



<http://researchspace.auckland.ac.nz>

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

Non-invasive method of measuring airway inflammation: exhaled nitric oxide

Dr Catherine Ann BYRNES

**FRACP, MBChB (New Zealand)
Graduate Certificate in Clinical Education (New South Wales)
Senior Lecturer & Honorary Consultant
Paediatric Respiratory Medicine**

The University of Auckland
Department of Paediatrics
Faculty of Medical & Health Sciences
Private Bag 92019
Auckland 1142
New Zealand

Ph: 64 9 3737 599 extn 89770
Fax: 64 9 3737 486
Email: c.byrn@uckland.ac.nz

-- DEC 2008

Background

LIBRARY

Nitric oxide (NO) was well known to be a component of air pollution, often in the form of nitrogen dioxide (NO₂). However its importance in biological systems altered dramatically with the discovery in 1987 that it was the 'endothelial-derived relaxing factor'. Since then there has been an explosion of research on NO demonstrating that this gaseous molecule was a widespread physiological mediator and was simultaneously recognised as a vital component of immune function contributing to macrophage-mediated cytotoxicity. NO was therefore a key molecule in modulating inflammation, including airway inflammation.

The aim of this thesis was:

1. To adapt a NO chemiluminescence analyser from measuring airway pollution to measuring exhaled air in human subjects.
2. To measure NO levels in exhaled air in adult subjects.
3. To evaluate whether altering measurement parameters altered the levels of NO obtained.
4. To adapt this technique from adults to measure exhaled NO in children.
5. To compare levels of NO from healthy children to groups of asthmatic children on either bronchodilator therapy only, or on regular inhaled corticosteroids.
6. To compare the levels of NO in a pilot group of asthmatic children before and after commencement of inhaled corticosteroids.

Methods

A Dasibi Environmental Corporation Model 2107 chemiluminescence analyser was adapted specifically requiring a reduction in response time, which was achieved by modification of the circuitry and re-routing of the analogue signal directly to a chart recorder, achieving a reduction of the response time by 80%. Addition of a number of analysers allowed the measurement of exhaled NO, carbon dioxide (CO₂), mouth pressure and flow for each exhalation from total lung capacity. Twenty adult subjects (in total) were then studied looking at direct (NO, CO₂, mouth pressure) versus t-piece (with the addition of flow) measurements making five exhalations from total lung capacity, at 3-minute intervals (direct/t-piece/direct or t-piece/direct/t-piece in series). The area of NO under the curve versus the peak of the NO trace was compared and the exhalation pattern of NO versus CO₂ was compared. Measurement conditions were altered to evaluate the effect of individual parameters on the exhaled NO result. This included separately assessing different expiratory flows, different expiratory mouth pressures, the effect of a high versus a low background NO level and the

effect of drinking water (of varying temperatures) prior to exhalation. Healthy control children were then enrolled to the study from a local school (Park Walk Primary School) and compared with asthmatic children enrolled from outpatient clinics at the Royal Brompton Hospital. The asthmatic children were further divided into those on bronchodilator treatment only and those on regular inhaled corticosteroid therapy. NO was also measured before and two weeks after commencing inhaled corticosteroid therapy in previously steroid-naive asthmatics.

Results

It was possible to modify a chemiluminescence analyser to enable measurement of exhaled NO. In 12 healthy subjects (mean age 32 years, 6 males) peak direct NO levels were 84.8 parts per billion (ppb) (standard error of the mean (SEM) 14.0ppb), significantly higher than 41.2ppb (SEM 10.8ppb) measured via the t-piece system. The exhaled NO rose to an early peak and plateau while the CO₂ levels continued to rise to peak late in the exhalation. The mean times to peak NO levels were 32.2 seconds (s) (direct) and 23.1s (t-piece), which was significantly different from the mean times to peak CO₂ levels at 50.5s (direct) and 51.4s (t-piece, $p < 0.001$). At peak NO level, the simultaneous CO₂ level of 4.9% (SEM 0.14%, direct) and 5.2% (SEM 0.18, t-piece) were significantly lower than the peak CO₂ achieved of 5.8% (SEM 0.21%, direct, $p < 0.001$) and 6.2% (SEM 0.28, t-piece, $p < 0.001$). There was no difference between repeat direct or t-piece measurements.

With regard to varying measurement conditions, the mean peak concentrations of NO decreased by 35ppb (95% confidence intervals 25.7-43.4) from a mean of 79ppb (SEM 15.4ppb) at an expiratory flow rate of 250mls/min to 54.1ppb (SEM 10.7ppb) at 1100mls/min. The mean peak concentration of NO did not change significantly when mouth pressure was increased in eight of ten subjects, although in two it did decrease in the highest pressure. The mean NO concentration with machine and subjects sampling from a low NO reservoir was 123ppb (SEM 19.4), which was an increase from results obtained before at 81.9ppb, SEM 10.2ppb, $p = 0.001$ 95% CI -19.9 to -62.7) and after at 94.2ppb (SEM 18.3ppb, $p = 0.017$, 95% CI 6.0-5.18) sampling with high ambient NO levels. The mean peak NO concentration decreased from 94.4ppb (SEM 20.8) to 70.8ppb (SEM 16.5, $p = 0.002$ 95% CI 12.9-33.1) with water consumption.

In 39 healthy pre-pubertal children with a mean age of 9.9 years (range 9-11 years, 23 girls) the mean direct exhaled NO level was 49.6ppb (SD 37.8ppb, range 11.5-197.2ppb) compared with mean exhaled NO via t-piece sampling of 29.2ppb (SD 27.1ppb, range 5.1-141.2ppb).

There was no significant difference between boys and girls for either the direct or the t-piece recordings. In comparison with normal children, 15 asthmatic children on bronchodilator therapy only had much higher levels of exhaled NO at 126.1ppb via the direct system (SD 77.1ppb, $p < 0.001$) and 109.5ppb via the t-piece system (SD 106.8ppb, $p < 0.001$). In 16 asthmatics on regular inhaled corticosteroids the mean peak exhaled levels were significantly lower at 48.7ppb via the direct method (SD 43.3ppb, $p < 0.001$) and via the t-piece system at 45.2ppb (SD 45.9ppb, $p < 0.01$). There was no difference between the normal children and the asthmatic children who were on regular inhaled corticosteroids ($p = 0.9$ direct, $p = 0.2$ t-piece). There was no significant difference in CO₂, mouth pressure, duration of expiration and expiratory flows between the three groups or between the two methods (direct and t-piece). In six asthmatic children the mean peak exhaled NO levels fell from a medium peak level of 124.5ppb to 48.6ppb when measured before and two weeks after commencement of inhaled corticosteroids on treatment.

Discussion

This research showed it was possible to modify an NO chemiluminescence analyser to enable measurement of exhaled NO in adult and paediatric subjects. Furthermore, it was possible to measure both healthy and asthmatic children. There were significant differences between the exhalation pattern of NO and CO₂ suggesting that NO was produced in the airways, not at alveolar level, unlike CO₂. The measurement of exhaled NO required a standardised approach as exhaled NO levels decreased with increasing expiratory flow, when measuring at a time of high ambient NO concentration, and with consumption of either hot or cold water immediately preceding exhalation (such as might be given if a subject was coughing). The findings with expiratory mouth pressure were less certain, with a difference seen in only two of ten subjects.

The levels of exhaled NO measured in children aged 9-11 years were lower than that measured in the adult subjects. There was no difference between boys and girls, or with other parameters such as having a personal history of atopy, a family history of atopy, or the presence of a smoker or furry pets within the house-hold. These findings may have altered with increased numbers in this group and could possibly be a type two statistical error. The results of exhaled NO in asthmatic children on bronchodilator therapy only were significantly elevated compared to both normal children and asthmatic children treated with regular inhaled corticosteroids. The exhaled NO level also fell significantly by two weeks following the commencement of inhaled corticosteroid treatment in steroid-naive asthmatic children. These

results suggested that the methods of measuring exhaled NO required standardization and that it could potentially be a non-invasive measure of airway inflammation to follow - particularly in children with asthma who were commencing inhaled steroid treatment.

Dedication

This is Dedicated:

To the strong women in my family from my great, great grandmother



to my sister Angela who always believes in me.

Acknowledgements

United Kingdom

Professor Andrew Bush invited me to a research position at the Royal Brompton Hospital and has been supportive from day one for both my research career and clinical training. His own approach I seek to emulate with his excellent clinical acumen, approach to children and their families. In addition, he maintains a huge research output and when working with him in this capacity his attention to detail and editing ability is superb. I, like most of the paediatric respiratory fraternity, count Andy, and his wife **Sue Bush**, as friends.

Professor Peter Barnes from the then National Heart & Lung Institute contributed valuable advice and supervised other research projects that I conducted. He rather bravely allowed me to join his laboratory as one of only three ‘medics’ among the 25 scientists from whom I learnt about statistics, information technology and attention to detail.

Dr Seinka Dinarevic assisted with the studies on the children and provided a link to the local school from where the children were recruited.

Park Walk Primary School, London who were approachable regarding the research and interested in assisting the study. The **children enrolled** from the school and from the Brompton Hospital clinics were terrific – humorous, enthusiastic, willing to help and could always be depended upon to question some factor about the testing or the research that I had not covered with them. They loved all the switches even more than I did.

Caroline Busst was the biomedical engineer who provided the main assistance with regard to modifying the analyser as we went through and assisted with troubleshooting whenever that was necessary. She brought completely different knowledge to mine to this project from an engineering and particularly electrical engineering capacity, which complimented the clinical and practical knowledge that I was able to offer.

I would like acknowledge the great ‘Fellows’ that I worked with at the Royal Brompton Hospital. We were embarking on clinical and research careers and it was great to be part of the group and they remain friends to this day; **Paul Munyard, Lara Shekerdemian, Jane Davies, Clare Hogg, Kate Brown and Adam Jaffe**.

New Zealand

The **Paediatric Department** at the Faculty of Health and Medical Sciences, University of Auckland all contributed particularly in the final days of submission assisting with proof-reading and formatting.

A very special thank you to **Jan Tate** the CF nurse specialist and friend who has always been very supportive and made my daily working life better, particularly at times of clinical overload. She also helped take the photos used in this thesis – late into the night.

Professor Innes Asher has always been supportive of myself in the clinical, research and teaching arenas and has helped greatly with her capacity, despite the many hats she currently wears, in offering advice and editing comments.

Dr Elizabeth Edwards who was my first research fellow, the first Ph.D student that I supervised which she completed before me and now both colleague and friend as well as fellow netball enthusiast.

Merrin Harger, project manager of subsequent research with whom I shared an office. She was amusing every day and left me one of her inspirational artworks when she left to embark on a new career as an artist (that we all were so talented).

Mirjana Jaksic, always generous with her own clinical time, I thank for supporting me in particular by picking up the occasional extra clinic, so that I could catch up.

Personal

My family has always been completely supportive in everything that I do and my parents, **Daphne Mary Pemberton Byrnes and Brian Liston Dominic Byrnes**, were thrilled to see me start at medical school all those many years ago, although, sadly, were not alive to see me graduate. And my special Aunt, **Veronica Commins**, who has supported my sister and myself through our careers and also, sadly, died before seeing this dedication to her years of generosity. My brothers, sisters and their families all offer their special supports – I feel lucky to be part of a large whanau, and I hope they will be pleased to see me emerge from my study.

It is a rare person who believes in you so wholeheartedly, even at times when you yourself have misgivings and I would like to thank my sister, **Angela Platt-Byrnes**, for ringing me every Saturday and every Sunday to ensure that I was ... and to encourage me to be ... sitting at my desk working on this thesis.

And to my own support network: **Dr Sue Armstrong-Wahlers, Dr Michael Wahlers** and **Trudi Fava**.

Contents

	Page
Abstract	II
Dedication	VI
Acknowledgements	VII
Contents	X
List of Tables	XV
List of Figures	XVI
List of Abbreviations	XVIII
Chapter 1: The burden of respiratory disease in New Zealand.....	1
1.1 Introduction.....	1
1.2 The burden of paediatric respiratory disease in New Zealand.....	2
1.3 The burden of asthma disease in New Zealand.....	5
1.4 The diagnosis of asthma – the quandaries in children	7
1.4.1 The asthma diagnosis in national and international guidelines.....	7
1.4.2 What is meant by ‘wheeze’?	7
1.4.3 Where does cough fit in?	10
1.4.4 Investigations	12
1.5 Treatment and concerns	21
1.5.1 Possible adverse effects of asthma treatment in children	21
1.5.2 A marker for inflammation would be useful.....	23
1.6 Asthma as an inflammatory disease.....	24
1.6.1 What has been learnt from autopsy studies?.....	24
1.6.2 Studies of inflammation using bronchoscopy and biopsy	25
1.6.3 Studies of inflammation using bronchoalveolar lavage.....	29
1.6.4 Inflammatory markers in induced sputum	31
1.6.4 (i) Studies in adult subjects.....	31
1.6.4 (ii) Induced sputum in children.....	34
1.6.4 (iii) Technical aspects of bronchoscopy, bronchial biopsy, bronchoalveolar lavage and/or induced sputum.....	36
1.6.4 (iv) Safety aspects of bronchoscopy, bronchial biopsy, bronchoalveolar lavage and/or induced sputum	38
1.6.5 Studies of inflammatory markers in blood and urine.....	41
1.6.6 Comparison of inflammation results between the different samples.....	44
1.7 Chapter summary	45
Chapter 2: Nitric oxide: pollutant to mediator.....	49
2.1 Introduction.....	49
2.2 Pollution, nitrogen oxides and disease.....	49
2.2.1 Concerns regarding pollution.....	49
2.2.2 Disease and pollution	51
2.2.3 Nitrogen oxides in pollution	56
2.3 The discovery of nitric oxide in biological systems	57
2.3.1 Vascular control	57
2.3.2 Immune function	60
2.3.3 Nitric oxide – a common pathway	61
2.3.4 Nitric oxide in physiological roles.....	62
2.3.4 (i) Cardiovascular	62
2.3.4 (ii) Nervous system – central and peripheral	64
2.3.4 (iii) Host defence.....	66
2.4 Chapter summary	68
Chapter 3: The synthesis, reactivity and control of nitric oxide.....	70

3.1	Introduction	70
3.2	Properties of nitric oxide	70
3.3	Reactions of nitric oxide.....	73
3.3.1	Reactive nitrogen species and superoxide reactions.....	73
3.3.2	Reactions with transition metals and metalloproteins	75
3.3.3	Nitrogen thiols and amines.....	76
3.3.4	Reaction with amino acids.....	77
3.3.5	Reaction with lipids.....	77
3.3.6	Reactions with genes	78
3.3.7	Making sense of the reactions	78
3.4	Nitric oxide synthase isoenzymes	79
3.4.1	Constitutive nitric oxide synthases	81
3.4.2	Inducible nitric oxide synthase.....	82
3.4.3	Control of the nitric oxide synthase isoenzymes.....	83
3.4.3 (i)	The constitutive forms.....	83
3.4.3 (ii)	The inducible form	85
3.4.4	Nicotinamide adenosine di-nucleotide phosphate oxidase and inducible nitric oxide synthase	87
3.4.5	Drugs & other agents that affect the isoenzymes and nitric oxide production.....	88
3.4.5 (i)	Drugs	88
3.4.5 (ii)	Nitric oxide synthase inhibitors.....	89
3.5	Chapter summary.....	91
Chapter 4:	Methods to measure nitric oxide	92
4.1	Introduction	92
4.2	L-arginine, L-citrulline and cyclic guanosine monophosphate	93
4.3	Methaemoglobin.....	93
4.4	Nitrite and nitrate.....	94
4.5	Nitric oxide.....	95
4.6	Chemiluminescence.....	97
4.6.1	Calibration	103
4.6.2	Safety and toxicity	104
4.7	Chapter summary.....	108
Chapter 5:	Methodological assessment of the chemiluminescence analyser	110
5.1	Introduction	110
5.2	The equipment and personnel.....	110
5.2.1	Chemiluminescence Analyser 'Model 2107'	110
5.2.2	Capnograph, pressure transducer, flow meter and chart recorder	111
5.2.3	Personnel	115
5.3	Direct versus reservoir measurement	116
5.4	Assessments of the analysers.....	117
5.5	Other measurements	120
5.6	Chapter summary.....	121
Chapter 6:	Methodological studies of exhaled nitric oxide in healthy adult subjects: direct versus t-piece testing	122
6.1	Introduction	122
6.2	Nitric oxide and the nitric oxide synthases in the lung	122
6.3	The need for methodological experiments	125
6.4	The aims of the exhaled nitric oxide methodological experiments.....	130
6.5	Setting up for the experiments.....	131
6.5.1	Set-up and calibrations	131
6.5.2	The exhalation protocol.....	136
6.5.3	Ethics and Consent	138

6.5.4	The subjects.....	138
6.5.5	Statistical analysis	138
6.6	Methodological experiment one.....	139
6.6.1	Hypotheses	139
6.6.2	Aims	139
6.6.3	Procedure	139
6.6.4	Results.....	140
6.6.4 (i)	Direct versus t-piece nitric oxide and carbon dioxide measurement	140
6.6.4 (ii)	Peak nitric oxide versus area under the nitric oxide curve	144
6.6.4 (iii)	Comparison of exhaled nitric oxide and carbon dioxide	146
6.7	Discussion: The origin of the exhaled nitric oxide	148
Chapter 7:	Methodological studies of exhaled nitric oxide in healthy adult subjects: investigating test parameters.....	153
7.1	Introduction.....	153
7.2	Ethics, subjects, calibrations and statistical analysis	154
7.3	Methodological experiment two - effect of expiratory flow.....	155
7.3.1	Hypothesis.....	155
7.3.2	Aim.....	155
7.3.3	Procedure	155
7.3.4	Results.....	156
7.4	Methodological experiment three – effect of pressure.....	158
7.4.1	Hypotheses	158
7.4.2	Aim.....	158
7.4.3	Procedure	158
7.4.4	Results.....	160
7.5	Methodological experiment four - effect of ambient nitric oxide.....	163
7.5.1	Hypothesis.....	163
7.5.2	Aims	163
7.5.3	Procedure	163
7.5.4	Results.....	164
7.6	Methodological experiment five – effect of water consumption	168
7.6.1	Hypothesis.....	168
7.6.2	Aim.....	168
7.6.3	Procedure	168
7.6.4	Results.....	169
7.7	Discussion: which measurement factors alter nitric oxide levels?	170
Chapter 8:	Exhaled nitric oxide in healthy and asthmatic children	178
8.1	Introduction.....	178
8.2	Ethics, consent, statistics and subjects	179
8.3	Methodology of exhaled nitric oxide measurement in healthy children.....	180
8.3.1	Hypotheses	180
8.3.2	Aims	180
8.3.3	Protocol	180
8.3.4	Results.....	182
8.4	Discussion: exhaled nitric oxide results in healthy children.....	186
8.5	Methodology of exhaled nitric oxide measurement in asthmatic children	191
8.5.1	Background	191
8.5.2	The asthmatic subjects	192
8.5.3	Protocol	193
8.5.4	Results.....	193
8.6	Discussion: exhaled nitric oxide in asthmatic children.....	196
Chapter 9:	Exhaled and nasal NO to today.....	202

9.1	Introduction	202
9.2	Technical factors affecting results across research groups.....	202
9.3	Standardisation	204
9.3.1	Single breath online measurement.....	205
9.3.1 (i)	Flow	206
9.3.1 (ii)	Mouth pressure	211
9.3.1 (iii)	Nasal clips and breath-holding	212
9.3.1 (iv)	The recorded measurement.....	213
9.3.1 (v)	The effect of ambient nitric oxide	213
9.4	Online spontaneous or tidal breathing measurement.....	214
9.5	Off-line measurement	216
9.6	Nasal nitric oxide measurement	220
9.7	Physiological alterations that may affect measurement	221
9.7.1	Size and gender.....	221
9.7.2	Age	222
9.7.3	Circadian rhythm	223
9.7.4	Menstrual cycle and pregnancy	223
9.7.5	Food and beverages	224
9.7.6	Summary of physiological factors that could alter nitric oxide levels	224
9.8	Nitric oxide levels in asthma and atopy	224
9.8.1	Does nitric oxide correlate with other asthmatic inflammatory markers?.....	225
9.8.2	Does nitric oxide correlate with lung function and bronchial hyper-responsiveness?	226
9.8.3	Can nitric oxide be used for diagnosis of asthma?	227
9.8.4	Is nitric oxide associated with symptoms and severity of asthma?	229
9.8.5	Can nitric oxide predict deterioration in asthma control?	231
9.8.6	What happens to nitric oxide during an acute asthma attack	233
9.8.7	What is the effect of atopy alone on nitric oxide?.....	233
9.8.8	Can nitric oxide be an outcome measure for assessing treatment?	235
9.8.8 (i)	Corticosteroids.....	235
9.8.8 (ii)	Anti-leukotriene receptor antagonists.....	237
9.8.8 (iii)	Long acting β_2 agonists.....	238
9.8.8 (iv)	Nedocromil sodium	239
9.8.8 (v)	Theophylline.....	239
9.8.8 (vi)	Novel medications	239
9.8.9	Summary of nitric oxide in asthma and atopy.....	240
9.9	Nitric oxide levels in primary ciliary dyskinesia.....	241
9.10	Nitric oxide levels in cystic fibrosis	242
9.11	Nitric oxide levels in bronchiectasis.....	245
9.12	Nitric oxide levels in upper respiratory tract infections	245
9.13	Nitric oxide levels in chronic obstructive pulmonary disease (COPD).....	246
9.14	Nitric oxide levels in smokers	247
9.15	Nitric oxide levels in interstitial lung diseases	249
9.16	Nitric oxide levels in exercise	250
9.17	Nitric oxide measurements in infants	252
9.17.1	Methodology.....	252
9.17.2	Levels of nitric oxide in infants with different respiratory diseases.....	256
9.17.3	Prenatal and maternal effects on nitric oxide levels	257
9.17.4	Effects of treatment on nitric oxide levels.....	257
9.17.5	Summary of nitric oxide findings in infants.....	257
9.18	Chapter summary.....	258
Chapter 10:	Reflections.....	262

Appendices.....	265
References.....	273

List of Tables

	Page
Table 1.1: Alternative diagnoses in children with wheeze.....	8
Table 3.1: Properties of nitric oxide.....	71
Table 3.2: Reactions with nitric oxide.....	72
Table 3.3: The characteristics of the nitric oxide synthase isoenzymes.....	81
Table 4.1: The companies providing chemiluminescence analysers adaptable for nitric oxide measurement in 1995.....	101
Table 5.1: The delay and response time of the NO, CO ₂ , mouth pressure and flow meter analysers used.....	118
Table 6.1: The published results of exhaled nitric oxide in adults.....	127
Table 6.2: Compete results for an individual subject.....	143
Table 6.3 NO and CO ₂ results from single exhalations in twelve adult subjects.....	143
Table 7.1: NO, CO ₂ and duration of each exhalation at different expiratory flows.....	157
Table 7.2: Comparison of the first and repeated last set of exhalations at the same expiratory flow.....	161
Table 7.3: NO, CO ₂ and duration of each exhalation at different expiratory mouth pressures.....	162
Table 7.4: Comparison of the first and repeated last set of exhalations performed at the same expiratory mouth pressure.....	162
Table 7.5: Exhaled NO results with high and low background NO concentrations.....	168
Table 7.6: Exhaled CO ₂ results with high and low background NO concentrations.....	168
Table 8.1: Coefficients of variation of peak NO, peak CO ₂ and mouth pressure measurements made by both the direct and t-piece systems, and of flow made by the t-piece system.....	185
Table 9.1: The recommended standard for single breath online NO measurement.....	206
Table 9.2: The recommended standards for single breath and tidal breathing off-line NO measurement.....	219
Table 9.3: The recommended standard for nasal NO measurement.....	220

List of Figures

	Page
Figure 1.1: Top 10 causes of avoidable admissions, 0-24 years, 1999.....	3
Figure 2.1: Diagram of blood vessels in cross section showing the endothelial layer.	59
Figure 3.1: The molecular structure of NO.....	70
Figure 3.2: The reaction to generate nitric oxide.....	71
Figure 3.3: The nitric oxide synthase reaction showing the generation of the constituent atoms of nitric oxide.....	80
Figure 3.4: The chemical structures of the nitric oxide synthase inhibitors.....	90
Figure 4.1: Chemical reaction between nitric oxide and ozone.....	98
Figure 4.2: Diagram of the chemiluminescence analyser.....	99
Figure 4.3: Relationship between chemiluminescence in millivolts to nitric oxide concentration in picomoles.....	100
Figure 4.4: Reaction equations used to release nitric oxide from other nitrogen compounds.....	103
Figure 5.1: Schematic diagram of how the analysers were placed.....	112
Figure 5.2: An example of tracing from the testing.....	113
Figure 5.3: Photographs of one of the children performing the exhalation.....	116
Figure 6.1: Recording of the calibrations for NO, CO ₂ and pressure analysers.....	133
Figure 6.2: Recording of the calibration of the flow rotameter and pneumotachograph.....	135
Figure 6.3: Schematic diagrams of the two different types of connections: direct and t-piece measurements.....	137
Figure 6.4: Example of the chart recording rolls for the direct versus t-piece measurements.....	140
Figure 6.5: Example of recording on one subject – direct and t-piece measurements.....	141
Figure 6.6: Mean exhaled NO levels measured by direct and t-piece systems.....	142
Figure 6.7: Example of a recording for the two systems.....	142
Figure 6.8: Mean exhaled CO ₂ levels measured by direct and t-piece systems.....	144
Figure 6.9: Comparisons of the peak NO with area under the curve.....	145
Figure 6.10a: Time to peak NO and peak CO ₂ measured by the direct system.....	146
Figure 6.10b: Time to peak NO and peak CO ₂ measured by the t-piece system.....	147
Figure 6.11a: CO ₂ levels at peak NO and at peak CO ₂ measured by the direct system.....	147
Figure 6.11b: CO ₂ levels at peak NO and at peak CO ₂ measured by the t-piece system.....	148
Figure 7.1: Exhaled NO results with increasing expiratory flows.....	157
Figure 7.2: Example of the tracing in one subject at two different expiratory flows.....	158
Figure 7.3: The effect of pressure on the calibration gas measurement.....	160
Figure 7.4: Exhaled NO results at increasing expiratory mouth pressure.....	161
Figure 7.5: Measurement of exhaled NO incorporating the reservoir system.....	165
Figure 7.6: Photographs of the changing ambient NO concentration recordings.....	166
Figure 7.7: Recording from one subject showing NO results with exhalations from low and high background NO.....	167
Figure 7.8: The effect of high and low pressure on the calibration gas measurement.....	167
Figure 7.9: The effect of consuming water on the subsequent exhaled NO levels measured.....	170
Figure 7.10: Hyperbolic relationship between exhaled NO and sampling flow rate.....	172
Figure 7.11: Exhaled NO determined at single breath plateau concentrations at increasing expiratory flows and at increasing expiratory mouth pressures.....	173
Figure 8.1: Peak exhaled NO results in healthy children.....	183
Figure 8.2a: Comparison of peak exhaled NO in boys and girls measured by the direct system.....	184
Figure 8.2b: Comparison of peak exhaled NO in boys and girls measured by the t-piece system.....	184

Figure 8.3a: Mean peak exhaled NO levels in control and asthmatic children measured direct to the analysers	194
Figure 8.3b: Mean peak exhaled NO levels in control and asthmatic children measured via the t-piece sampling system	195
Figure 8.4a: The effect of commencing inhaled corticosteroid therapy on peak exhaled NO levels in asthmatic children measured via the direct method	196
Figure 8.4b: The effect of commencing inhaled corticosteroid therapy on peak exhaled NO levels in asthmatic children measured via the t-piece sampling system.....	196
Figure 9.1: A two-compartment model of NO exchange	208
Figure 9.2: Plateau nasal NO increasing with age.....	223
Figure 9.3: Diagram of the measurement of lung function and NO in an infant.....	253
Figure 10.1: Medline publications with a focus on nitric oxide research per year 1980-2006	262

List of Abbreviations

<	less than
>	greater than
ABPA	allergic bronchopulmonary dysplasia
ATS	American Thoracic Society
ATP	adenosine triphosphate
BAL	bronchoalveolar lavage
BH ₄	tetrahydrobiopterin
BTS	British Thoracic Society
C3, C5	complement factor 3, complement factor 5
cAMP	cyclic adenosine 3', 5' monophosphate
CAT1, CAT2, CAT2B, CAT 3	cationic amino acid proteins
CB	chronic bronchitis
CD3+	cell marker for T lymphocyte
CD4+	cell marker for T helper lymphocyte
CD8+	cell marker for T cytotoxic lymphocyte
CF	cystic fibrosis
cGMP	cyclic guanosine monophosphate
cNOS	constituent nitric oxide synthase
CNS	central nervous system
Co	cobalt
CO	carbon monoxide
CO ₂	carbon dioxide
COPD	chronic obstructive pulmonary disease
CSF	central spinal fluid
Cu	copper
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECMO	extracorporeal membrane oxygenation
ECP	eosinophil cationic protein
ECRHS	European Community Respiratory Health Survey
EDRF	endothelial derived relaxing factor

ELISA	Enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EPN/EPX	eosinophilic neuraminadase
EPO	eosinophilic peroxidise
ERS	European Respiratory Society
ERSTF	European Respiratory Society Task Force
FAD	flavin adenosine dinucleotide
Fe	iron
FEF _{25-75%}	forced expiratory flow at 25 to 75 percent of forced vital capacity
FEV ₁	forced expiratory volume in 1 second
FEV _{0.5}	forced expiratory volume in half a second
FMN	flavin mononucleotide
FVC	forced vital capacity
GINA	Global Initiative for Asthma
GM-CSF	Granulocyte macrophage – colony stimulating factor
GTP	guanosine 5-triphosphate
H ₂ O	water
H ₂ S	hydrogen sulphide
HNO ₂	nitrous acid
HRCT	high resolution computerised tomography
ICAM1	intracellular adhesion molecule 1
IFN γ	interferon gamma
IgA	immunoglobulin A
IgG	immunoglobulin G
IgE	immunoglobulin E
IHCS	inhaled corticosteroids
IL1, IL2, IL3, IL4, IL5, IL6, IL8, IL9, IL10, IL11, IL12, IL13, IL17	interleukin 1, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, interleukin 8 interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, interleukin 17
iNOS	inducible nitric oxide synthase
ISAAC	International Study of Asthma and Allergies in Childhood
Kb	kilobases
L/min	litres per minute
LABA	long acting β_2 agonist

LADA	N ^w N ^w dimethyl L-arginine
LFA1	lymphocyte function-associated antigen 1
L-NAME	N ^w arginine methyl ester
L-NMMA	N ^w monomethyl L-arginine
L-NOARG	N ^w -nitroarginine
LNIO	N- imino-ethyl-ornithine
LPC	lysophosphotidylcholine
LPS	lipopolysaccharide
LTC4, LTD4, LTE4	leukotriene C4, leukotriene D4, leukotriene E4
MBP	major basic protein
mg/ml	milligrams per millilitre
mls/min	millilitres per minute
Mn	manganese
mRNA	messenger ribonucleic acid
N ₂	nitrogen
N ₂ O	nitrous oxide (“laughing gas”)
N ₂ O ₃	dihydrogen trioxide
N ₂ O ₄	dihydrogen tetraoxide
NADPH	nicotinamide adenosine di-nucleotide phosphate
NANC	non adrenergic, non cholinergic (nerves)
NF-κB	nuclear factor-kappa B
NHANES 1	National Health and Nutrition Examination Survey 1
nL/min	nanolitres per minute
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NO +	nitrosoium cation
NO-	nitroxyl anion
NO ₂	nitrogen dioxide
NO ₂ -	nitrite
NO ₃ -	nitrate
NO ₂ Tyr	3-nitrotyrosine
NOS	nitric oxide synthase/s
NOx	nitrogen oxides (usually NO, NO ₂ and NO ₃)
NZPAG	New Zealand Paediatric Asthma Guidelines
O ₂	oxygen

O ₂ ⁻	superoxide anion
O ₃	ozone
OH ⁻	hydroxyl anion
ONOO ⁻	peroxynitrite
ONOOH	peroxy nitrous acid
PaO ₂	pulmonary artery oxygen
PCD	primary ciliary dyskinesia
PC ₂₀	provocation concentration producing 20% fall in the forced expiratory volume in one second
PD ₂₀	provocation dose producing 20% fall in the forced expiratory volume in one second
PEF	peak expiratory flow
Pmo	mouth pressure
ppb	parts per billion
ppm	parts per million
PM ₁₀	particulate matter with a diameter of less than 10µm
redox	reduction oxidative reactions
ROC	receiver-operator curve
RS	sulphur thios
RSV	Respiratory Syncytial Virus
SD	standard deviation
SEM	standard error of the mean
sGC	soluble guanylate cyclase
SIGN	Scottish Intercollegiate Guidelines Network
SLE	systemic lupus erythematosis
SO ₂	sulphur dioxide
TB	tuberculosis
TGFβ	transforming growth factor beta
T _H 1	T helper lymphocyte cell type 1
T _H 2	T helper lymphocyte cell type 2
T _H 3	T helper lymphocyte cell type 3
TNFα	tumour necrosis factor alpha
TNFβ	tumour necrosis factor beta
type I (nNOS)	Type I neuronal nitric oxide synthase
type II (iNOS)	Type II induced nitric oxide synthase

type III (eNOS)	Type III endothelial nitric oxide synthase
UK	United Kingdom
$\mu\text{g/ml}$	micrograms pre millilitre
UNICEF	United Nations International Children's Fund
USA	United States of America
UV light	ultraviolet light
V/Q	ventilation and perfusion ratio
Zn	zinc