</td>
</tr>
<tr>
<td>Mean body mass index ± SD (kg/m²)</td>
<td>19.5 ± 3.1</td>
<td>19.9 ± 3.3</td>
<td>20.0 ± 3.0</td>
<td>19.8 ± 6.4</td>
<td>20.2 ± 3.1</td>
</tr>
<tr>
<td>Mean body surface area ± SD (m²)</td>
<td>1.49 ± 0.18</td>
<td>1.51 ± 0.19</td>
<td>1.55 ± 0.18</td>
<td>1.47 ± 0.27</td>
<td>1.50 ± 0.18</td>
</tr>
</tbody>
</table>
7.2 Exhaled Nitric Oxide in Chinese Children

Since the distribution of exhaled nitric oxide levels of the population of normal controls was skewed to the right as shown in figure 7.2, logarithmic transformation of eNO values were performed prior to further statistical comparison between groups (Figure 7.3).
Figure 7.2: Histogram of levels of exhaled nitric oxide in the study population. The theoretical normal distribution is given by the line.

Figure 7.3: Histogram of log transformed levels of exhaled nitric oxide in the study population. The theoretical normal distribution is given by the line.
Figure 7.4: Box-plots showing median and inter-quartile ranges of exhaled nitric oxide (eNO) in Chinese controls. Y-axis is shown in log scale.

The mean eNO (median; IQR) in males was 17.4 ppb (11.3; 7.6-20.9) while it was 12.1 ppb (8.8; 5.9-12.8) in females. The eNO level was higher in males than in females ($P < 0.001$).
Figure 7.5: Box-plots showing median and inter-quartile ranges of exhaled nitric oxide (eNO) in Chinese controls, allergic non-asthmatics and asthmatics.

Y-axis is shown in log scale. (* $P < 0.001$)
Figure 7.5 shows the comparison of eNO levels for Chinese controls, allergic non-asthmatics and asthmatics. For asthmatic males, the mean eNO (median; IQR) was 43.4 ppb (33.3; 12.0-56.0); for asthmatic females, 35.0 ppb (18.0; 10.5-51.1). For both males and females, the eNO levels of the asthmatics were higher than that of normal controls of the same sex ($P < 0.001$). The mean (median; IQR) eNO of Chinese children with “current asthma” (14 males and 6 females) was 56.0 ppb (47.1; 29.1-67.5).

For the allergic non-asthmatic Chinese males, the mean eNO (median; IQR) was 27.6 ppb (19.0; 11.0-40.5); for females, 18.5 ppb (12.3; 8.7-25.4). The eNO level for this group of subjects was also higher in males than in females ($P = 0.003$). For both Chinese male and female, the eNO level of asthmatics was higher than that of allergic non-asthmatics ($P = 0.048$ and $P = 0.027$ respectively) as shown in figure 7.5. In addition, the eNO level of allergic non-asthmatics was also different from that of normal controls of the same sex ($P < 0.001$).
Figure 7.6: Box-plots showing median and inter-quartile ranges of exhaled nitric oxide (eNO) in Chinese controls and clinic asthmatics. Y-axis is shown in log scale.
A total number of 91 asthmatics (54 males) were recruited from the asthma clinic for eNO measurement. The mean age (range) was 12.3 years (6-21). Figure 7.6 shows the comparison of eNO levels for Chinese controls and clinic asthmatic subjects. The exhaled NO level was again significantly higher in subjects with asthma than in non-asthmatic normal controls. For asthmatic males, the mean eNO (median; IQR) was 52.0 ppb (45.5; 28.6-73.4); for asthmatic females, 52.3 ppb (47.2; 32.4-70.0). For both males and females, the eNO levels of the asthmatics were higher than that of normal controls of the same sex ($P < 0.001$). These results are similar to those found in the asthmatic children recruited from schools.
Table 7.2 shows the correlation between the eNO levels and the anthropometric measurements in Chinese normal controls. Exhaled NO levels did not show any significant correlation with height, weight, body mass index (BMI) or body surface area (BSA).

Table 7.2: Correlation of exhaled nitric oxide level and anthropometric measurements in Chinese normal controls.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
<th>Combined</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 368</td>
<td></td>
<td>n = 384</td>
<td></td>
<td>n = 752</td>
<td></td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Height</td>
<td>-0.086</td>
<td>0.098</td>
<td>-0.032</td>
<td>0.541</td>
<td>0.050</td>
<td>0.167</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.049</td>
<td>0.346</td>
<td>0.018</td>
<td>0.727</td>
<td>0.043</td>
<td>0.242</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.007</td>
<td>0.895</td>
<td>0.028</td>
<td>0.581</td>
<td>0.017</td>
<td>0.643</td>
</tr>
<tr>
<td>Body surface area</td>
<td>-0.057</td>
<td>0.279</td>
<td>0.013</td>
<td>0.800</td>
<td>0.050</td>
<td>0.168</td>
</tr>
</tbody>
</table>
7.3 Exhaled Nitric Oxide in Caucasians and Other Ethnic Groups

Table 7.3 shows the results of the current study compared with the population reference values reported in the literature. Chinese controls were found to have slightly higher eNO levels when compared with the levels of Caucasians reported in the literature (Dotsch et al., 1996; Silvestri et al., 1999; Baraldi et al., 1999a; Franklin et al., 1999; Latzin et al., 2002; Jouaville et al., 2003).

Table 7.3: Comparison of reference values of eNO in normal control children.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>n</th>
<th>Median age (years)</th>
<th>Mean eNO (ppb)</th>
<th>Expiratory flow (ml/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dotsch et al. (1996)</td>
<td>37</td>
<td>Not given</td>
<td>3.0</td>
<td>Not given</td>
</tr>
<tr>
<td>Silvestri et al. (1999)</td>
<td>22</td>
<td>Not given</td>
<td>4.0</td>
<td>Not given</td>
</tr>
<tr>
<td>Baraldi et al. (1999)</td>
<td>159</td>
<td>6-15 (range)</td>
<td>8.7</td>
<td>Not given</td>
</tr>
<tr>
<td>Franklin et al. (1999)</td>
<td>157</td>
<td>9.7 (mean)</td>
<td>10.3</td>
<td>50</td>
</tr>
<tr>
<td>Latzin et al. (2002)</td>
<td>63</td>
<td>12.2</td>
<td>11.9</td>
<td>45</td>
</tr>
<tr>
<td>Jouaville et al. (2003)</td>
<td>96</td>
<td>10.3</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>Current study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese male</td>
<td>368</td>
<td>14.4</td>
<td>17.4</td>
<td>50</td>
</tr>
<tr>
<td>Chinese female</td>
<td>384</td>
<td>14.4</td>
<td>12.1</td>
<td>50</td>
</tr>
</tbody>
</table>
The eNO levels of Caucasians and other ethnic groups in the current study are summarized in table 7.4. For Caucasian male controls in our study, the mean eNO (median; IQR) was 14.1 ppb (12.5; 8.8-19.1); for Caucasian females, 14.6 ppb (10.7; 6.0-13.5). Although the level was higher in males than in females, the difference did not reach statistical significance ($P = 0.075$).

For the allergic non-asthmatic Caucasians ($n = 23$), their mean eNO (median; IQR) was 24.9 ppb (14.1; 9.5-38.2). The mean eNO (median; IQR) of asthmatic Caucasians ($n = 65$) was 32.9 ppb (15.8; 10.3-44.3). Their eNO were higher than the level of Caucasian controls (which were $P = 0.023$ and $P < 0.001$) respectively.

We have also compared the eNO levels of Chinese and Caucasian controls. For both sexes, there was no significant difference between the levels in Chinese and Caucasians.

For the control subjects of other Asian races ($n = 24$), their mean eNO (median; IQR) was 17.6 ppb (10.2; 8.6-25.0). Further subgroup analysis was not performed because of the small sample size.
Table 7.4: Exhaled NO of normal controls in Caucasians and other Asian groups.

<table>
<thead>
<tr>
<th></th>
<th>Caucasians Controls</th>
<th>Other Asian groups Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 57 (25 males)</td>
<td>n = 24 (14 males)</td>
</tr>
<tr>
<td>Male</td>
<td>12.5 (8.8-19.1)</td>
<td>13.6 (8.9-30.9)</td>
</tr>
<tr>
<td>Female</td>
<td>10.7 (6.0-13.5)</td>
<td>9.0 (7.9-13.1)</td>
</tr>
<tr>
<td>All</td>
<td>11.7 (7.6-14.6)</td>
<td>10.2 (8.6-25.0)</td>
</tr>
</tbody>
</table>
Asthma is a common condition affecting 10-30% of children in developed
countries. Despite the availability of effective therapeutic agents, many of the
asthmatics continue to suffer significant morbidity related to sub-optimal treatment
(Rabe et al., 2004). Although international guidelines recommend that treatment
should be adjusted according to the level of severity reflected by the symptoms
reported by patients along with lung function testing, many patients tend to
underreport their symptoms resulting in underestimation of the severity by the
physicians.

Airway inflammation is one of the most important components of asthma.
There are different methods to assess the degree of airway inflammation in asthmatic
patients. Over the past decades, there have been a lot of research studies showing
that exhaled nitric oxide may represent a useful noninvasive marker of airway
inflammation. A recent prospective trial has also confirmed that the measurement
of eNO might help the clinicians to use lower maintenance doses of inhaled steroid
in managing patients with persistent asthma (Smith et al., 2005).
In order to utilize exhaled nitric oxide measurement for the diagnosis and monitoring of asthma, normal reference range of eNO needs to be established. In the study of the current thesis, we aim at establishing the normal reference range of eNO in Chinese schoolchildren. In addition, we also determine the possible influence of gender and anthropometric measurements on the eNO levels.

In this study with a large community sample of schoolchildren, a validated online monitoring system, NIOX® (Aerocrine AB, Stockholm, Sweden), was employed and measurements were made according to ATS and ERS guidelines. We have shown that the eNO level of normal controls is significantly higher in boys than in girls. In line with what has been published in the literature (Kharitonov et al., 1994), Chinese children with asthma or symptoms of wheeze had significantly higher eNO level than in controls. Furthermore, the results of the present study also confirm that there is a strong correlation between exhaled NO levels and symptoms of other allergic diseases such as rhinitis.

This is one of the largest studies to investigate the eNO level in a community sample of schoolchildren. In Chinese children, the ISAAC questionnaires have been validated against methacholine induced bronchial hyperresponsiveness (BHR)
(Lai et al., 1997). Having a physician diagnostic label of "asthma" had a sensitivity of 0.88 and a specificity of 0.90 in predicting asthma associated BHR. For the purpose of epidemiological study, the ISAAC questionnaires can be used for separating "asthmatics" from "normal controls". Many recent studies have demonstrated the potential of eNO measurement as a promising noninvasive marker for the diagnosis and assessment of asthma. Establishment of normal reference level is important for determining the utility of eNO measurement in different populations. Many research studies in the past decade were directed at investigating the relationship of eNO and other invasive markers of airway inflammation in asthmatics. Administration of oral or inhaled corticosteroids has been found to decrease eNO level in treated patients (Jatakanon et al., 1998a; Little et al., 2000). Silverstri et al. demonstrated that the eNO level was significantly related to the absolute number or percentage of blood eosinophils in asthmatic patients (Silvestri et al., 1999). Furthermore, Piacentini et al. has showed that FEV₁ has a significant inverse relationship with eNO level in asthmatic children (Piacentini et al., 2000). Exhaled NO level has also been found to have a significant relationship with serum eosinophil cationic protein (Strunk et al., 2003). Taken together, all these studies suggested that eNO level does reflect the degree of airway inflammation and may be useful in asthma monitoring. Among the methods of
monitoring airway inflammation, only eNO measurement is a truly noninvasive method providing real-time results in the clinic setting.

Although there have been a few population studies of the reference values of eNO in children, the sample sizes of these studies were too small to reveal the possible effects of gender and anthropometric measurements on the eNO levels (Latzin et al., 2002; Franklin et al., 2003; Jouaville et al., 2003). The current study clearly demonstrated a gender difference of eNO in Chinese schoolchildren. In the small group of Caucasian subjects in our study, we have also found a trend of higher eNO in males than in females but the difference did not reach statistical significance ($P = 0.075$). Further larger studies in Caucasians and other ethnic groups are needed to confirm whether such gender difference documented in the Chinese population is universal or not. The exact reasons for the possible gender differences are unknown.

There are differences in the dimensional, structural, functional, immunological, hormonal characteristics and disease manifestations between the airways of male and female (Becklake and Kauffmann, 1999), and these differences may lead to varying levels of eNO in males and females. The lungs of girls are generally smaller than
those of boys. One explanation for this gender difference could be that males have larger lung volumes including large expiratory dead space volumes relative body indices. Expiratory NO originates mainly in the dead space compartments and the size of this compartment may influence the total NO airway diffusing capacity, a factor that may influence the eNO level. Difference in flow within the intrathoracic airways may be one of the reasons for the gender difference. As the level of eNO is flow-dependent (lower eNO level with higher flow), to generate the same flow at the mouth level, the flow in the small airways should be higher in the girls as their airways are relatively smaller than those in the boys. As a result, the eNO concentration at the mouth level at a constant flow would be lower in females.

Previous studies have demonstrated that atopic individuals have higher eNO level even when they do not have asthma or symptoms of wheeze (Franklin et al., 2003; Jouaville et al., 2003). Data from a recent ISAAC study in Chinese schoolchildren showed that the prevalence of atopy as demonstrated by skin prick testing (SPT) was about 30% which is similar to those reported in England and Australia (Wong et al., 2001). Difference in the rate of atopy among boys and girls may also be partly responsible for the gender difference. Dietary difference is another possible reason affecting the eNO levels. Exhaled NO level has been found
to be significantly elevated after intake of a nitrate-rich meal (Olin et al., 2001). Nitrate is found to be present in relatively larger amounts in green vegetables such as lettuce and spinach. Studies of the possible food components in Chinese diet, which may affect the eNO level, are necessary.

For the subjects of other Asian races in the current study, there were 24 control subjects (14 males) and their mean eNO was 17.6 ppb, which is comparable to the Chinese controls. The sample size of this group is rather small such that further subgroup analyses were not performed. Additional studies with larger sample of other Asian races are required to determine if their eNO levels are different from those of the Caucasians or Chinese. In addition, the eNO levels of the allergic non-asthmatic children in both Chinese and Caucasians were intermediate between the levels of the controls and asthmatics. The differences between the controls and their asthmatic counterparts were statistically significant in Chinese schoolchildren. Although there appears to be a big overlap of the eNO levels between normal controls and “asthmatics” in this study, one should note that the group of “asthmatic” children recruited from the schools included all those with a diagnostic label of asthma even if they did not have any current symptoms and some children (32 %) in this category were on anti-inflammatory therapy. Therefore, a proportion of these
children might no longer have significant airway inflammation resulting in lower eNO levels. As shown in the figure 7.5, children with a physician’s label of asthma along with symptom of wheeze within the past 12 months had the highest eNO level (median = 47.1 ppb). Measurement of a large group of confirmed asthmatic not on anti-inflammatory therapy will be necessary to fully assess the utility of eNO measurement as a diagnostic tool for asthma in children.
In order to improve asthma diagnosis and monitoring, a simple and reliable biomarker of airway inflammation is needed. Research studies in the past decade have confirmed that exhaled nitric oxide represents an accurate and reliable marker of airway inflammation. Exhaled NO levels correlate well with other known markers of airway inflammation obtained by invasive and noninvasive means. Clinical studies have confirmed that eNO predicts asthma exacerbations and its level will decrease with the use of anti-inflammatory therapy such as inhaled steroid.

We have successfully recruited a large number of schoolchildren for the measurement of exhaled nitric oxide. We have demonstrated that eNO level is higher in boys than in girls. As reported in the literature, the eNO level was much higher in the asthmatics when compared with the normal controls. Children with other allergic diseases will also have higher level of eNO even when they do not have asthma or wheeze. The results of our study have established the normal reference range of exhaled NO in Chinese boys and girls. We have also confirmed
that anthropometric measures such as weight, height, BMI and body surface area do not have significant influences on the level of eNO.

Several questions related to eNO remain unanswered. We have only tested a small group of Caucasian children and children of other Asian racial groups. Our sample did not have enough power to detect any gender difference of eNO within these racial groups. Studies with larger sample of these children are needed. The difference of eNO level between Chinese males and females is interesting although the exact reasons for the difference remain to be explored. This gender difference has important clinical implication because different cutoff levels should be used for males and females in the differentiation of asthmatics from normal controls.

In order to maximize the participation rate of our study, we did not include skin prick testing (SPT) and blood testing for IgE measurements. As a result, we are not able to determine the relationship of atopy (as documented by SPT or serum IgE) and eNO level in our subjects. Further studies including these objective measures of atopy are needed to determine the influence of atopy on the eNO levels. Furthermore, genetic studies of the NOS polymorphism are required to reveal their possible influence on the eNO levels in different individuals (van's Gravesande et al.,)
2003; Wechsler et al., 2000; Wang et al., 2004). In addition, we should also extend our study to a younger age group in future studies to determine if children under 11 years of age will have similar eNO levels when compared with the older children.

Finally, measurements of the eNO levels in large groups of confirmed asthmatics including atopic and nonatopic asthma are needed to determine the optimal cutoff values in differentiating asthmatics from normal controls in Chinese children.
Appendix 1  Questionnaires (Chinese Version)
Appendix 2  Questionnaires (English Version)

References
香港中文大學
醫學院兒科學系
學生健康問卷調查2004

請回答以下問題，並在適當的答案空格加上「✓」號或填上答案。如果選擇錯誤，請將選錯答案劃去，並在適當的答案空格內塡上。

學校名稱： __________________________ 班別： ________________

今天日期： _______年 ______月 ______日

學生姓名：（中文） __________________ （英文） __________________

年齡： ______歲 出生日期： ______年 ______月 ______日

性別： 1️⃣ 男 2️⃣ 女

種族： 1️⃣ 華人 2️⃣ 非華人

請註明： ________________

出生地點： ________________________________

● 如果你不是在香港出生，你曾在香港居住多久？ ________年

地址：（英文） ________________________________

住址電話： ________________ 父親手提電話： ________________

母親手提電話： ________________ 學生手提電話： ________________

請回答以下問題，並在適當的答案空格加上「✓」號。

● 過去2星期內，你有沒有上呼吸道感染
  （例如：傷風、感冒等症狀）？

  1️⃣ 有 2️⃣ 沒有

醫生量度（學生不用塡寫）

檢查時間： ________________

身高： ______（厘米） 體重： ______（千克） 最近一餐進食時間： ______

eNO： __________ Avg FENO： __________ Avg VNO： __________
請回答以下問題，並在適當的答案空格加上「✓」號。

1. 你的胸部過往有沒有曾經發出
   喘聲、氣緊或 He He 聲？
   【如果你的答案是「沒有」的話，請跳往問題（6）繼續作答。】
   1. 有
   2. 沒有

2. 過去 12 個月內，你的胸部有沒有發出
   喘聲、氣緊或 He He 聲？
   【如果你的答案是「沒有」的話，請跳往問題（6）繼續作答。】
   1. 有
   2. 沒有

3. 過去 12 個月內，你曾有過多少次氣緊？
   1. 沒有
   2. 1-3 次
   3. 4-12 次
   4. 12 次以上

4. 過去 12 個月內，你平均有多少晚是
   因為這些氣緊而從睡眠中醒過來？
   1. 從沒有因氣緊而從睡眠中醒過來
   2. 平均每星期少於 1 晚
   3. 平均每星期 1 晚或多於 1 晚

5. 過去 12 個月內，你有沒有因為氣喘到太嚴重
   而影響說話能力，每次吸氣只能講一至兩個字？
   1. 有
   2. 沒有

6. 你過往有沒有曾經透過醫生或醫院得知患上哮喘？
   1. 有
   2. 沒有

7. 過去 12 個月內，你有沒有服用過任何
   醫治氣喘（哮喘或氣管敏感）藥物？
   1. 有
   2. 沒有

8. 過去 12 個月內，你運動時或運動之後，
   胸部有沒有發出喘聲或咳嗽？
   1. 有
   2. 沒有

9. 過去 12 個月內，除了患上傷風或肺部受感染的時候，
   你晚上有沒有乾咳？
   1. 有
   2. 沒有
當你沒有患上傷風或感冒的時候，問題 10-14 所提及的情況有沒有出現？

10. 你有沒有曾經患有打噴嚏、流鼻水或鼻塞問題？
   （注意：傷風或感冒的時候不計算在內）
   【如果你的答案是「沒有」的話，請跳往問題（14）繼續作答。】
   □ 有  □ 沒有

11. 過去 12 個月內，你有沒有患有打噴嚏、流鼻水或鼻塞問題？
    （注意：傷風或感冒的時候不計算在內）
    【如果你的答案是「沒有」的話，請跳往問題（14）繼續作答。】
    □ 有  □ 沒有

12. 過去 12 個月內，上列鼻部不適出現時，
    你有沒有同時出現流眼水和眼睛癢癢的問題？
    □ 有  □ 沒有

13. 過去 12 個月內，上列鼻部不適怎麼影響你的日常生活？
    □ 毫無影響  □ 有些影響  □ 相當影響  □ 嚴重影響

14. 你過去有沒有曾經透過醫生或醫院得知患上鼻敏感？
    □ 有  □ 沒有

15. 你有沒有有一些痕疹持續半年或以上都未消散？
    【如果你的答案是「沒有」的話，請跳往問題（20）繼續作答。】
    □ 有  □ 沒有

16. 過去 12 個月內，這種痕疹有沒有發作？
    【如果你的答案是「沒有」的話，請跳往問題（20）繼續作答。】
    □ 有  □ 沒有

17. 這種痕疹有沒有影響以下身體部位：手肘內側、膝頭後面，
    腳踝前面、臀部下端、頸、耳朵或眼睛四周？
    □ 有  □ 沒有

18. 過去 12 個月內，這種痕疹有沒有完全消散？
    □ 有  □ 沒有

19. 過去 12 個月內，你平均有多少晚
    是因為這種痕疹而不能入睡？
    1 □ 過去 12 個月內從沒有
    2 □ 平均每星期少於 1 晚
    3 □ 平均每星期 1 晚或多於 1 晚

20. 你過去有沒有曾經透過醫生或醫院得知患上濕疹？
    □ 有  □ 沒有
錄影帶問題

請留意影帶片段，並在適當的答案空格加上「✓」號。

片段一：片段一是一個年輕人在靜止狀態下的情況。

<table>
<thead>
<tr>
<th>問題一</th>
<th>你在一生中，曾否出現過像片段中這種呼吸狀態？</th>
<th>有</th>
<th>沒有</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>如果有：在去年出現過？</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>如果有：每月出現1次或多次？</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

片段二：片段二是兩個年輕人正在運動的情況。

一個是穿深色衫，而另一個是穿淺色衫。

<table>
<thead>
<tr>
<th>問題二</th>
<th>你在一生中，曾否出現過像片段中穿深色衫的男孩那樣在運動時或運動後這種呼吸狀態？</th>
<th>有</th>
<th>沒有</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>如果有：在去年出現過？</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>如果有：每月出現1次或多次？</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

片段三：片段三是一個年輕人在夜間醒過來的情況。

<table>
<thead>
<tr>
<th>問題三</th>
<th>你在一生中，曾否出現過像片段中這樣在夜間醒過來？</th>
<th>有</th>
<th>沒有</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>如果有：在去年出現過？</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>如果有：每月出現1次或多次？</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

片段四：片段四也是一個年輕人在夜間醒過來的情況。

<table>
<thead>
<tr>
<th>問題四</th>
<th>你在一生中，曾否出現過像片段中這樣在夜間醒過來？</th>
<th>有</th>
<th>沒有</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>如果有：在去年出現過？</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>如果有：每月出現1次或多次？</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

片段五：片段五是另外一個年輕人在靜止狀態下的情況。

<table>
<thead>
<tr>
<th>問題五</th>
<th>你在一生中，曾否出現過像片段中這種呼吸狀態？</th>
<th>有</th>
<th>沒有</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>如果有：在去年出現過？</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>如果有：每月出現1次或多次？</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---問卷完---

***多謝您參與此項調查***
Please tick “✓” or write your answers to these questions in the space provided. Most questions require you to tick “✓” your answer in a box. If you make a mistake put a cross in the box and tick the correct answer.

School: ___________________________ Class: _______

Today’s Date: __________ / __________ / __________
Day  Month  Year

Name: ___________________________ Age: _______

Date of Birth: __________ / __________ / __________
Day  Month  Year

Sex: 1 □  Male  2 □ Female

Ethnicity / Race: 1 □ Chinese  2 □ Non-Chinese

Please specify: ___________________________

Place of Birth: ___________________________

● If you were not born in Hong Kong, how many years have you lived in Hong Kong?
___________ yrs

Home Address: ___________________________

Home Tel: __________  Mom / Dad’s Mobile: __________

Please tick “✓” your answers to this question in the space provided.

● Have you had upper respiratory infection (e.g. symptoms of cold or the flu) in the past 2 weeks? 1 □ Yes 2 □ No

Medical Officer Use Only

Time: __________

Height: ________(cm)  Weight: ________(kg)  Last Meal: ________
eNO: _______ _______ _______  Avg F_eNO: _______  Avg V_NO: _______
Please tick "✓" your answers to these questions in the space provided.

1. Have you ever had wheezing or whistling in the chest at any time in the past?  
   [IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 6]  
   1 [ ] Yes  
   2 [ ] No

2. Have you had wheezing or whistling in the chest in the past 12 months?  
   [IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 6]  
   1 [ ] Yes  
   2 [ ] No

3. How many attacks of wheezing have you had in the past 12 months?  
   1 [ ] None  
   2 [ ] 1 to 3  
   3 [ ] 4 to 12  
   4 [ ] More than 12

4. In the past 12 months, how often, on average, has your sleep been disturbed due to wheezing?  
   1 [ ] Never woken with wheezing  
   2 [ ] Less than one night per week  
   3 [ ] One or more nights per week

5. In the past 12 months, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?  
   1 [ ] Yes  
   2 [ ] No

6. Have you ever had asthma?  
   1 [ ] Yes  
   2 [ ] No

7. Have you had taken any medications for asthma treatment in the past 12 months?  
   1 [ ] Yes  
   2 [ ] No

8. In the past 12 months, has your chest sounded wheezy during or after exercise?  
   1 [ ] Yes  
   2 [ ] No

9. In the past 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection?  
   1 [ ] Yes  
   2 [ ] No
Questions 10-14 are about problems which occur when you DO NOT have a cold or the flu.

10. Have you *ever* had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu?  
   □ Yes  □ No  
   *IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 14*

11. In the past 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu?  
   □ Yes  □ No  
   *IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 14*

12. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?  
   □ Yes  □ No

13. In the past 12 months, how much did this nose problem interfere with your daily activities?  
   □ Not at all  □ A little  □ A moderate amount  □ A lot

14. Have you *ever* had hay fever?  
   □ Yes  □ No

15. Have you *ever* had an itchy rash which was coming and going for at least six months?  
   □ Yes  □ No  
   *IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 20*

16. Have you had this itchy rash at any time in the past 12 months?  
   □ Yes  □ No  
   *IF YOU HAVE ANSWERED “NO” PLEASE SKIP TO QUESTION 20*

17. Has this itchy rash at any time affected any of the following places:  
   the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?  
   □ Yes  □ No

18. Has this rash cleared completely at any time during the past 12 months?  
   □ Yes  □ No

19. In the past 12 months, how often, on average, have you been kept awake at night by this itchy rash?  
   □ Never in the past 12 months  □ Less than one night per week  □ One or more nights per week

20. Have you *ever* had eczema?  
   □ Yes  □ No
Video Questionnaire

Please pay attention to the video and tick “✓” your answers to these questions in the space provided.

SCENE 1: The first scene is of a young person at rest.

QUESTION 1: Has your breathing been like this, at any time in your life? Yes No
   if YES: has this happened in the past year? 1 2 1 2
   if YES: has this happened one or more times a month? 1 2 1 2

SCENE 2: The second scene is of two young people exercising. One is in a dark shirt and the other is in a white shirt.

QUESTION 2: Has your breathing been like the boy’s in the dark shirt during or following exercise at any time in your life? Yes No
   if YES: has this happened in the past year? 1 2 1 2
   if YES: has this happened one or more times a month? 1 2 1 2

SCENE 3: The third scene is of a young person waking at night.

QUESTION 3: Have you been woken at night like this at any time in your life? Yes No
   if YES: has this happened in the past year? 1 2 1 2
   if YES: has this happened one or more times a month? 1 2 1 2

SCENE 4: The fourth scene is also of a young person waking at night.

QUESTION 4: Have you been woken at night like this at any time in your life? Yes No
   if YES: has this happened in the past year? 1 2 1 2
   if YES: has this happened one or more times a month? 1 2 1 2

SCENE 5: The final scene is of another person at rest.

QUESTION 5: Has your breathing been like this, at any time in your life? Yes No
   if YES: has this happened in the past year? 1 2 1 2
   if YES: has this happened one or more times a month? 1 2 1 2

-The End-

***Thank you for taking part in this survey***
References


ten Hacken NH, van der Vaart H, van der Mark TW, Koeter GH, Postma DS. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. *Am J Respir Crit Care Med* 1998;158:902-7.


