Title Page

Full Title: The effect of oxygen, temperature and hydrogen sulphide on the human pulmonary circulation

Total number of volumes: One

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Qualification for which the thesis is submitted: Doctor of Medicine

Institution: University of Hull and the University of York

Department: Hull York Medical School

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Abstract

Introduction: The human pulmonary circulation is poorly understood at a physiological level which is a shame given that the pathology affecting it, particularly pulmonary artery hypertension, can have detrimental effects not only in the lungs but on the heart. Pulmonary artery hypertension in its acute or chronic form carries a high mortality. Few centres have the luxury to utilise human tissue to study this phenomenon. My thesis looks at the effect of certain stimuli such as oxygen, temperature and hydrogen sulphide to discern their role in governing pulmonary artery reactivity at both the tissue and organ level.

Methods: Tissue was supplied from lungs taken from patients with lung cancer following resection of the tumour during surgery. I used a combination of isolated arterial ring models in organ baths and isolated perfused lung models to study the factors governing pulmonary arterial tone and pulmonary artery pressures at a tissue and organ level respectively.

Results: At the tissue level, hypoxia caused nitric-oxide independent dilation of human pulmonary arteries whilst hyperoxia caused a vasoconstriction. This hyperoxic vasoconstriction is dependent on both voltage gated calcium-channels in the cell membrane as well as release from intracellular calcium stores. It is also dependent on oxygen-free radicals. Hypothermia blunts this vasoconstrictive response to hyperoxia as well as endothelin-1 and potassium chloride-mediated pulmonary smooth muscle contraction. Hydrogen sulphide dilates pulmonary arteries. At the organ level, oxygen changes either via the perfusate or the ventilator do not affect pulmonary artery pressures. Both hypothermia and hydrogen sulphide reduce both pulmonary artery pressures and bronchial pressures.

Conclusions: Compensatory mechanisms within the pulmonary circulation may compensate for hypoxic vasodilation and hyperoxic vasoconstriction or there may be a systemic component to entities such as “hypoxic pulmonary vasoconstriction” seen in animal models. Hydrogen sulphide may provide a possible treatment avenue for pulmonary artery hypertension.

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Abbreviations

2-APB: 2-Aminoethoxydiphenyl borate

ATP: Adenosine Triphosphate

CO2: Carbon Dioxide

COPD: Chronic Obstructive Pulmonary Disorder

CPB: Cardiopulmonary Bypass

CSE: Cystathionine γ-lyase

DPI: Diphenyleneiodonium

ET-1: Endothelin-1

EVLP: Ex-vivo Lung Perfusion

GTP: Guanosine Triphosphate

H2S: Hydrogen Sulphide

HPAR: Human Pulmonary Artery Rings

HPAS: Human Pulmonary Artery Strips

HPV: Hypoxic Pulmonary Vasoconstriction

H-R: Hypoxia-Reoxygenation

ILPM: Isolated Lung Perfusion Model

I-R: Ischaemia-Reperfusion

KATP: ATP-sensitive potassium channels

KCl: Potassium Chloride

L-NAME: L-NG-Nitroarginine Methyl Ester

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

OSA: Obstructive Sleep Apnoea

PAH: Pulmonary Artery Hypertension

PAP: Pulmonary Artery Pressure

PAEC: Pulmonary Artery Endothelial Cell

PASMC: Pulmonary Artery Smooth Muscle Cell

ROCK: Rho-Associated Protein Kinase

ROS: Reactive oxygen species

SNP: Sodium Nitro-Prusside

Section A: Acknowledgements, Ethics, Presentations, Prizes and Publications of MD study

“If everybody is thinking alike, then somebody isn't thinking”

— General George S. Patton Jr. (November 11, 1885 – December 21, 1945)

Dedication

I dedicate this for my loving parents, Rajini-Maria and Senathirajah, and sister, Shaumya.

Acknowledgements

Firstly, I would like to thank my supervisors Professor Alyn Morice and Mr Mahmoud Loubani for their support, constructive criticism and allowing me the freedom to think for myself and try out new ideas. I don't think I will ever meet someone like Mr Loubani again in my life and I will be eternally indebted to him for bringing me to Hull as a research fellow. Secondly I would like to thank Robert Bennett for teaching me the techniques in the laboratory. Thirdly, I would like to thank my Thesis Advisory Panel: Dr Anne-Marie Seymour, Dr Jack Kastelik, and Dr Simon Hart for their invaluable advice and encouragement. Fourthly, I would like to thank Mr Neil Cartwright for inspiring my research and clinical commitments to cardiothoracic surgery and Mr Constantine Simintiras for his intra and extra-curricular support pertaining to my research. Fifthly, I would like to thank Peter Gardiner from Clinical Skills Ltd for help illustrating the diagram for my introduction. Next, I would like to thank Mr Guvendik for sponsoring my MD. I would also like to thank Mr Steven Griffin, Mr Mubarak Chaudhry, Mr Michael Cowen and Mr Alexander Cale for supplying tissue from their operations for my study and their patience. Dr Ajith Vijayan was very supportive of me during my time here. In addition, I would like to thank Sister Leslie Rusling and Mrs Rachel Howell on ward 27 for having put up with a lot during my time as a research fellow. Finally, I would like to thank all at HYMS especially Elaine Brookes and Julie Crawford for their help and organisational efforts for my MD, and James Illingworth from Research and Development at Castle Hill Hospital for making my life easier.

Declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources. I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised.

Funding

The funding for this project came from charitable funds donated to Mr Loubani, Mr Griffin and Mr Guvendik at the department of cardiothoracic surgery at Castle Hill Hospital.

Content of Ethics

(a) : Ethical approval document 12/LO/1233

(b) : Ethical approval document 12/SW/0365

(c) : Ethical approval document 12/NW/0042

Ethical approval for the use of human bronchial tissue and pulmonary artery tissue

Health Research Authority

National Research Ethics Service

NRES Committee London - Fulham
HRA NRES Centre Manchester
Barlow House
3rd Floor, 4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7821
Facsimile: 0161 625 7299

18 July 2012

Dr Priyadharshanan Ariyaratnam
Cardiothoracic Department
Castle Hill Hospital
Cottingham
HU16 5JQ

Dear Dr Ariyaratnam

Study title:	**Mechanisms of in-vitro responses of human pulmonary arteries and bronchi to hypoxia and reoxygenation**
IRAS Project Number:	**111712**
REC reference:	**12/LO/1233**

The Proportionate Review Sub-committee of the NRES Committee London - Fulham reviewed the above application on 16 July 2012.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Investigator CV	CV - Professor Alyn Morice	01 July 2012
Other: Letter from funder - Hull & East Yorkshire Hospitals		10 July 2012
Other: CV - Dr Priyadharshanan Ariyaratnam		01 July 2012
Participant Consent Form: Patient Agreement to investigation or treatment		
Participant Information Sheet	1	01 July 2012
Protocol	1	01 July 2012
REC application	3.4	10 July 2012
Referees or other scientific critique report	Email from Dr Simon Hart	10 July 2012

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators

- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/LO/1233	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Signed on behalf of:
Dr Charles Mackworth-Young
Chair

Email: shehnaz.ishaq@northwest.nhs.uk

Enclosures: List of names and professions of members who took part in the review

"After ethical review – guidance for researchers"

Copy to: Mr James Illingworth, Research & Development Department, Hull & East Yorkshire NHS Trust.

Professor Alyn Morice,
Academic Medicine
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

NRES Committee London - Fulham

Attendance at PRS Sub-Committee of the REC meeting on 16 July 2012

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Kanagasabai Ganeshaguru	Lay member	Yes	
Dr Shaun Griffin	Director of Communications and Public Affairs	Yes	
Dr. Charles Mackworth-Young (Chairman)	Consultant Physician	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Shehnaz Ishaq	Committee Co-ordinator

Ethical approval for the use of human saphenous vein tissue and internal mammary artery tissue

NHS
Health Research Authority

NRES Committee South West - Cornwall & Plymouth
Bristol Research Ethics Committee Centre
Level 3
Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 342 1330
Facsimile: 0117 342 0445

05 December 2012

Dr Priyadharshanan Ariyaratnam
Cardiothoracic Registrar and Research Fellow
Hull and East Yorkshire Hospitals Trust
Department of Cardiothoracic Surgery
Castle Hill Hospital
Cottingham
HU16 5JQ

Dear Dr Ariyaratnam

Study title:	**Mechanisms of in-vitro responses of isolated human systemic arteries and veins to Hypoxia and Re-Oxygenation**
REC reference:	**12/SW/0365**
IRAS project ID:	**118554**

Thank you for your letter responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Charlotte Allen, nrescommittee.southwest-cornwall-plymouth@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Approved documents

The documents reviewed and approved by the Committee are:

Document	*Version*	*Date*
Investigator CV		
Other: CV - Academic Supervisor		
Other: Letter from Funder		10 July 2012
Other: Email - clarification of tissue storage		29 November 2012
Participant Consent Form	1	01 November 2012

Participant Information Sheet	3	30 November 2012
Protocol	3	30 November 2012
REC application	3.4	22 November 2012
Referees or other scientific critique report		15 November 2012
Response to Request for Further Information		
Response to Request for Further Information	Email	30 November 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/SW/0365	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Canon Ian Ainsworth-Smith
Chair

Email: nrescommittee.southwest-cornwall-plymouth@nhs.net

Enclosures: *"After ethical review – guidance for researchers" (via email)*

Copy to: *Mr James Illingworth, Research & Development Department*

Ethical approval for the use of human lungs for ex-vivo lung perfusion studies

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Lancaster
HRA NRES Centre - Manchester
Barlow House
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14 January 2013

Dr Priyadharshanan Ariyaratnam
Cardiothoracic Registrar and Clinical Research Fellow
Hull and East Yorkshire Hospital Trust
Department of Cardiothoracic Surgery
Castle Hill Hospital
Cottingham
HU16 5JX

Dear Dr Ariyaratnam

Study title:	**Responses to Hypoxia and Re-oxygenation in Isolated Human Lungs**
REC reference:	**13/NW/0042**
IRAS project ID:	**115045**

The Proportionate Review Sub-committee of the NRES Committee North West - Lancaster reviewed the above application on 10 January 2013.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Carol Ebenezer, nrescommittee.northwest-lancaster@nhs.net.

Ethical opinion

Point out that the application was not in lay language for future reference

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

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Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Investigator CV	Ariyaratnam	
Investigator CV	Morice	
Other: Letter from funder		
Other: critique		
Participant Consent Form: for investigation or treatment		
Participant Information Sheet	1	04 January 2013
Protocol	1	03 January 2013
REC application	3.4	04 January 2013

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

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information is available at National Research Ethics Service website > After Review

13/NW/0042	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

pp.

Dr Lisa Booth
Chair

Email: nrescommittee.northwest-lancaster@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Mr James Illingworth, Research & Development Department*

A Research Ethics Committee established by the Health Research Authority

Journals, Conferences, Prizes

Peer reviewed journals

(1) P Ariyaratnam, M Loubani, AR Cale, M Chaudhry, ME Cowen, MA Jarvis, S Griffin, AH Morice. The Effect of deep hypothermia on the Human Pulmonary Circulation. *J Therm Biol* February 2014, (4) 20-24

(2) P Ariyaratnam, M Loubani, AH Morice. Hydrogen Sulphide dilates human pulmonary arteries: a possible role in pulmonary hypertension*? Microvascular Research*. November 2013, Volume 90, Pages 135–137.

(3) P Ariyaratnam, M Loubani, AH Morice. Hypoxic Pulmonary Vasoconstriction in Humans. *Biomedical Research International* August 2013 (Biomed Res Int. 2013;2013:623684. doi: 10.1155/2013/623684. Epub 2013 Aug 20).

(4) P Ariyaratnam, M Loubani, RT Bennett, SC Griffin, MA Chaudhry, ME Cowen, L Guvendik, AR Cale, AH Morice. Hyperoxic vasoconstriction of human pulmonary arteries: a novel insight into acute ventricular septal defects. *ISRN Cardiology*. 2013 Mar

31; 2013: 685735. doi: 10.1155/2013/685735. Print 2013. PMID: 23606985.

Conferences presented

(1) European Association of Cardiothoracic Surgeons (EACTS) annual conference, Barcelona, October 2012

(2) Yorkshire Deanery annual Cardiothoracic meeting, Cottingham, December 2012

(3) Society of Cardiothoracic Surgeons of Great Britain and Ireland, Brighton, March 2013

(4) HYMS Postgraduate Conference, Hull University, June 2013

(5) European Association of Cardiothoracic Surgeons (EACTS) annual conference, Vienna, October 2013

Prizes

(1) European Young Investigator Award (EACTS) for Thoracic Surgery, Barcelona, October 2012 (£3000).

(2) European Young Investigator Award (EACTS) for Thoracic Surgery, Vienna, October 2013 (£3000).

Section B: Introduction

Pulmonary circulation

The pulmonary circulation is a unique and curious labyrinth. It is a coliseum where not only a complex anatomical arrangement plays out but also a physiological spectacle is on display which defies classical systemic circulatory logic. It is this however, which makes it a fun and challenging arena to watch as a spectator.

Pulmonary circulatory anatomy and embryology

The pulmonary circulation's primary function in humans is to carry deoxygenated blood, which has returned to the right side of the heart, to the lungs where it becomes oxygenated and carbon dioxide is removed, and then finally to return it to the left side of the heart for it to pump it to the body [1, 2] (Figure1).

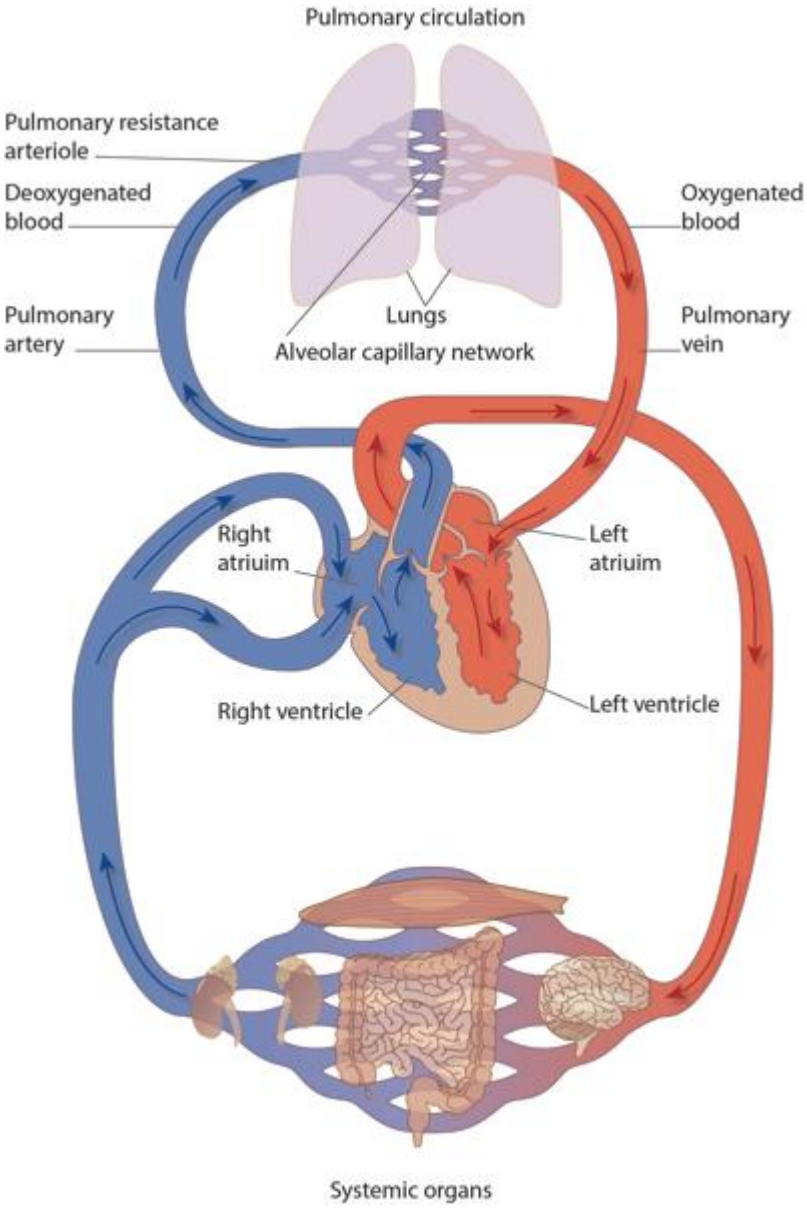


Figure 1: The Pulmonary Circulation

The complexity of the pulmonary circulation comes from its interaction with the lungs. The lungs are not receiving pulmonary arterial blood for nourishment. This comes from the bronchial circulation which is a product of the systemic circulation [3, 4]. Rather the lungs are doing the pulmonary artery a favour by getting rid of the gaseous waste from the body and

replenishing it with oxygen. *The pulmonary artery is always hypoxic* and *gas exchange occurs at the capillary bed* [5, 6]. The importance of this cannot be overstated yet many of the animal studies that investigators conduct to study gas exchange and the pulmonary circulation omit this set-up from any of the thought processes. So for instance, they study the effect of oxygen on the pulmonary artery, completely ignoring the fact that oxygenation occurs at the capillary-alveolar level.

Embryology of the pulmonary circulation

Embryologically, the pulmonary circulation develops not as a continuum but in discrete spatio-temporal sections that unite. What I mean by this is that the pulmonary arteries and veins develop independently of each other and this macro-circulation (pulmonary arteries and veins) in turn develop temporally independently of the microcirculation (capillaries).

The main pulmonary artery's birth begins with the creation of the pulmonary valve during the fourth and fifth week of gestation which stimulates the division of the common ventricular outflow tract (conotruncus) into the pulmonary artery and the aorta [7, 8]. The division of the pulmonary artery follows the division of the primordial lung bud into the bronchi-alveolar units [9, 10]. This highly emotional bond between the pulmonary artery and the bronchi-alveolar units is highlighted by the fact that agenesis of the pulmonary artery leads to agenesis of the lung parenchyma [11].

The pulmonary vein, on the other hand, is less intimately linked to the alveolar development. Whilst it is true that in the ancient times of the

primordial lung bud, venous drainage developed from this bud to the vitelline and cardinal venous systems, once these systems regressed, a new pulmonary venous system developed [12,13]. Cells from the early left atrium began to migrate towards the developing lung giving rise to the pulmonary vein in the 5th week of gestation [14, 15]. This close developmental relationship between the left heart and the pulmonary vein may explain why arrhythmias of the left heart can be obliterated by surgery on the pulmonary vein [16, 17].

Whilst the macro-circulation is mature between weeks 5 and 10, the microcirculation (capillaries) does not develop until after 26 weeks gestation [18, 19]. That is only after the alveolus matures.

This developmental origin of the pulmonary circulation may also explain why scientists have yielded the pulmonary artery and the capillaries as having significance to gas-exchange-vasoreactive phenomena such as hypoxic pulmonary vasoconstriction, compared to pulmonary veins, as the development of the pulmonary artery and capillaries are intimately linked to the development of the alveoli whilst the pulmonary vein is more associated to the development of the left atrium.

Pulmonary circulatory physiology

Circulatory dynamics

The pulmonary circulation, despite being rogue from the circulation is still in series with it and is therefore reliant on systemic blood flow not only coming to the right side of the heart but leaving the left side of the heart. Thus, if there is increase in the afterload of the left ventricle (e.g. aortic valve stenosis) or there is an increase in pressure in the left atrium (mitral valve stenosis), then there will be higher pressures in the pulmonary artery [20, 21].

Determinants of pulmonary circulatory tone

The smaller resistance arterioles of the pulmonary artery contain the most smooth muscle [22]. Despite this, pulmonary arteries as a whole have less smooth muscle than the systemic arteries. This makes them more distensible and compressible [23]. Because of this, pulmonary arteries are subject more to the influences of extrinsic forces such as gravity, alveolar compression, lung volumes, body position as well as intravascular pressures from the flow of blood [24]. For example, at the apex of the lung, the alveolar pressure is high and the pulmonary artery pressure may be low as a result of the heart pushing uphill to the apex and there may be a degree of pulmonary artery collapse at this level. However, at the base of the lung, the pulmonary arterial pressure is high and so trumps the alveolar pressure and the pulmonary artery stays open creating a relative dead space at the apex compared to the base. This is a physical effect determining regional pulmonary blood flow [25]. But there are other determinants.

Pulmonary arterial flow is determined by the cardiac output from the right ventricle which is determined by heart rate, contractility and preload. This is the systemic component.

There is a local non-physical determinant of pulmonary flow such as that which is exists in the systemic circulation to control oxygen demand. For example when one exercises, there is an increase in blood flow to the striated muscles of the body as a result of local neuroendocrine vasodilatory mechanisms [26, 27]. The same thing is postulated to exist in the pulmonary circulation with regard to certain factors.

Factors which acutely increase pulmonary artery tone are agonists of smooth muscle contraction such potassium chloride and endothelin [29, 30]. Factors which are known to decrease pulmonary artery tone are smooth muscle relaxants such as nitric oxide and acetylcholine [31, 32, 33]. These are released or inhibited as a result of certain stimuli.

There are other factors such as oxygen and temperature. Oxygen is strange because it could be a factor or it could be a stimulus which releases factors. For oxygen to be a factor it must act directly on pulmonary arteries but this is flawed as oxygen exchange is far from the pulmonary artery. Hence, it is more likely logically to be a stimulant. Temperature is almost certainly a stimulant that modulates factors but there is evidence that it can directly affect the infrastructure of cells. I will discuss more about these two next.

Oxygen and the pulmonary circulation

Humans are addicted to oxygen. If you believe in evolution, when the earth did not have much oxygen, species relied on anaerobic respiration to

produce energy [34, 35] As this is not a very efficient process and as oxygen became more readily available, we adapted cells and eventually organs (lungs) to extract this element to provide a more effective method to generate energy [36, 37].

Now one of the problems with addiction to a substance is that one develops ingenious methods to get that substance whilst refraining from activities that don't go to achieving that goal [38]. This happens with oxygen as well. It is postulated that when units in the lung can't get oxygen, these units are cut off to gas exchange so that pulmonary arterial blood can go to areas of the lung that does have oxygen. Although the short-term goal is achieved, the longer term effects, in this case pulmonary hypertension, can be counterproductive.

Hypoxic pulmonary vasoconstriction: definition and effects

Hypoxic pulmonary vasoconstriction (HPV) is this process whereby local vasoconstriction of blood vessels supplying hypoxic areas of lung cause diversion of pulmonary arterial blood flow to well oxygenated areas of lung [39, 40]. This ingenious mechanism preserves systemic oxygenation and has been shown to be the case in various animal models.

The problem arises when the hypoxic stimulus is not removed either due to the living conditions of the organism (high altitude where oxygen levels are low) or as a result of intrinsic lung disease [41, 42]. Some people think that this vasoconstriction leads to an increase in pulmonary artery resistance and subsequent pulmonary hypertension [43] whilst others feel that the original

HPV may actually be of dubious significance in established PAH secondary to chronic hypoxia [44].

Despite the enormous efforts that have gone into studying HPV, there is no definite answer to its mechanism or whether it is universal across species. Rats demonstrate this phenomenon but ferrets and sea lions do not demonstrate HPV [45, 46]. In fact, they demonstrate hypoxic pulmonary vasodilation.

Further to this the results in humans on the subject are variable. Of course, humans are not like animal models as usually we experiment on healthy animals or where we have selectively caused an illness in them (gene knockout etc.) whereas humans are not genetically engineered (yet) and so things are more complex.

The first part of my thesis will look at the human evidence of HPV and was subsequently published as the first review of its kind regarding this.

Pulmonary hypertension: Definition, causes and significance

Definition

Pulmonary artery hypertension (PAH) is defined by the European Society of Cardiology as having a pulmonary artery pressure at rest equal to or greater than 25mmHg as measured by right heart catheterisation [47].

Classification

Below is a table (Table 1) classifying pulmonary artery hypertension with regard to clinical associations [48].

Table 1: Classification of PAH based on aetiology

Primary Pulmonary Artery Hypertension	**PAH associated with lung disease**	**PAH associated with heart disease**	**PAH associated with blood vessel blockage**	**PAH of unknown origin**
Idiopathic Genetic	Chronic Obstructive Pulmonary Disorder Interstitial Lung Disease	Systolic dysfunction Diastolic dysfunction Valvular heart disease	Acute pulmonary embolus Chronic thromboembolic disease	Haematological disorders Systemic disorders Metabolic disorders

Causes and pathology

From the above table regarding the classification of PAH, it is apparent that there is no single cause of pulmonary hypertension. It is evident that it exists under different conditions whether this is as a result of intrinsic problems with the lung or as a result of problems in the pulmonary circulation after the lung as in the case of heart disease.

Pulmonary artery hypertension has different underlying structural changes in the pulmonary artery depending on whether it is acute or chronic.

As an illustration, consider thromboembolic pathologies. An acute pulmonary embolus blocking off the pulmonary artery or an arteriole will cause pulmonary artery hypertension as a result of the offending embolus blocking pulmonary flow and increasing the pressure proximal to the blockage as well as the result of the embolus irritating the artery and causing

localised vasoconstriction. Although there is a reactive post-inflammatory intimal hyperplasia, there is no chronic remodelling of the pulmonary artery [49, 50]. Compare this to chronic thromboembolic disease where the resulting PAH is as a result of the clot resorption and resultant remodelling such as intimal and smooth muscle hyperplasia of the pulmonary artery [51, 52].

The same is true of hypoxia. Chronic hypoxia is associated with pulmonary artery remodelling (smooth muscle hyperplasia, increased connective tissue deposition) and the resultant PAH is a combination of this remodelling, vasoconstriction and polycythaemia [53, 54] whilst acute hypoxia is associated with presumed localised vasoconstriction of pulmonary arteries which is reversible [55, 56].

Effects

Pulmonary artery hypertension leads to increased strain on the right ventricle due to the increased afterload on the right ventricle [57, 58, 59]. The ensuing remodelling of the right ventricle will initially compensate to maintain forward flow of blood through the pulmonary circulation. However, over time, the ventricle will fail and the tricuspid valve will begin to leak leading to decompensated heart failure. Untreated, the patient will die over months or years [60, 61].

Acute PAH is a little more dangerous as the right heart has not had time to remodel and compensate [62, 63, 64]. Hence a massive pulmonary embolus will likely result in acute right heart failure and death over hours or days if left untreated [65].

Implications for cardiothoracic surgery

What is the importance of this phenomenon to me as a cardiothoracic surgical trainee?

There are two angles.

The first angle is from a therapeutic angle. The management of chronic pulmonary artery hypertension is complex. Quite often treating the underlying problem in secondary pulmonary artery hypertension will lead to the resolution of the PAH [66, 67]. However, even removing the insult may not reverse the structural pathological changes in the pulmonary artery and is not relevant in primary pulmonary artery hypertension [68, 69]. Hence, medical strategies may be required directed at the pulmonary artery hypertension per se, such as selective vasodilators of the pulmonary circulation [70, 71]. These may fail and a surgical treatment becomes necessary. The first of these surgical interventions are pulmonary endarterectomies performed for chronic thromboembolic diseases [72, 73]. The second is heart-lung transplantation: there is no point in replacing the failed heart on its own as the pathological pulmonary artery will make the donor heart equally miserable [74, 75].

The second is the implication of PAH on surgical outcomes. In the case of chronic PAH, we know that the presence of this before undertaking cardiac surgery has a significant mortality risk on these patients [76, 77]. The other aspect is that PAH developing during cardiac surgery has a significant impact as these patients now become a high morbidity and mortality risk [78, 79].

It is this last part that intrigues me most. Why do patients who have little or no PAH prior to cardiac surgery, suddenly develop PAH during and immediately following cardiac surgery?

Pulmonary artery hypertension following cardiac surgery

There are 2 reasons. One is that following cardiac surgery, the myocardium of the left ventricle takes a hit either because of ischaemia during the operation (myocardial infarction) or inadequate cardiac protection rendering it more susceptible to transient loss of function (myocardial stunning) [80, 81]. Either way, the failed left heart is unable to clear blood returning to it from the lungs hence it fills back into the pulmonary circulation leading to pulmonary oedema and pulmonary artery hypertension.

The second is less obvious. It is postulated that events during cardiopulmonary bypass (CPB) leads to changes in pulmonary artery tone once CPB is turned off.

CPB is a tool used by cardiac surgeons to direct deoxygenated venous return away from the right heart and instead to a machine which removes CO2 and adds O2 (i.e. performs the function of the lungs) and then returns this to the aorta effectively bypassing the heart and lungs. This is done so we can operate on the heart whilst maintaining circulatory support [82, 83, 84].

As you can see from this, the lungs can be turned off safely. During this time the alveolus of the lung is hypoxic. Hence, it could be this period of hypoxia which causes global vasoconstriction leading to PAH once lung perfusion is restored. Or could it be the re-oxygenation which leads to the PAH after oxygenation is restored? Or could it be as a result of the reperfusion of the lung via the pulmonary circulation and all those metabolites and free-radicals which cause the PAH?

Hypoxia-Reoxygenation in the systemic circulation

Well let's look at hypoxia-reoxygenation first. There has been a lot of work in the last 10 years discerning the effect of "ischaemia reperfusion" in cardiac surgery. Ischaemia is the process whereby the delivery of nutrients (via blood flow) is inadequate to match the metabolic demands of the tissue [85, 86, 87]. Cardiac surgeons are clever as over 60 years ago they discovered that if you arrest the heart using cardioplegia ("cardio" =heart "plegia"=to paralyse) during CPB, then even if you stop the blood flow (to operate on the heart), there is no metabolic activity and so the heart by definition will not become ischaemic [88, 89].

However, such practice is not perfect and we know that there are periods where the heart will become ischaemic due to human error or the patient's heart's intrinsic ability to resist cardioplegia [90]. During this ischaemic period, the heart muscle may become necrosed. For many years, we believed this was the cause of cardiac dysfunction following cardiac surgery or even a heart attack in the community. However, there has been evidence

that it is the reperfusion after CPB or after putting a coronary stent in someone after a heart attack that causes cardiac dysfunction.

How does this reperfusion cause myocardial injury? One reason could be due to oxygen free radicals (such as superoxide anion, hydroxyl radical, and peroxynitrite). These oxygen free radicals do a number of things including reacting with fatty acids leading to damage to membrane-bound enzyme systems and the cell membrane integrity of the endothelium. They are also involved in the depletion of nitric oxide which is essential for the maintenance of the artery endothelium as well as stimulating the release of platelet activating factor from the endothelium thereby promoting neutrophil aggregation and further free radical production [91, 92, 93]. This is all bad for the endothelium.

Another theory suggests that the mechanical reperfusion of the artery may lead to endothelial damage and dysfunction stimulating vasoconstriction in addition to plugging by reactive leucocytosis [94, 95].

But what has this to do with hypoxia-reoxygenation (H-R)? Well, H-R specifically relates to the effect of oxygen in all this and so removes other factors such as shear stress and plasma-based compounds, red cells etc. from the equation.

H-R is actually less widely studied likely because it removes the complexity of reperfusion injury and the dynamic interaction between various systems such as the inflammatory cascade, clotting cascade and free radicals.

However, studies have given important insights. For example, studies on human umbilical veins have shown that isolated endothelial cells subjected

to anoxia-reoxygenation release superoxide anions into the extracellular medium at a concentration reliant on the duration of both anoxia and reoxygenation [96, 97].

This is important as it is not necessarily the reoxygenation which produces free radicals. Hypoxia pre-exposure seems to increase cellular ROS production, likely from mitochondrial electron transport complexes as evidenced by downstream effects of these ROS being inhibited by blockers of mitochondrial electron transport such as rotenone in cardiomyocytes [98, 99].

Hypoxia, also appears to be decrease activity or expression of antioxidant defences. This down-regulation of antioxidant enzymes may also contribute to reoxygenation injury [100, 101].

Reoxygenation injury can therefore be understood in terms 'preconditioning' effects occurring in hypoxia that predispose to cellular dysfunction and cell death during reoxygenation.

The source of these reactive oxygen species is interesting and ranges from production by xanthine oxidase and NADPH oxidases under conditions of re-oxygenation [102].

Hypoxia-reoxygenation in the pulmonary circulation

The lungs do not have an intrinsic activity during CPB as the patient is anaesthetised and so the lungs will not inflate or deflate without a ventilator

device. Hence, one can argue that the lungs are not prone to ischaemia during this time. But this may not be true. We know that the heart is quiescent during cardioplegia as we have an ECG monitor. However, the same cannot be sure that the lung is metabolically inactive during this period. Despite this, we may have a non-argument here because I have already mentioned that the bronchial circulation provides the nutrients to the lung so this is not interrupted during CPB and so even if the lung is metabolically active, like the brain, kidneys etc., it will receive its nutrients. However, the significance of the bronchial circulation to nutrient supply is debatable and one can presume that the pulmonary artery still has a significant input into this.

Ischaemia-reperfusion models of the rabbit lung, for instance, is associated with increased pulmonary vascular resistance and reduced alveolar perfusion in conjunction likely as a result of an inflammatory response rather than H-R per se [103].

In relation to H-R, specifically, studies on human epithelial cells have revealed that culture in normoxia after hypoxia, produced increased ROS and increased lactate dehydrogenase [104].

The effect of this hypoxia-reoxygenation has been demonstrated in isolated canine lungs which showed an increase in vascular permeability and oedema formation. This vascular permeability increase was likely to due to an increase in the resistance of the post-capillary resistance [105]. However, this seems odd as the pulmonary vein is not an active vessel and lacks smooth muscle.

As mentioned previously, free radical production is dependent on the presence of substrates or enzymes such as xanthine dehydrogenase/oxidase. An interesting study looking at pulmonary artery smooth muscle cells from pigs showed that pigs lack xanthine dehydrogenase/oxidase. In these cells, H-R had no effect on cell cytotoxicity or the production of ROS [106].

Isolated rat lung models, for instance have demonstrated that glutathione, a marker of oxidative stress, is released in the pulmonary circulation and the lung during times of H-R. Isolated rat pulmonary endothelial cells reveal that in response to H-R they secrete significant amounts of cytokine induced neutrophil chemo-attractant and monocyte chemo-attractant protein-1[107].

Hence it seems apparent that H-R has significant cellular implications but the physiological effect on contractility still remains elusive.

The first section of my thesis will have a look at the evidence for the contractile effects of oxygen changes in the human pulmonary circulation looking at mechanistic data including the role of calcium. This will lay the foundation for my experimental models which comprise the following chapters.

Effect of temperature on the human pulmonary circulation

In cardiac surgery and lung transplantation, there is a period of hypothermia. It is used in two scenarios. Firstly in routine cardiac surgery (coronary artery surgery and valvular surgery), the body is cooled on CPB to 32 degrees

Centigrade (C) to reduce the metabolic activity of the body and thus reduce the incidence of ischaemia whilst on CPB [108, 109]. The second reason it is employed is so the body can be safely drained of blood so we can "arrest" the circulation and do open vascular surgery without any blood to obscure our view. This second method can only be done in "deep hypothermia" where the body is cooled down to at least 12 C. The theory goes that the brain can safely have circulatory arrest for 20-30 minutes so long as the temperature is at deep hypothermia [110, 111, 112].

There has been evidence that rewarming from deep hypothermia can have severe consequences on the pulmonary circulation [113]. However this is anecdotal.

More interesting to me however, is that this complex surgery on the heart or aorta which necessitate deep hypothermic circulatory arrest quite often results in pulmonary artery hypertension following the operation [114,115]. However this has been put down to events during CPB such as I-R and H-R described above.

But could it be due to rewarming from deep hypothermia?

Hypothermia on the cardiovascular system

Hypothermia was widely believed to have a depressant role in smooth muscle contraction and metabolic activity as evidenced by studies on the vascular system [116, 117], digestive system [118] as well as in skeletal muscle [119].

This would lead many to believe that hypothermia diminishes the responsiveness of the vascular system. This is not true, quite the contrary in fact.

A nice study demonstrated that myocardial strips taken from non-failing hearts in pigs and humans had an increased contractile response to stimulants at moderate hypothermia (32C). This same study showed that in anaesthetised pigs cooled to 32C, whilst their heart rate increased, their cardiac output and stroke volume increased [120].

Rewarming on the vascular tone

It's all well and good cooling something down but what about rewarming? Is there an alteration in physiology from hypothermia back to normal? Do the changes that occur during hypothermia persist upon rewarming? Does rewarming do something new to hypothermia?

In many ways this story of Hypothermia-Rewarming follows a similar story to Hypoxia-Reoxygenation.

Some of these questions have been answered. The changes that we saw in response to hypothermia go to the other extreme upon rewarming. So we saw that hypothermia increased myocardial contractility but now after hypothermia/rewarming, myocardial contractility is significantly reduced in rat models [121].

Similar studies on human resistance arteries such as the radial artery demonstrate that rather than a reduced contractility, Subsequent rewarming from 22 degrees C to 37 degrees C re-established contraction of these

arteries at precooled levels and led to an elevation of the basal tension [122]. Hence, do different vessels respond differently to hypothermia/rewarming?

Whole animal models which show that cooling to 25°C followed by rewarming resulted in a reduced systolic, but not diastolic left ventricular function [123]. This phenomenon has been attributed to rewarming as hypothermia depressed both systolic and diastolic function in dogs but rewarming corrected the diastolic dysfunction but not the systolic dysfunction [124].

Could it be that it is this left ventricular dysfunction which is to blame for pulmonary artery hypertension rather than the direct effect of hypothermia/rewarming on the pulmonary artery?

That is what I will be investigating.

Linking hypothermia to ischaemia-reperfusion and hypoxia-reoxygenation

Studies have shown that changes that exist in contractility during hypothermia, exist in conjunction with I-R. One study showed that in rats, hypothermia improved myocyte contractility and sensitivity to Ca^{2+} under conditions of normal perfusion and following reperfusion after a period of ischemia [125]. Hence temperature changes may not only affect the vascular tone of blood vessels directly but may also modulate the response of arteries to other factors such as oxygen tension.

This was the original research into hypothermia in cardiac surgery because it found that hypothermic synchronized retro-perfusion applied after coronary occlusion and before reperfusion significantly improved cardiac function during occlusion whilst it also minimized complications of reperfusion and reduced myocardial infarction [126].

Effect of Hydrogen Sulphide on the pulmonary circulation

Well that allows a lot to work on regarding causes of pulmonary artery hypertension following cardiac surgery. But how can we treat it?

As mentioned, we can treat the underlying cause but is that quick enough? Surgeons are notorious for having no patience when it comes to decision making (although the complete opposite is true for their technical skills which flourish under patience): they want the answer fast.

There are fast drugs out there that reduce pulmonary artery hypertension in both the acute and chronic phase.

Firstly there are the calcium channel blockers such as nifedipine which were used in the initial treatment era of pulmonary artery hypertension. They have a modest reduction in pulmonary artery pressures whilst having a significant deleterious effect on systemic circulation pressures and contractility of the myocardium [127].

Next there are the endothelin receptor antagonists [128]. These are not only licensed for primary pulmonary artery hypertension but also for heart failure from which either pulmonary artery hypertension is a cause, effect or an association.

More promising are the phosphodiesterase-inhibitors such as sildenafil which work by increasing the bioavailability of nitric oxide which is usually a very fragile gas but which is perhaps one of the most potent vasodilators known. The neat thing about this compound is that it may improve cardiac dysfunction too by associated or unrelated treatment of the pulmonary artery hypertension [129].

There are also the prostacyclins which can be inhaled and swallowed as well as given parentally [130]. Recently, there has been interest in soluble guanylate cyclase stimulators in clinical trials which have also shown promising results [131].

All of the above agents have a modest effect in the reduction of pulmonary artery pressures ranging from 5-10% when the baseline pressures are almost twice the normal range. What we need is an agent that significantly reduces pulmonary pressures whilst preserving systemic pressures.

I think there may be a potential candidate in hydrogen sulphide.

Hydrogen sulphide: what is it?

Hydrogen sulphide is a remnant of the abyss from which we came. Its smell is repulsive (akin to bad eggs apparently) not because it is something new which we fear but it is something from our past that we are trying to forget [131].

H2S is synthesized naturally in the body from L-cysteine mainly by the activity of two enzymes, cystathionine-g-lyase and cystathionine-b-synthetase. H2S is metabolized by oxidation in mitochondria or by methylation in the cytosol. It is excreted mainly by the kidney as free or conjugated sulphate [132].

Hydrogen sulphide is considered a dangerous substance. In 1950 in Poza Rica, Mexico, there was an accidental discharge of hydrogen sulphide rich gas which in the worst affected region resulted in 22 dying [133]. The mechanisms by which H2S is believed to exert such damage is believed to be as a result that at relatively high concentrations of H2S (about 50 μM), it affects the activity of the respiratory chain, causing a complete inhibition of the complex IV [134, 135]. But anything in large enough quantities can kill including oxygen, water, warfarin the list goes on.

It wasn't until 15-20 years ago that scientists began to exploit H2S at "therapeutic" doses. Findings have demonstrated that at low concentrations (<20 μM), H2S stimulates oxidative phosphorylation and increases ATP biosynthesis [136].

Interestingly, H2S has recently been thought to be an oxygen sensor and may be a remnant of the evolutionary pathways associated with switching from anaerobic to aerobic respiration [137].

Hydrogen sulphide as a vasodilator

Furthermore, H2S has been found to be a potent dilator of animal systemic vessels. These vessels are not only the large conductance vessels such as the aorta but also the smaller resistance vessels, such as the mesenteric arteries, which determine blood pressure [138, 139].

This is likely to be mediated by the activation of ATP-sensitive potassium channels (KATP) as several effects of H2S are mimicked by other KATP-openers and abolished by KATP-blockers [140]. However, other pathways have been suggested including cross talk between H2S and Nitric Oxide as inhibition of NO attenuates the vasodilatory effects of H2S [141].

Hydrogen sulphide in the lungs

Within the pulmonary circulation, H2S content and CSE activity are significantly enhanced in isolated rat lung subjected to ischemia/reperfusion injury. In fact, preventive perfusion with H2S attenuates such an injury, reducing malondyaldehyde (MDA) production and stimulating superoxide dismutase and catalase activity [142].

With regard to pulmonary hypertension, the endogenous CSE/H2S pathway has been found to be down-regulated in hypoxic PAH (HPH), resulting in a decreased endogenous H2S production in rat lung tissues due to oxidative stress [143].

With regard to the airways, again animal models have demonstrated that it exerts a dilatory role. Mouse bronchial rings, for instance, dilate significantly to 'therapeutic' doses of H2S [144].

Hydrogen as a possible therapeutic agent for PAH

Hence, it seems from the above information on H2S that it is an important vasodilator and may be important in the role of lung physiology. It therefore may be an important agent to study not only for its role as a potential pulmonary vasodilator but also as a bronchodilator so as to help with oxygen delivery in the context of pulmonary hypertension.

Associations and Impact of pulmonary hypertension

Although cardiac surgical risk stratification systems such as the EUROSCORE incorporate pulmonary artery hypertension as a significant predictor of mortality, there is little in the way of how PAH affects other outcomes in surgery such as respiratory and gastro-intestinal complications.

In addition, as we have alluded to, the mechanisms of PAH are complex and largely unknown.

Therefore, I thought it may be useful to have a supplemental chapter of the predictors/associations of PAH in our cardiac surgical patients over the last 20 years as well as outcomes of the presence of pre-operative PAH following cardiac surgery.

Aim

The aim of this, thesis, therefore, is to investigate the reactivity of human pulmonary arteries to various stimuli.

I wanted to focus not only on the possible factors which may control pulmonary pressures in humans (oxygen and temperature) but also identify possible therapeutic strategies to combat pulmonary artery hypertension in humans.

The first chapter is the first review of hypoxic pulmonary vasoconstriction exclusively from human studies.

The second chapter builds on the previous by conducting detailed experimental data concerning the gaps in our knowledge of the impact of changes in oxygen tone upon the pulmonary circulation in humans at the tissue and organ level as well as factors that may influence this.

The third chapter concerns the effect that temperature may play on pulmonary vascular tone. I looked at the effect that deep hypothermia and normothermia play in regulating pulmonary arterial tone at the tissue and organ level.

The fourth chapter explores one potential therapeutic strategy to combat rises in pulmonary artery tone: Hydrogen Sulphide. I describe the effect this peculiar compound has on the pulmonary circulation.

The supplemental chapter tackles the issue of PAH in the per-operative period and the impact this has on the outcomes of patients undergoing cardiac surgery at our institution.

The Epilogue brings all these chapters together.

Section C: Methods

Introduction

The methods describe herein are generic for the relevant chapters which follow. Detailed experimental protocols for specific studies will be discussed in more detail in the appropriate chapter.

Literature review Chapter

The chapter entitled “Hypoxic pulmonary vasoconstriction in humans” (Chapter 1 part A) was published as a review article in Biomedical Research International.

It followed the theme of a ‘narrative review’ [145]. A narrative review is an important contribution to the literature as it brings together diverse information on a particular condition to make it easier to read not only for the lay clinician or scientist but also experts in the field. It is often difficult to see the wood from the trees particularly for such a complex topic as hypoxic pulmonary vasoconstriction (HPV).

Laying the groundwork for my thesis, I realised that much of the data concerning the pulmonary circulation pertained to animal studies. Whilst these were complex, detailed and quite exhilarating in their methods and endeavours, the clinician can be forgiven for asking “what is the relevance

in humans?". This is why I undertook this literature review for my thesis and submitted it as the first paper exploring HPV particularly in humans.

The narrative review was conducted as follows:

(1) A focussed question was asked "What is the evidence for HPV in humans".

(2) The following sources of information were used

  (a) PUBMED online database

  (b) EMBASE/Excerpta Medica

  (c) Cochrane Database of Systemic reviews

(3) The search term was "Hypoxic Pulmonary Vasoconstriction in humans"

(4) Inclusion criteria:

  (a) All primary basic science articles on HPV in humans from 1950 to the present

  (b) All primary clinical articles on HPV in humans from 1950 to the present

  (c) All Journals and languages as long as they were translated into English

(5) Exclusion criteria:

  (a) Animal studies on HPV

(6) Analysis of data

  (a) Evaluating purpose of study reviewed

  (b) Providing a synopsis of the content of the study

  (b) Critiquing the research design or methods used in each study

  (c) Presenting a brief review of the important findings

(7) Synthesis of data: Article findings were sorted into (a) effects of hypoxia/hyperoxia on human tissue (b) effects of hypoxia/hyperoxia on clinical parameters in humans

(8) Formulating a conclusion and anticipating future directions

The PRISMA diagram for this literature review is given in Figure 2.

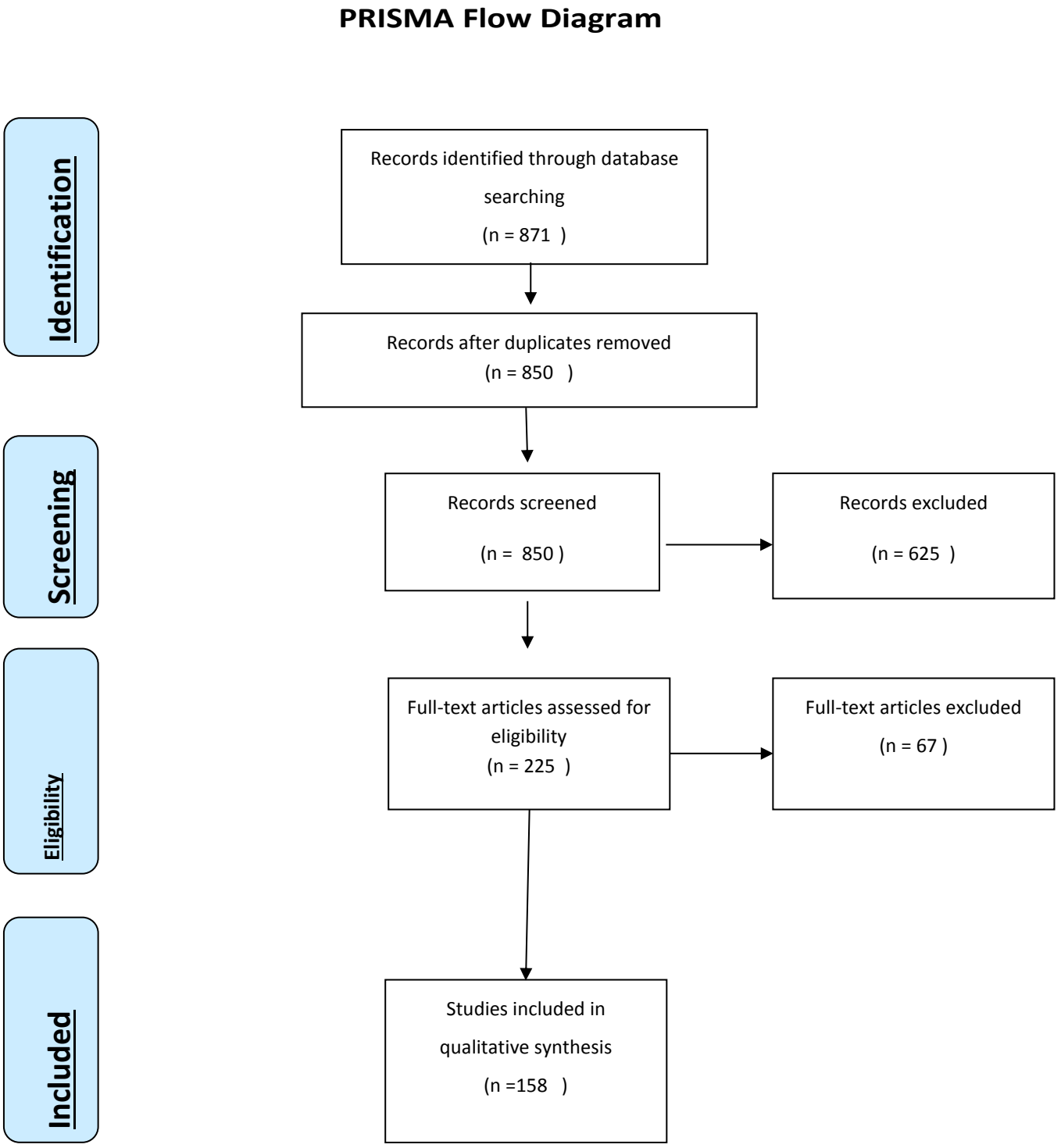


Figure 2: PRISMA for HPV review chapter

Human tissue experiments

Ethical approval for these experiments was obtained and the documentation for this is given at the beginning of the thesis.

As a condition of the ethical approval, all tissue is anonymised and so this precludes analysis of patient characteristics as factors affecting the vasoactivity of isolated arteries and isolated perfused lungs.

Isolated Ring Models

The chapters in my thesis pertaining to studying the effects of oxygen, temperature and hydrogen sulphide on the human pulmonary artery used techniques studying isolated human pulmonary artery rings in organ baths and is described in this section.

Why use isolated rings?

Isolated rings allow evaluation of drugs and conditions on the human pulmonary artery at a tissue level. It may be able to evaluate whether the sensing mechanism for a response to a stimuli seen at a clinical level resides in the tissue itself if the response can be reproduced. However, it would not be able to ascertain whether the sensing mechanism for the response is definitely in the tissue studied. For example, take the case of a pulmonary artery ring exposed to hypoxia and the resultant constriction. Although we can say that there must be an oxygen-sensing mechanism within the

pulmonary artery which allows it to give a response, it does not mean that hypoxic pulmonary vasoconstriction occurs as a result of pulmonary artery oxygen sensing: it could be because oxygen sensing occurs at another place e.g. the alveolar-capillary bed and the signal is conducted to the pulmonary artery.

Isolated rings have been used to study animal models of disease for as long as the last two generations can remember [146, 147]. Interestingly, many experimental results diverge mainly because of the conditions used to test certain agonists. For example, HPV cannot be elucidated in some experiments unless a certain degree of pre-constriction is provided [148].

Human experiments are rarer for the simple reason that the tissue is utilised from resected lungs taken from patients with lung disease mainly bronchial carcinomas. Hence, much of the results can be debated as to whether they are representative of normal physiology despite the fact that the tissue is normally trimmed from areas of tumour-free zones. However, it is a validated method that has yielded interesting results [149, 150].

Method for isolated rings

Informed consent was obtained for human lung tissue obtained from patients undergoing surgery for bronchial carcinoma at Castle Hill Hospital, Cottingham, UK. Ethical approval for this project was obtained from the Regional Ethics Committee.

Extralobar (mean internal diameter 4mm) and medium sized intralobar (mean internal diameter 2 mm) pulmonary arteries were dissected from healthy areas of lung resections. Arteries were cut into 5mm thickness rings.

Vessels were mounted between stainless steel wires connected to an isometric force transducer (Dynamometer UFI Devices, UK) (see Figure 3). The rings were immersed in a 25ml water jacketed organ bath (Radnoti, USA) containing Krebs-Henseleit (Krebs) solution warmed to 37°C. Oxygen was bubbled at varying concentrations (0%-hypoxia, 21%-normoxia and 95%-hyperoxia, balance N2) and CO2 was kept constant at 5% to maintain a pH of 7.4.

A resting tension of 1-2g was applied and the vessels were allowed to equilibrate for 60 minutes. The endothelial integrity of each vessel was confirmed by significant vasodilatation to acetylcholine (ACh, 1 mM) before the experimental protocol. Smooth muscle viability was confirmed by exposure to potassium chloride (30 mM KCl) at the end of the experiment. Any arteries not responding to KCl were excluded from the analysis (Please see figure 3).

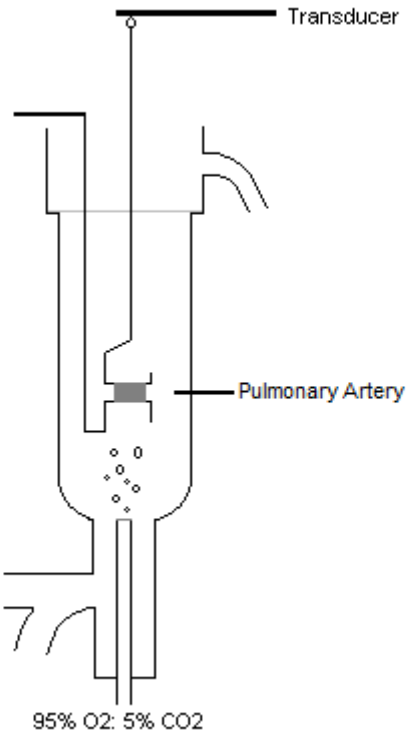


Figure 3: Isolated Pulmonary Artery Ring set-up

Sample size for isolated pulmonary artery rings

Power calculations, based on preliminary data from our laboratory, showed that a total number of 6 patients was able to detect a 20% difference in the vasomotor responses of pulmonary arteries to oxygen, hydrogen sulphide and temperature, with $\alpha = 0.05$ and statistical power 80%. This gave us a total of 18 patients for the baseline changes to the above conditions.

We used 5 modulators (Nifedipine, Dantrolene Sodium, DPI, 2-APB and L-NAME) to study the mechanistic insights into responses to oxygen. Preliminary studies showed that Power calculations, based on preliminary

data from our laboratory, showed that a total number of 6 patients was able to detect a 50% difference in the vasomotor responses of pulmonary arteries to these changes, with $\alpha = 0.05$ and statistical power 80%. This gave us a total of 30 further patients.

We used 3 modulators (Potassium Chloride, Endothelin-1 and Sodium Nitroprusside) to study the mechanistic insights into responses to hypothermia. To compare differences between two groups (those exposed to hypothermia and those maintained in normothermia), power calculations, based on preliminary data from our laboratory, showed that a total number of 3 patients in each group was able to detect an 80% difference in the vasomotor responses of pulmonary arteries to hypothermia, with $\alpha = 0.05$ and statistical power 80%. This gave us a total of (3 X 3 X 3) 18 further patients.

Hence a minimum of 64 patients were required. We obtained rings from 89 patients.

New (fresh) rings were used to check each condition (oxygen changes, temperature and hydrogen sulphide).

A flow diagram of how these rings used are given below (Figure 4):

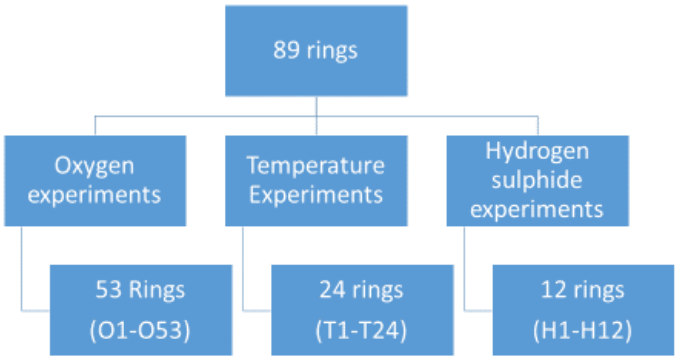


Figure 4: Flow diagram for distribution of pulmonary artery rings used

Isolated perfused lung models

The chapters in my thesis pertaining to studying the effects of oxygen, temperature and hydrogen sulphide on the human pulmonary circulation as a whole used techniques studying isolated human perfused lung models and is described in this section.

Why use perfused lung models?

Isolated Perfused Lung Models (IPLM) serve as a model to evaluate the effect of substances such as drugs and gasotransmitters as well as conditions such as temperature and acidic/alkaline on the organ as a whole. This is important as it checks whether the effects seen at a tissue level hold true at an organ level too.

In addition it evaluates whether effects seen in the lung are as a result of intrinsic pulmonary mechanisms or whether there is a systemic component. For example, hypoxic pulmonary vasoconstriction seen from derivative parameters such as pulmonary blood flow in clinical studies may or may not occur at the isolated lung level implying that for HPV to occur, it must involve the lungs in conjunction with systemic input and signalling pathways.

IPLM have been well described in animals where the animals are usually free off disease [151, 152]. In humans, like the isolated ring models, there is the restriction that we are using diseased lungs. This raises two concerns. Firstly, there are the ethical challenges. This lung will be sent for histological analysis after the experiments; there is therefore, the risk that the experiments may inadvertently affect the staging by destroying lung tissue or making analysis difficult. This will affect the subsequent treatment of the patient (chemo/radiotherapy etc.). Secondly, the diseased lungs may already have a degree of abnormal vasoactivity such as pulmonary artery hypertension as many of these patients with have chronic lung.

However, the model is useful as it moves away from animal studies to human models. The model is validated from previous experiments as well as clinical studies which study ex-vivo lung perfusion used for optimisation of lungs prior to transplantation [153].

Method for isolated perfused lungs

Informed consent was obtained for human lung tissue obtained from patients undergoing surgery for bronchial carcinoma at Castle Hill Hospital, Cottingham, UK. Ethical approval for this project was obtained from the Regional Ethics Committee.

The ex-vivo Krebs perfused human lung system used in the present study is shown in Figure 5. The lung sample was suspended in a polycarbonate collection reservoir (Sorin Biomedica, Quedgeley, Gloucester, UK) from a force transducer (Thames Side Maywood Instruments Ltd, Tilehurst, Berkshire, UK) to allow continuous measurement of lung weight. The pulmonary arterial and bronchial systems were cannulated. The bronchial cannula was connected to a piston ventilator (Harvard Apparatus Ltd, Edenbridge, Kent, UK) to allow ventilation with room air. A respiration rate of 10 breaths/min and tidal volume of 100–300 ml were set according to the size and airway resistance of the lung sample and maintained for the duration of the experiment. The resultant mean airway pressure was 15 (4.7) mm Hg. The pulmonary artery cannula was then connected to the perfusion circuit which consisted of connective PVC tubing (Cobe Laboratories,

Quedgeley, Gloucester, UK), which had been primed with 1 litre oxygenated Krebs bicarbonate solution to ensure that all air had been evacuated. The perfusate comprised Krebs bicarbonate solution aerated with 95% O2/5% CO2 and was circulated to the pulmonary arteries from a second polycarbonate perfusate reservoir (Baxter Healthcare, Compton, Berkshire, UK) via a peristaltic pump (Watson-Marlow, Falmouth, Cornwall, UK). The perfusate temperature and pH were continuously recorded using in line temperature (Terumo UK, Knowsley, Merseyside, UK) and pH (Philips Medical Systems, Leeds, West Yorkshire, UK) probes. The temperature was controlled by a heat exchanger (Sorin Biomedica) and the pH at 7.4 via a pH controller (Medical Physics, Hull Royal Infirmary, Hull, UK) which regulated the flow of CO2 into the perfusate. Pulmonary venous drainage flowed freely into the collection reservoir before being returned to the perfusate reservoir for re-circulation. Perfusion pressure was continually monitored via a pressure transducer (Datex-Ohmeda Ltd, Hatfield, Hertfordshire, UK) and maintained at 16.4 (5.8) mm Hg by regulating the perfusion flow rate. This was determined for each sample on the basis of the size and vascular resistance of the sample, and ranged between 100 and 500 ml/min. The perfusate PO2 was varied using a membrane oxygenator (D 905 EOS) to create hypoxic, normoxic and hyperoxic perfusates. The concentration of oxygen in the ventilator was varied using hypoxic, normoxic and hyperoxic gas cylinders (British Oxygen Company).

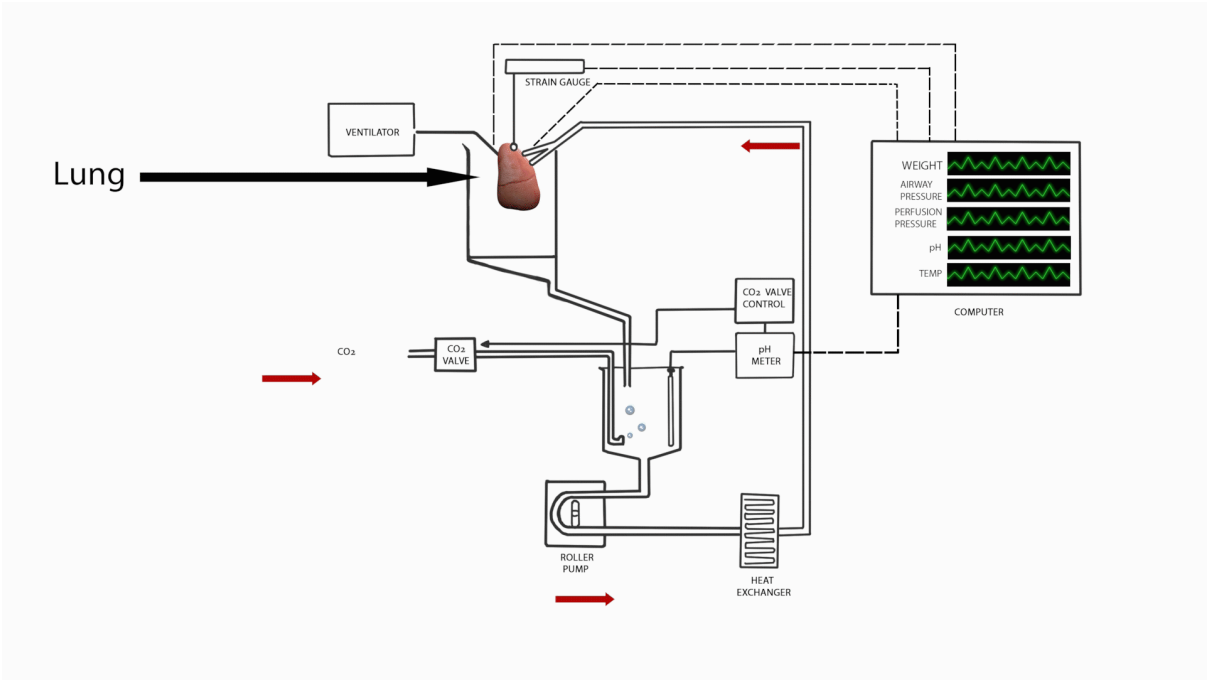


Figure 5: Isolated perfused Lung Model set-up

Sample Size Calculation for Isolated Perfused Lung Models

Power calculations, based on preliminary data from our laboratory, showed that a total number of 3 patients was able to detect a 40% difference in the vasomotor responses of pulmonary arteries to oxygen, hydrogen sulphide and temperature, with $\alpha = 0.05$ and statistical power 80%. This gave us a total of 3 patients for each of for the baseline changes to the above conditions (oxygen, temperature and hydrogen sulphide). An extra 3

patients were required to compare lungs maintained in 37C compared to lungs maintained in 17 degrees C and rewarmed to 37 degrees C.

New lungs were used to check each condition (oxygen changes, temperature and hydrogen sulphide).

A flow diagram of how many lungs were used in each experiment condition is given below (Figure 6).

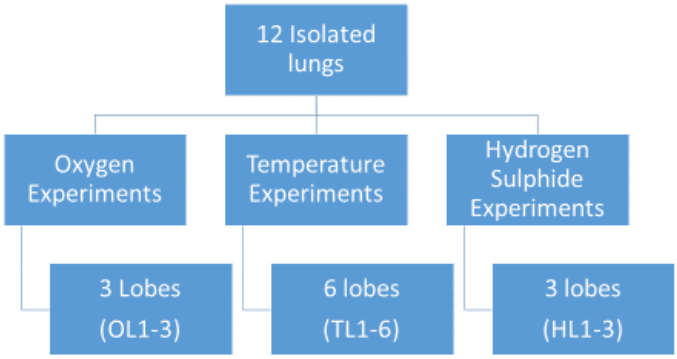


Figure 6: Flow diagram for distribution of perfused lungs used

Protocol for oxygen experiments

Informed consent was obtained for human lung tissue obtained from patients undergoing surgery for bronchial carcinoma at Castle Hill Hospital, Cottingham, UK. Ethical approval for this project was obtained from the Regional Ethics Committee (Rec reference 12/LO/1233).

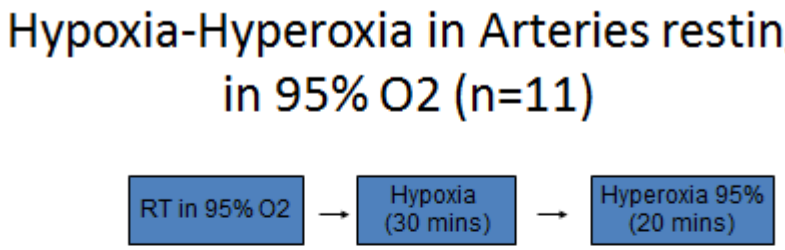
Extralobar (mean internal diameter 4mm) and medium sized intralobar (mean internal diameter 2 mm) pulmonary arteries were dissected from healthy areas of lung resections Arteries were cut into 5mm thickness rings.

Vessels were mounted between stainless steel wires connected to an isometric force transducer (Dynamometer UFI Devices, UK) (see Figure 3). The rings were immersed in a 25ml water jacketed organ bath (Radnoti, USA) containing Krebs-Henseleit (Krebs) solution warmed to 37°C. Oxygen was bubbled at varying concentrations (0%-hypoxia, 21%-normoxia and 95%-hyperoxia, balance N2) and CO2 was kept constant at 5% to maintain a pH of 7.4.

A resting tension of 1-2g was applied and the vessels were allowed to equilibrate for 60 minutes. The endothelial integrity of each vessel was confirmed by significant vasodilatation to acetylcholine (ACh, 1 mM) before the experimental protocol. Smooth muscle viability was confirmed by exposure to potassium chloride (30 mM KCl) at the end of the experiment. Any arteries not responding to KCl were excluded from the analysis.

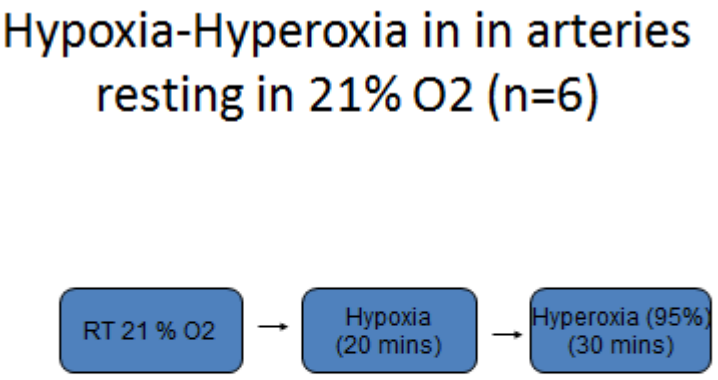
Effect of H-R on vessels maintained in 95% O2

Vessels (n=11) were equilibrated for 60 minutes in *hyperoxic* conditions and resting tension recorded. Vessels were then exposed to 30 minutes of hypoxia and subsequently re-oxygenated with hyperoxia for 30 minutes and tension recorded.



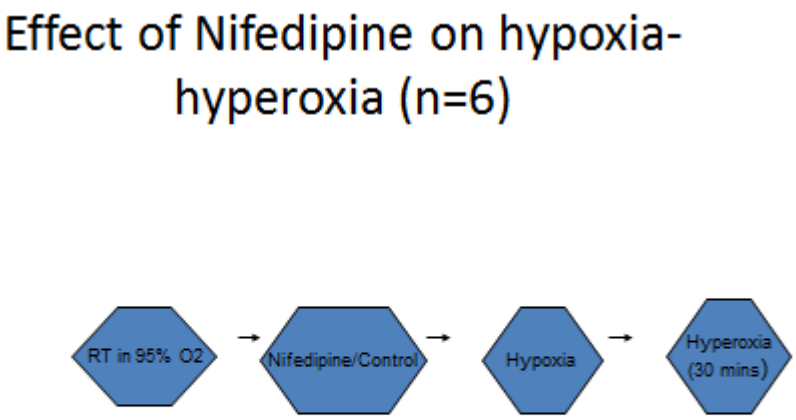
Effect of Hypoxia-Hyperoxia on vessels maintained in 21% O2

Vessels (n=6) were left to equilibrate for 1 hour in *normoxic* conditions and resting tension recorded. Vessels were then exposed to 20 minutes of hypoxia and subsequently re-oxygenated with hyperoxia for 30 minutes and tension recorded.



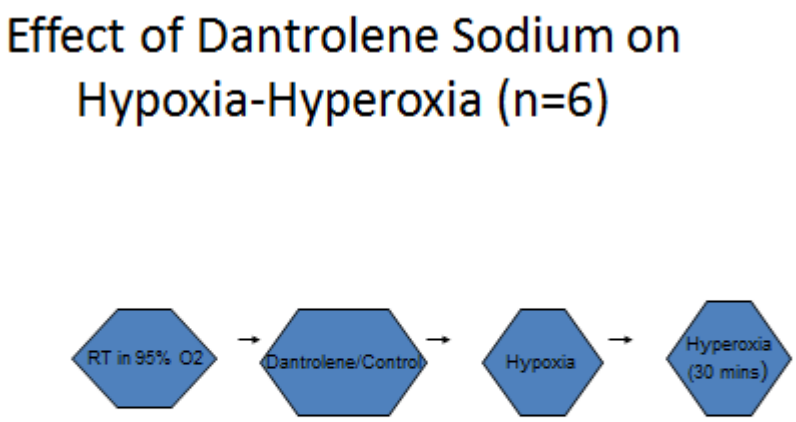
Effect of calcium L-type channels on the vasoconstrictive response

Vessels (n=6) were left to equilibrate for 1 hour in *hyperoxic* conditions at 37C and resting tension recorded. Vessels were then exposed to 20 minutes of hypoxia and reoxygenated with hyperoxia for 30 minutes. Nifedipine (5µM) was added and the experiment was repeated.



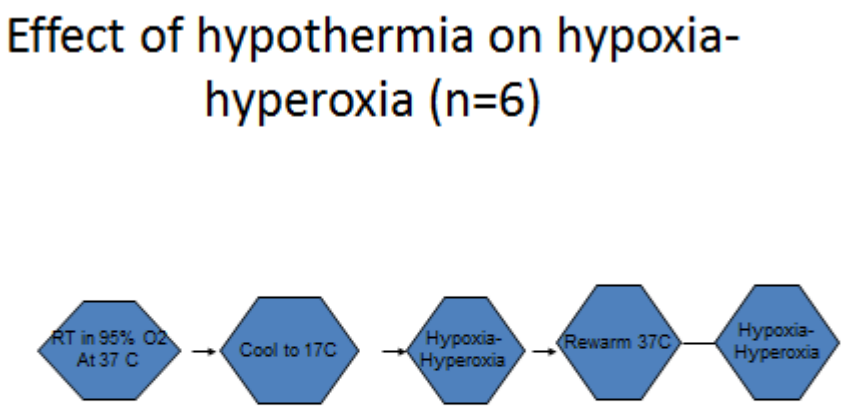
Effect of dantrolene on the vasoconstrictive response

Vessels (n=6) were left to equilibrate for 1 hour in *hyperoxic* conditions and resting tension recorded. Vessels were then exposed to 20 minutes of hypoxia and reoxygenated with hyperoxia for 30 minutes. Dantrolene Sodium (10µM-200µM) was added and the experiment was repeated.



Effect of hypothermia on the H-R response

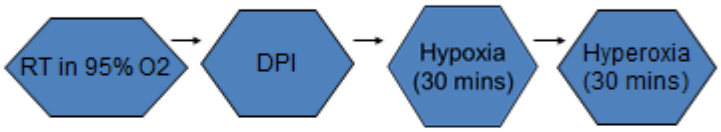
Vessels (n=6) were left to equilibrate for 60 minutes in *hyperoxic* conditions at 17C and resting tension recorded. Vessels were constricted with 30mM KCl and washed out. Vessels were then exposed to 20 minutes of hypoxia and subsequently re-oxygenated with hyperoxia for 30 minutes and tension recorded. Arteries were then rewarmed to 37C, constricted with 30mM KCl and washed out. H-R as above was repeated at 37C.



Effect of DPI on re-oxygenation and KCl-mediated contraction

Vessels (n=6) were left to equilibrate for 1 hour in *normoxic* conditions and resting tension recorded. Vessels were then exposed to 20 minutes of hypoxia and reoxygenated with hyperoxia for 30 minutes. 2-APB (100µM-500µM) was added and the experiment was repeated. 30mM of KCl was added to the organ baths and allowed to reach maximum tension. Arteries were then washed out and left to re-equilibrate in normoxia and resting tension recorded. DPI (100nm) was added to the organ bath (n=6). Vessels were then exposed to 30 minutes of hypoxia. Vessels were re-oxygenated with hyperoxia for 30 minutes. Without a washout, arteries were exposed to 30mM KCl to establish any effect of DPI on the maximal contraction to KCl.

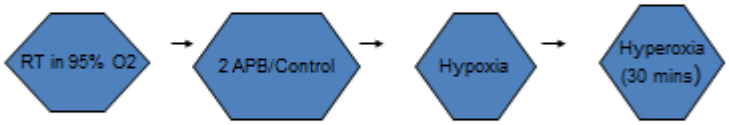
Effect of DPI on hypoxia-hyperoxia
(n=6)



Effect of 2-APB on hypoxia-reoxygenation

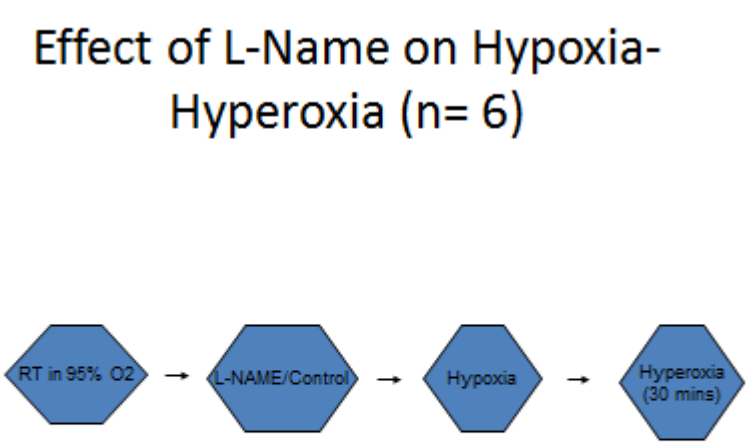
Vessels (n=6) were left to equilibrate for 1 hour in *hyperoxic* conditions and resting tension recorded. Vessels were then exposed to 20 minutes of hypoxia and reoxygenated with hyperoxia for 30 minutes. 2-APB (100µM-500µM) was added and the experiment was repeated.

Effect of 2-APB on hypoxia-hyperoxia
(n=6)



Effect of L-NAME on hypoxia-reoxygenation

Vessels (n=6) were left to equilibrate for 1 hour in hyperoxic conditions and resting tension recorded. Vessels were then exposed to 30 minutes of hypoxia and subsequently re-oxygenated with hyperoxia for 30 minutes and tension recorded. L-NAME (1mM) was added and hypoxia-reoxygenation was repeated.



Isolated perfused and ventilated lung models

Effect of H-R on lobes maintained in 21% O2

Lobes (n=3) were left to equilibrate for 1 hour in *normoxic* conditions at 37C and pulmonary artery pressures were recorded. Lobes were then exposed to 20 minutes of hypoxia and subsequently re-oxygenated with hyperoxia for 30 minutes and pulmonary artery pressures recorded.

Reagents used

Krebs bicarbonate solution consisted of 113.8 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.2 mM KH2PO4, 11.4 mM glucose and 2.4 mM CaCl2 dissolved in deionised water. Reagents were from Fisher Scientific (UK) except Nifedipine (Tocris USA), Dantrolene sodium (Tocris, USA) and acetylcholine chloride (Sigma-Aldrich, UK). Gas

mixtures were from the Linde Group (UK). Oxygen saturations in the organ baths were measured using an oxygen probe (ArrowLab (™)).

Protocol for temperature experiments

Study model and design

Ethical approval for this project was obtained from the Regional Ethics Committee (12/LO/1233). Informed written consent was obtained from each patient to use surplus tissue from lung resection surgery for research.

Extralobar (mean internal diameter 4mm) and medium sized intralobar (mean internal diameter 3 mm) pulmonary arteries were dissected from healthy areas of lung resections from patients with lung cancer. Arteries were cut into 5mm thickness rings.

Vessels were mounted between stainless steel wires connected to an isometric force transducer (Dynamometer UFI Devices, UK). The rings were immersed in a 25ml water jacketed organ bath (Radnoti, USA) containing Krebs-Henseleit (Krebs) solution at either 17 degrees centigrade (17C) for deep hypothermia or 37 degrees centigrade (37C) for normothermia. The temperature was maintained at either 17C or 37C by a heat exchanger (Sorin Biomedica) (See Figure 3).

Experimental protocols

A resting tension of 1g was applied and the vessels were allowed to equilibrate for 60 minutes. Arteries were pre-constricted with 30nM KCl prior to the experiment. Any arteries not responding to Potassium Chloride (KCl) were excluded from the analysis.

Effect of Endothelin-1, KCl and SNP on vessels maintained in 37C

(i) *Endothelin-1*: Vessels (n=4) were left to equilibrate for 60 minutes in normoxic conditions at 37C and resting tension recorded. 100pM-1nM Endothelin-1 was added to the organ baths and allowed to reach maximum tension.

(ii) *KCl*: Vessels (n=4) were left to equilibrate for 60 minutes in normoxic conditions at 37C and resting tension recorded. 300µM-30mM of KCl was added to the organ baths and allowed to reach maximum tension.

(iii) *SNP*: Vessels (n=4) were left to equilibrate for 60 minutes in normoxic conditions at 37C and resting tension recorded. 30mM of KCl was added to the organ baths and allowed to reach maximum tension. 1µM-100µM SNP was then added to the organ bath and allowed to reach maximum tensions.

The time taken to reach maximum tension in each case was a mean of 10 minutes.

Differential effect of Endothelin-1, KCl and SNP at 17C warmed to 37C

The above experiments (i-iii, each with n=4) were repeated at 17C and then the arteries were then washed out. They were then rewarmed to 37C and the experiments repeated again at 37C.

(b) *Isolated perfused and ventilated lung models*

Effect of hypothermia on the isolated lungs

Lobes (n=3) were left to equilibrate for 60 minutes in *normoxic* conditions at 17C and resting pulmonary artery pressures recorded. Lobes were constricted with 30mM KCl and washed out. Lobes were then rewarmed to 37C, constricted with 30mM KCl and washed out. This constriction to KCl was compared with lobes (n=3) subjected to 30mM KCl maintained at 37C without cooling or rewarming.

Reagents used

Krebs bicarbonate solution consisted of 113.8 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.4 mM glucose and 2.4 mM $CaCl_2$ dissolved in deionised water. Reagents were from Fisher Scientific (UK) except SNP (Sigma-Aldrich, UK) and Endothelin-1 (American Peptide Company, CA, USA). Krebs was bubbled with 95% oxygen and 5% carbon dioxide to stabilise and normalise to PCO2 and pH of the buffer.

Protocol for hydrogen sulphide experiments

Isolated human pulmonary artery rings

Ethical approval from the local ethics committee was obtained (12/LO/1233) to measure isometric in large and medium sized pulmonary artery rings (mean diameter) 4mm obtained from lung resections for patients with bronchial carcinoma (n=12).

Smooth muscle viability was confirmed by contraction to potassium chloride (30 mM KCl) and endothelial integrity was confirmed with dilation to 1mM Acetylcholine.

Vessels were mounted between stainless steel wires connected to an isometric force transducer (Dynamometer UFI Devices, UK). The rings were immersed in a 25ml water jacketed organ bath (Radnoti, USA) containing Krebs-Henseleit buffer solution warmed to 37°C, continuously bubbled with 95% O2 and 5% CO2 to maintain a pH of 7.4 (See Figure 3). Vessels were pre-constricted with KCl (30mM) as this technique has been used in previous studies to investigate the vasodilatory effects of certain compounds in the context of acute pulmonary artery hypertension [223]. The effect of hydrogen sulphide (H2S) was investigated in a dose-dependent manner using sodium hydrogen sulphide. We used a range of 20µM-500µM of H2S as this has been used in previous studies to investigate its effect on human systemic arteries [224, 225].

Isolated human perfused lungs

Informed consent was obtained for human lung tissue obtained from patients (n=3) undergoing surgery for bronchial carcinoma at Castle Hill Hospital, Cottingham, UK. Ethical approval for this project was obtained from the Regional Ethics Committee (13/NW/0042).

Hydrogen sulphide (50µM-500µM) was added to the circulating perfusate and the resultant pulmonary artery and bronchial pressures were measured.

Reagents used

Krebs bicarbonate solution consisted of 113.8 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.2 mM KH2PO4, 11.4 mM glucose and 2.4 mM CaCl2 dissolved in deionised water. Reagents were from Fisher Scientific (UK) except sodium hydrosulfide hydrate which was from Sigma-Aldrich (USA). Gas mixtures were from the Linde Group (UK). Oxygen saturations in the organ baths were measured using an oxygen probe (ArrowLab (™)).

Preliminary Measurements and Statistical analysis for oxygen, temperature and hydrogen sulphide experiments

Tensions were recorded on Lab Chart (ADInstruments, UK). These were recorded at the end of the time intervals indicated. We chose these specific time intervals as preliminary results demonstrated that the time taken for

maximum dilation to hypoxia was between 15 and 20 minutes and the time taken to maximum constriction to hyperoxia was between 20 and 30 minutes (Table 2).

Table 2: Preliminary results to determine time-frames

PA ring	Time taken to maximum dilation to hypoxia (mins)	Time taken to maximum constriction to hyperoxia (mins)
Preliminary ring 1	15.5	20.5
Preliminary ring 2	18.5	22.5
Preliminary ring 3	17	27

Other studies have measured tensions at maximum contractions and dilations rather than at certain time-points [154]. However others have used measurements at certain time points [155]. There does not appear to be differences between the methods in regard to outcomes and we employed the method of discrete time-frames as it allows for consistent recording.

Contractions or dilations of pulmonary artery are expressed as a percentage of the resting tension. Data are presented as means +/- standard error of the mean (SEM). Statistical analysis was by paired student t-tests for repeated measurement analysis and by unpaired student t-tests to compare treatment groups. A p-value of <0.05 was considered significant.

The distribution of the data was tested for normality first prior to parametric statistical tests being employed.

All statistical analysis was performed using SPSS statistics 20 (IBM Chicago, IL, USA).

Retrospective clinical chapter methods

The final appendix chapter of my thesis is a small retrospective study evaluating the predictors and effects of raised pulmonary pressures in our cardiac surgical population.

Retrospective studies are important for two reasons. Firstly, when clinical trials are not possible, it may be the case that retrospective studies can help define prognostic factors so that a therapeutic strategy may be implemented depending on the predicted risks. Secondly, retrospective studies are relatively inexpensive and faster to conduct than others studies.

I obtained the data from the national cardiac database system (Dendrite) which features characteristics entered prospectively when a patient undergoes cardiac surgery. Local approval from the audit review board is not necessary as data is anonymised on this central database and is subject to the regulations of the National Cardiac Surgery Database: due to the retrospective nature the need for patient consent was waived, on the understanding that individual patients are not identified.

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This study was conducted on a population of 14,652 consecutive cardiac surgery patients who underwent operation between April 1995 and March 2013. All data were collected prospectively during the patient admission and entered onto a cardiac surgery database as part of routine clinical practice. Methods of data collection and definitions have been previously published [156] and are also available from www.nwheartaudit.nhs.uk. Validation of data involved checking each record for completeness. Any data identified by a trained audit officer as different between that recorded in the medical records and that recorded in the database were flagged back to the relevant surgical team for correction, if required. All records entered onto the databases were also cross-checked against theatre books to ensure inclusion of all cases.

Patient characteristics were defined in accordance with the Society of Cardiothoracic Surgeons of Great Britain and Ireland minimum dataset.

Continuous data are shown as median values with 25th and 75th percentiles. Categorical variables are shown as a percentage and comparisons were made with $\chi 2$ tests as appropriate. Standard statistical tests were used to calculate odds ratios and 95% confidence intervals. A multivariate logistic regression analysis was undertaken on the development dataset, using the forward stepwise technique, to identify independent risk factors for raised pulmonary artery pressures.

Section D: Experiments and results

Chapter 1: Role of oxygen in determining pulmonary artery tone

Part A: Hypoxic pulmonary vasoconstriction in humans

Abstract to chapter part

Hypoxic pulmonary vasoconstriction is the elegant theory put forward more than six decades ago to explain regional variations in perfusion within the lung in certain animal species in response to localised restrictions in oxygenation. Although considerable progress has been made to describe the phenomenon at the macroscopic level and explain it at the microscopic level, we are far from a universal agreement about the process in humans. This review attempts to highlight some of the important evidence base of hypoxic pulmonary vasoconstriction in humans and the significant gaps in our knowledge that would need bridging.

Introduction

Hypoxic Pulmonary Vasoconstriction (HPV), although regarded as a physiological process that preserves systemic oxygenation, is also regarded as a pathophysiological entity predisposing to heightened pulmonary artery tones and subsequent pulmonary artery hypertension [43].

Whilst the process is established in some species, it is far from conclusive in humans where the exact site and nature of the response to hypoxia is the subject of much controversy and debate.

Reflected in this is, although thorough and extensive, is the domination of much of the published reviews concerning this phenomenon with animal models of the disease process with little in the way of human data [39].

In this review, we seek to concentrate on the existing evidence for HPV within humans looking at responses all the way from isolated human pulmonary cells to clinical studies in patients.

Human Isolated Pulmonary Artery Smooth Muscle Cells

Acute hypoxia

Pulmonary Artery Smooth Muscle Cells (PASMCs) are located across the full length of the pulmonary arterial tree from large pulmonary arteries to (albeit to a lesser degree) the smaller arterioles [22].

Isolated PASMC react to hypoxia without either the influence of the surrounding lung parenchyma or systemic transmitters. This is an important concept as it has led some researchers to postulate that the PASMC may be

the oxygen sensor to varying oxygen tensions [56]. This concept, however, is fraught with problems not least because the pulmonary arteries, even the smaller pre-capillary arterioles, are distant from the alveolus where gas exchange occurs [157].

Despite this, the mechanisms underlying the hypoxic response in PASMCs continue to be intriguing.

Calcium plays an important role in this response. Intracellular calcium is increased in isolated human PASMC subjected to acute hypoxia (<5 minutes).

For example, Tang et al, using a mixture of functional pharmacology and gene knockout techniques, have shown that this acute hypoxic-calcium increase in human PASMCs is dependent to a lesser degree on voltage gated calcium channels (inhibition of which attenuated the hypoxic calcium related increases by 30%), and to a greater degree on other trans-membrane channels such as the Transient Receptor Potential (TRP) channels (inhibition of which attenuated the response by 60%) [158]. Further to this, the subtype of TRP channel plays an important role. Inhibition of store operated TRP channels such as TRPC1, which function dependently on depletion of intracellular calcium stores, attenuated the hypoxic-calcium response to lesser degree than TRPC6 channels which are ligand operated.

Meng et al have shown that this hypoxic-calcium increase is inhibited by Arachidonic Acid (AA) which considerably attenuated the calcium increase [159]. Breakdown of AA by Cyclo-oxygenase-2 (COX) enhanced the hypoxic-calcium response suggesting AA itself mediates the attenuation of

the hypoxic-calcium response rather than its derivatives. Interestingly, one of the inhibitors of this AA mediated inhibition is diacylglycerol which coincidentally is a ligand of the TRPC6 channel possibly suggesting a link between AA and receptor operated TRP channels in regulating the calcium response to hypoxia in human PASMCs.

Indeed, TRP channels are known to be important in the proliferation of human pulmonary vascular smooth muscle [160]. In addition, in patients with pulmonary artery hypertension, TRP channels have been found to be over-expressed [161, 162]. However, the role of TRP channels in acute vasoconstriction in human pulmonary arteries is less certain.

Other important messengers besides calcium are reactive oxygen species (ROS) developed in response to changes in oxygen tension. Mehta et al have shown that under sustained hypoxic conditions (1-4 hours), ROS are decreased within human PASMCs [163]. They also noted that normoxic ROS synthesis is predominately mitochondrial in origin within these cells, suggesting that the mitochondria may play a central role in the regulation of the acute hypoxic response in these cells.

Remarkably, they compared the response to human coronary artery smooth muscle cells which also showed a reduction in ROS but as we know, systemic arteries dilate to hypoxia in contrast to that suggested in their pulmonary cousins. Why this differential contractile response to a similar intracellular phenomenon exists within the same species is still a mystery.

These results concur with other studies which show decreased ROS production in response to acute hypoxia but increased ROS in chronic hypoxia in isolated human pulmonary artery smooth muscle cells [164].

It must be noted, however, that the aforementioned experiments avoided testing PASMC contractions in isolation per se, seeking rather to establish the intracellular signalling responses. Hence one cannot confirm if the respective changes in messaging pathways manifested as a contraction or dilatation. This is important as many of the cultured cells have come from companies with little details in the manuscripts about the site of origin of these cells: be they from larger pulmonary conductance arteries or from smaller arterioles or a mixture. Larger conductance arteries, as we know from animal and human studies, respond differently to hypoxia compared to resistance arteries. Understandably, it is technically difficult to assess contractility in single cells but there are methods that have been used in animal models of HPV including tension forces generated by cells grown on a flexible growth surface (polymerized polydimethyl siloxane) manifesting as wrinkles and distortions of the surface under the cells or from measurements of myosin light chain phosphorylation [165].

Chronic hypoxia

The plot thickens when one moves away from acute hypoxia to more chronic hypoxic insults. Wu et al have shown that although acute hypoxia (5-10 minutes) stimulates a reduction in ROS, chronic hypoxia (48 hours) actually increases ROS production in human PASMCs [166]. This points to a potential difference at the subcellular level response to acute and chronic

hypoxia which may explain the phenotypical changes seen in acute hypoxia (vasoconstriction) and chronic hypoxia (vascular remodelling).

Key regulators of vascular remodelling in response to chronic hypoxia include the Rho GTPase family of proteins. They are involved in cell adhesion, migration and proliferation. An aesthetic study by Wojciak-Stothard et al demonstrated in human PASMCs that Rho B levels were significantly increased in acute hypoxia (30 minutes-4 hours) [167]. This increase coincided with an increase in cytoskeleton remodelling within human PASMCs (represented by an increase in stress fibre formation). The authors further showed that this increase could be mimicked in normoxic conditions by inducing overexpression of Rho B implying Rho B's significant role in this process.

To cement the role played by the Rho GTPase family, Yu et al have shown that Rho kinase A (ROCK) expression was increased in sustained periods of acute hypoxia (4-12 hours) in human PASMCs, implying hypoxia may affect the Rho A/ROCK pathway, implicit in smooth muscle proliferation which may account for the hypoxic pulmonary artery hypertension seen in chronic models secondary to remodelling [168].

It is worth noting that the degree of acute hypoxia (0% O2-5% O2) and definitions of acute hypoxia and chronic hypoxia are variable which may give rise to inconsistencies. Part of the problem for this is that what may be acute in some animals may be chronic in others and this may account for the variability in human studies.

Hence future experiments may focus on establishing the actual differential contractile responses of isolated cells cultured from various sites of the pulmonary arterial tree with consistent degrees and durations of hypoxia.

Human Isolated Pulmonary Artery Endothelial Cells

Isolated human pulmonary artery endothelial cells (PAECs) are a major source of Nitric Oxide (NO) production in the pulmonary circulation via endothelial Nitric Oxide Synthase (eNOS). Experiments looking at the effect of hypoxia on endothelial cells have predominately been concerning more prolonged hypoxic exposure (48 hours). Takemoto et al have told a continuation of the Rho story by demonstrating this chronic hypoxia is associated with an increase in ROCK expression with a simultaneous *decrease* in eNOS mRNA and protein expression in human PAECs [17]. The fact that eNOS increased by blocking ROCK with its selective inhibitor, hydroxyfasudil, demonstrated that eNOS may be dependent on ROCK.

Ghrelin is known to have protective effects on endothelial cells which are notoriously fragile entities. Yang et al have shown that hypoxia for 24 hours reduces human PAEC viability and this is prevented by pre-treatment with ghrelin [169]. Sub analysis revealed that ghrelin increased NO secretion and eNOS phosphorylation in hypoxic conditions.

As NO is a potent vasodilator, eNOS inhibition would logically suggest that the vasoconstrictive-vasodilation balance may be shifted in chronic hypoxia towards constriction by reducing NO production.

Despite this, Beleslin-Čokić et al have shown that chronic hypoxia (48 hours) actually causes an *increase* in NO production within human PAECs [170]. However, consistent with the above data, they did show a decrease in eNOS. This increase in Nitric Oxide has been confirmed in another study by Krotova et al which showed that hypoxia increased NO in human lung microvascular endothelial cells [171]. So where would this increase in NO come from? It appears that there is an increase in other NOS enzymes notably inducible NOS (iNOS). Thus, it appears that the body may try to compensate for the constrictive remodelling seen in PASMCs in response to chronic hypoxia by inducing the release of dilatory mediators such as NO from PAEC via iNOS.

With regard to proliferation of PAECs, in contrast to PASMCs which have been shown to proliferate under prolonged periods of hypoxia (2-7 days), Yu et al have shown that human PAECs do not proliferate under hypoxic conditions [172]. This implies that human PASMCs may thrive under hypoxic stimuli as opposed to human PAECs which succumb to their fragility under similar conditions.

Studies have also demonstrated that PAECs, under hypoxic conditions, increase their permeability [173]. Perivascular oedema, may contribute to changes in lung vascular resistance, but this would be due to passive compression rather than active hypoxic vasoconstriction.

Figure 7 and Figure 8 give an overview of the mechanisms by which of acute and chronic hypoxia affect human pulmonary cells.

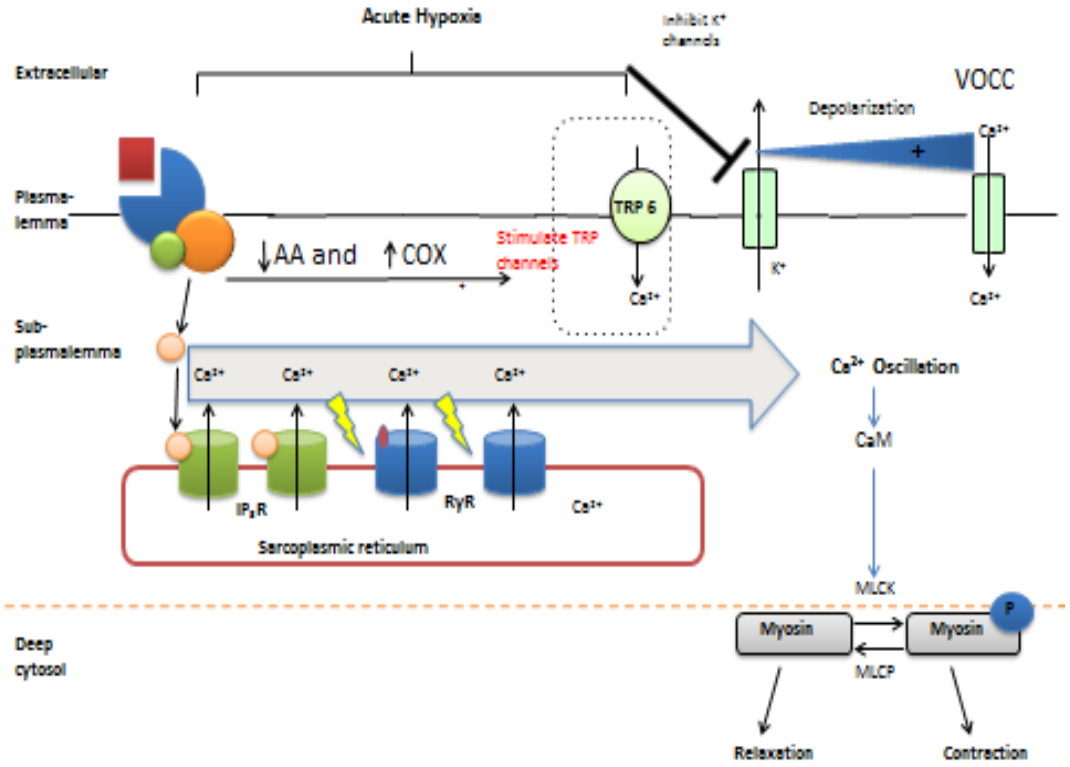

Figure 7: Model for Acute Hypoxic Vasoconstriction in Humans

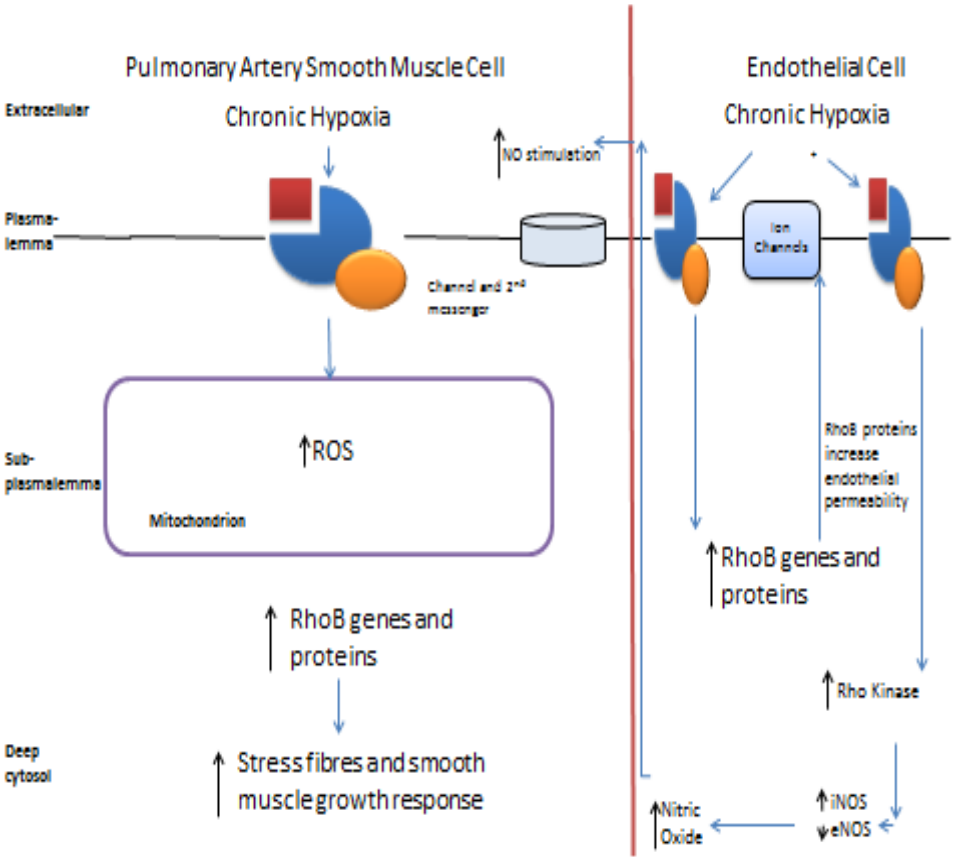

Figure 8: Model for chronic hypoxic remodelling in human pulmonary arteries

Human pulmonary artery rings and strips

Studies on human pulmonary artery strips (HPASs) and rings (HPARs) have been less consistent than studies on isolated cells. The majority of tissue was taken from healthy sections of lung from patients who underwent lobectomies for lung cancer.

Hoshino et al demonstrated that in HPASs, very little response occurred to acute hypoxic stimulation (<5 mins) when arteries (<5mm diameter) were allowed to rest naturally with a tension of 2g [174]. However, when arteries were pre-stimulated with histamine, they constricted to hypoxia. The response was significantly attenuated by compounds such as HA 1004 which is an inhibitor of cyclic nucleotide-and calmodulin-dependent-protein kinases as well as attenuation by depletion of intracellular calcium.

This necessity for pre-stimulation appears to be a common theme with HPARs as well. Demiryurek et al have shown that pre-contracted rings constrict to acute hypoxia in a manner that is dependent on the presence of the endothelium as denuding the endothelium resulted in a markedly reduced hypoxic vasoconstrictive response [175].

However, Ohe et al have shown that smaller HPARs (<2mm) needn't have any degree of pre-stimulation and were able to constrict to hypoxia in their natural resting state in a calcium-dependent fashion [176].

We have shown that un-stimulated larger HPAR (mean diameter 4mm) dilate to hypoxia (0% O2) in a Nitric Oxide-independent manner and constrict to hyperoxia (95% O2) in a voltage-gated calcium-dependent fashion [177].

It is unclear why, therefore, a certain degree of pre-stimulation is warranted in some studies and not others particularly as we have just seen that human PASMC have changes in intracellular calcium and other 2nd messenger components without recourse to pre-stimulation.

One reason for the variability of results could be because of the patients from whom the samples are taken from. These are lung cancer patients with varying degrees of other pulmonary and systemic disorders. Studies have shown there are considerable differences in the responsiveness of HPARs in patients with different pulmonary disorders. For example, Cases et al showed that in patients on bronchodilator therapies had greater contraction to noradrenaline and greater relaxation to acetylcholine compared to patients without bronchodilator requirements [178].

This appears to be supported by the fact that Pienado et al have found that in patients with COPD (i.e. those on long-term bronchodilator therapy), there is an increase in the expression of certain potassium channels such as BK_{Ca} within HPARs which was positively correlated to a greater degree of constriction in response to hypoxia (again in the presence of pre-constriction) [179].

It is apparent, therefore, that further work needs to be done on human pulmonary artery rings to elucidate if conditions other than pre-stimulation or pre-existing lung disease (and associated pharmacological agents) play a role in varying the response to hypoxia.

Isolated lung models

One way in which the problems of site of HPV and isolated responses of the pulmonary circulation can be overcome would be with isolated perfused and ventilated human lung models. Although extensively researched in animals, isolated lung models have yet to be substantiated in humans. By ventilating the airways with varying concentrations of oxygen and monitoring airway and pulmonary artery pressures, one can investigate the overall pulmonary arterial response across the vascular the tree without systemic cardiac output interference and the contribution of systemic hormonal effects.

A curious contribution to raised pulmonary arterial pressures may be the compression of the surrounding parenchymal tissue in response to hypoxia which has been demonstrated in animal and human studies [180]. By measuring changes in weight in the isolated lung and bronchial dilation, one could theoretically evaluate how much compressive effect oedema and bronchial pressures play respectively, on the surrounding pulmonary vasculature.

An indirect evaluation of reaction to oxygen changes in the isolated lung have come as a by-product of ex-vivo lung perfusion (EVLP) strategies for donor lung optimisation prior to transplantation into patients. EVLP allows for improvement in lung physiology in lungs that would otherwise not be considered for transplantation in an age of limited donor supply. George et al have shown that pulmonary artery pressures rise upon reperfusion of explanted lungs from patients in EVLP and that this rise is greatest when the initial period of ischaemia was greatest [181]. However, there is as yet, little data on the effect of hypoxia-reoxygenation via ventilating the explanted

lung with varying degrees of oxygen and this would be interesting to look at to evaluate the subsequent effect on pulmonary artery pressures.

Acute hypoxic challenges in patients

Measurement of changes in pulmonary artery pressures in response to varying the inspired oxygen concentration (FIO2) in ventilated patients has yielded valuable results in the cumulative effect of hypoxia-reoxygenation on both the pulmonary and systemic circulation.

Historically, in the 1950s and 1960s when a lot of the interest took off, there was actually a lot of conflicting evidence governing the effects of unilateral hypoxia (ventilating one lung with hypoxia and the other with normoxia or hyperoxia) on the pulmonary circulation. Fishman et al from New York developed a method in 1955 combining broncho-spirometry, with each lung breathing a specifically selected oxygen mixture, cardiac catheterization, and arterial cannulation to apply the Fick principle to measure blood flow within each lung as well as total blood flow in addition to pulmonary artery pressure in 6 anaesthetised male patients undergoing lung resection [182]. They found that by controlling one lung with a hyperoxic FIO2 (25-33%) and subjecting another to normoxia followed by hypoxia (10-12%) for 25 minutes, there was *no* alteration in blood flow to either lung or any changes in pulmonary vascular pressures.

This is in contrast to Defares et al from Sweden, who in 1958, utilised a similar technique but this time in 12 normal subjects and they found that the blood flow to the hypoxic lung fell from 55% to 33% during a similar

period and concentration of hypoxia from Fishman's study [183]. They reasoned that this discrepancy between results could be attributed to the fact that Fishman used patients diagnosed with tuberculosis or suspected bronchogenic carcinomas whilst Defares' patients were healthy volunteers.

Defares' group later repeated the experiment in the lateral decubitus position as opposed to the subject in the supine position (as in the case of the experiments above). They showed that this hypoxic redistribution of blood flow is not powerful to overcome the gravitational effects of blood diversion from the upper lung to the lower lung in the lateral thoracotomy position [184].

One of the leaders in this experimental field, Hedenstierna, compared flows in the hypoxic (FIO2= 8%-12%) lung to the contralateral hyperoxic (FIO2=100%) lung in patients and found that although there was a significant reduction in the relative blood flow to the hypoxic lung (without a change in total cardiac output), there was no change in the pulmonary artery pressure [185]. Interestingly, they found that giving one lung hyperoxia and another normoxia, made no difference to relative lung flows and pulmonary artery pressures which contradicts some animal models demonstrating hyperoxic vasodilation and others implying the oxygen free radical release from hyperoxia may stimulate vasoconstriction [186, 187].

The results of regional blood flow redistribution have been repeated by Morrell et al without cardiac output studies and without recourse to general

anaesthesia but using radio-labelled isotopes and scintigraphic lung imaging under local anaesthetic conditions by utilising bronchoscopic techniques of brief periods of selective lobar occlusion [188]. The results have been similar to general anaesthetic studies. However, a potential confounding factor is that the partial pressure of carbon dioxide increased in the occluded lobe/segment and this may contribute to a vasoconstrictive response in addition to the regional hypoxia.

From the above experiments, there is an obvious implication that reduced regional perfusion in hypoxia equates to a hypoxic vasoconstriction although this has not been demonstrated directly in these studies.

With regard to modulators of this hypoxic response, as the majority of these studies have been done by anaesthetists, they had a preconditioned disposition to investigate the effect of anaesthetic reagents on this redistribution effect. Hedenstierna's group had measured regional pulmonary blood flow in response to unilateral hypoxia in the presence of clinical doses of the maintenance anaesthetic agent isofluorane (1% and 1.5%) and found it had no effect on the hypoxic redistribution of blood flow [189].

One cannot help but be sceptical from the above data with regard to a local modulation of HPV. If blood flow is indeed redistributed in response to hypoxia with hypoxic segments and normoxic/relatively hyperoxic segments dilating, then there must be some degree of central control either

within the pulmonary circulation or within the body at large. However, contrary to this theory of a compensatory vasodilation response in the ventilated/hyperoxic lung would be the notion that the normal healthy human lung has a negligible resting tone hence would not be able to dilate further as evident by the lack of vasodilatory response to inhaled Nitric Oxide in those subjects breathing air [190].

With regard to global hypoxia, a study by Talbot et al showed that if patients received global hypoxia for 4 hours via a hyperbaric chamber without anaesthesia, there was an increase in the tricuspid pressure gradient as measured by echocardiography [191]. Tricuspid pressure gradient is a validated measure of pulmonary vascular tone although it is dependent on numerous factors not least the requirement for a certain degree of tricuspid regurgitation. Nevertheless, this small study consisting of 9 patients seems to demonstrate that global hypoxia, as opposed to regional hypoxia, would cause a net increase in pulmonary vascular tone.

Cargill et al using a similar method of measuring changes in pulmonary vascular tone, found that rendering healthy volunteers globally hypoxic for *brief periods* (30 minutes) by inhaling hypoxic gas mixtures, the tricuspid pressure gradient increased [192]. This increase was significantly attenuated by infusing patients with Brain Natriuretic Peptide but not Atrial Natriuretic Peptide prior to the hypoxic challenge.

This rise in PVR in response to global hypoxia appears to be supported by a similar study by Dorrington et al in which 6 healthy volunteers received a

more prolonged period of global hypoxia 5hours-8 hours in a hyperbaric chamber, measuring pulmonary vascular resistance (PVR) in a more invasive fashion utilising a pulmonary artery catheter [193]. They found that PVR increased more than two fold within a couple of hours of hypoxic exposure and this was reversed upon normoxia.

Frostell et al, in awake healthy subjects, demonstrated that global inhalation of a hypoxic gas mixture for only 6 minutes resulted in an increase in the mean pulmonary artery pressure [194]. Importantly, however, this was accompanied by a significant increase in cardiac output, implying that HPV may not be the only response to hypoxia but there is a systemic cardiac response which also contributes to the raised pulmonary artery pressures (PAP) as a consequence of hypoxia. Frostell also found that this rise in PAP was attenuated by Nitric Oxide although whether this is antagonising HPV or merely vasodilating independently, remains to be elucidated.

Global hypoxia appears therefore to impact on pulmonary artery pressures to a greater degree than regional hypoxia as well oxygenated districts of the lung can compensate for suspected HPV whilst global hypoxia, it would seem, by a mixture of increased cardiac output (systemic control) and raised pulmonary tone (pulmonary control) shifts the balance towards reversible pulmonary hypertension.

Studies on Chronic Airway Disease Patients

It is widely believed that the pulmonary artery hypertension associated with chronic hypoxia is due more to vascular remodelling, hypervolaemia, polycythaemia and increased blood viscosity rather than HPV per se. The initial famous observations documented by Penaloza and Arias-Stella demonstrated that although Peruvians in general are born with right ventricular hypertrophy and elevated resting pulmonary artery pressures, those that remain at sea level demonstrate a rapid reversal of these phenomenon whilst those remaining at high altitudes show little regression of these characteristics [195, 196]. Autopsy of these individuals revealed that this PAH was likely due to the thickening of the muscular layers of the pulmonary arterial tree. They measured partial pressures and saturations in these inhabitants and concluded there was a direct causal relationship between hypoxia and PAH.

Although this is an important finding, it may be an associative phenomenon rather than a causal one. For example, it is not as straight forward as this as oxygen concentration is not the only change upon moving to higher altitudes, there are changes in other atmospheric and ecological factors. Further to this, other humans who live in high altitudes such as Tibetans demonstrate neither raised pulmonary artery pressures nor any structural abnormalities of the pulmonary arterial tree [197]. This difference may be due to evolutionary factors as Tibetans have populated the high altitudes for a much longer time, and are therefore much better adapted to the resultant hypoxic conditions, as compared to Peruvians. However, this explanation still remains hypothetical.

Studies in patients with chronic lung disease have demonstrated possible existence of HPV controlling regional lung blood flow. For example Santos et al have shown that in patients with Chronic Obstructive Pulmonary Disorder (COPD), the dispersion of blood flow improved dramatically upon administration of 100% oxygen, the authors thereby stipulating that the HPV pre-existing in these patients was alleviated [198]. Although this is a derivative finding that HPV exists in these patients, it is interesting to note that even in the chronic stages of hypoxia, HPV appears to be at least partially reversible.

Another interesting group of patients are those with obstructive sleep apnoea (OSA). Boyson et al demonstrated that patients who have episodes of apnoea during the night have associated rises in pulmonary artery pressures and this was in company of small fluctuations in oxygen saturations [199]. However, OSA patients have pulmonary artery hypertension during the daytime as well when they are not apnoeic [200], suggesting hypoxia associated with periods of apnoea is not a simple answer to the rises in PAP but other complex physiological and structural factors may be involved.

Conclusions and future directions

Hypoxic Pulmonary Vasoconstriction is a peculiar phenomenon where rather than the standard negative feedback mechanisms in place in the systemic circulation to improve oxygen delivery in times of scarcity, the lung seeks rather to shut things down completely. Animal studies have

provided the basis to investigate basic and complex pathways that may explain this entity.

However, there are emerging inconsistencies. For example, there has been a pre-occupation with the oxygen sensing mechanism residing in the normally hypoxic pulmonary artery when it is actually miles away (relatively) from the alveolus where gas exchange occurs. Recent animal studies have provided insights into the sensory apparatus living in the capillary-alveolar network which would make more logical sense and this should stimulate human research into this area [157].

In addition, as there are significant inter-species differences in the responses of the pulmonary arterial tree to hypoxia and some species contradict HPV completely [200], there needs to be a greater drive to build upon the existing valuable human data to pinpoint the exact response of human pulmonary arteries to hypoxia and under what conditions.

The two main problems with this are firstly there is a scarcity of human tissue and the centres that can obtain tissue from surgery are not necessarily the centres with the cutting edge technology to investigate the samples. Secondly, we have seen that the variation in the response to oxygen even within humans may be due to the differences between ‘healthy’ patients and those with significant pulmonary disease; invasive investigations of healthy subjects would present many ethical and logistical considerations [201]. Hence, those exemplary researchers with valuable expertise in animal models of HPV and technological methods must be allowed to liaise with clinicians who have access to the primary patient samples.

In conclusion, despite the advancements made in discerning HPV in nature, the mechanisms behind the cellular, tissue, organ and whole body response to hypoxia in humans remain in its infancy.

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Part B: Effect of hypoxia and hyperoxia in isolated human pulmonary arteries and isolated perfused lung models

Abstract to chapter part

Introduction: Acute rises in pulmonary artery pressures following cardiopulmonary bypass may be attributed to hypoxia-reoxygenation (H-R) and reperfusion events taking place in the hitherto isolated pulmonary circulation. We investigated these events in human isolated perfused lung models in addition to within isolated human pulmonary artery ring whilst evaluating the role of calcium and temperature in the response to these events.

Methods: Human lobar pulmonary artery rings (*n*=18) and lobes (*n*=6) were obtained from resections for patients with bronchial carcinoma. Fresh rings were mounted in organ baths containing normoxic Krebs solution and subsequently exposed to brief periods of hypoxia followed by hyperoxia in the presence or absence of (a) a cell membrane voltage-gated calcium channel antagonist, nifedipine, (b) an intracellular calcium store-antagonist, dantrolene sodium, and (c) an inhibitor of reactive oxygen species (Diphenyleneiodonium) whilst tension was recorded. Isolated perfused human lung models consisted of lobes ventilated via a bronchial cannula and perfused with Krebs via a pulmonary artery cannula. A membrane oxygenator allowed oxygen tensions to vary whilst CO2/pH remained constant and pulmonary artery pressures were recorded.

Results: Re-oxygenation caused a net vasoconstriction of isolated human pulmonary artery rings ($p<0.05$). This was inhibited by nifedipine and dantrolene ($p<0.05$). Diphenyleneiodonium abolished the hyperoxic vasoconstrictive response ($p<0.05$) but it did not affect the constriction of these arteries to potassium chloride. In contrast, isolated perfused lungs

showed no changes in pulmonary artery pressures in response to hyperoxia either via the ventilator or the perfusate ($p>0.05$).

Conclusions: Re-oxygenation causes vasoconstriction of isolated human pulmonary arteries dependent on extracellular and intracellular calcium signalling. However, re-oxygenation either via the bronchi or the perfusate does not cause an elevation in pulmonary artery pressures in isolated perfused human lungs, thus demonstrating relaxatory compensatory mechanisms within the whole lobe to counteract reoxygenation-induced pulmonary vasoconstriction. This is important, not only as pathophysiological observation concerning cardiopulmonary bypass but also has implications for lung optimisation in ex-vivo lung perfusion prior to lung transplantation.

Introduction

In this study, we hypothesised that hypoxia-reoxygenation would predispose human pulmonary arteries to heightened tones and this would be reflected both at the isolated arterial level as well as within the whole lung. We also hypothesised that calcium may be important in this response and that rewarming would further augment the risk of pulmonary hypertension.

Acute rises in pulmonary artery pressures are associated with a high peri-operative morbidity and mortality in cardiac surgical patients [202]. Although the evidence shows that the common final pathway is likely to predispose to acute right heart failure [203], there is less consensus as to how this acute pulmonary artery hypertension originally occurs.

Ischaemia-reperfusion injury following cardiopulmonary bypass has been extensively studied in animal models not only in the heart [204] but also within the context of systemic end-organ damage for example in the case of renal and hepatic injury [205, 206]. However, little is known of the effects on the lung. The limited animal studies that do concern the pulmonary system, have shown that rather than ischaemia-reperfusion per se, it is actually the hypoxia-reoxygenation events occurring during reperfusion of the pulmonary circulation and re-instating lung ventilation that may carry a burden on the reactivated lung [207, 208]. Despite this, human data is still lacking in this area.

Calcium, both from the extracellular compartment as well as intracellular stores, is known to play an important role in smooth muscle contraction

within arteries in response to changes in oxygen tension [209, 210]. However, little is known of its role in the human pulmonary circulation and it would be interesting to discern its role in re-oxygenation events.

Vasoconstrictive mediators, in response to oxygen changes, may involve reactive species-dependent NADPH-oxidase pathways [211].

Results

Effect of H-R on vessels maintained in 95% O2

All vessels dilated in response to hypoxia. The mean vasodilatory response was 18.2% +/-1.4 ($p<0.005$). Upon re-oxygenation vessels responded with a mean vasoconstriction of 13.1% +/-1.3 ($p<0.05$). Subsequent H-R produced similar ($p>0.05$) vasodilatory responses to hypoxia (17.4% +/-1.5) and vasoconstrictive responses to hyperoxia (14.5% +/-1.0) (see Table 3 & Figure 9).

Table 3: Response to H-R in pulmonary arteries maintained in hyperoxia

Pulmonary Ring	Resting tension (grams)	tension (grams) to hypoxia round 1	% hypoxic dilation round 1	tension to hyperoxia round 1 (grams)	% hyperoxic vasoconstriction round 1	tension to hypoxia round 2 (grams)	% hypoxic vasodilation round 2	tension to hyperoxia round 2 (grams)	% hyperoxic constriction round 2
O1	2.20	1.68	23.62	1.94	15.33	1.65	14.82	1.90	15.12
O2	1.90	1.48	22.17	1.77	20.02	1.56	11.83	1.77	13.39
O3	1.90	1.50	21.25	1.80	20.58	1.43	20.69	1.69	18.34
O4	2.30	1.86	18.99	2.10	12.62	1.88	10.60	2.17	15.42
O5	1.60	1.31	17.93	1.42	8.35	1.16	18.21	1.25	7.56
O6	1.90	1.55	18.26	1.70	9.78	1.40	17.99	1.53	9.47
O7	2.10	1.62	22.97	1.84	14.04	1.62	12.19	1.89	16.42
O8	1.90	1.74	8.27	1.93	10.83	1.48	23.27	1.71	15.49
O9	1.80	1.52	15.57	1.74	14.62	1.35	22.61	1.59	18.25
O10	2.00	1.73	13.61	1.87	8.04	1.59	14.85	1.86	17.28
O11	2.10	1.73	17.39	1.92	10.84	1.45	24.54	1.64	12.92

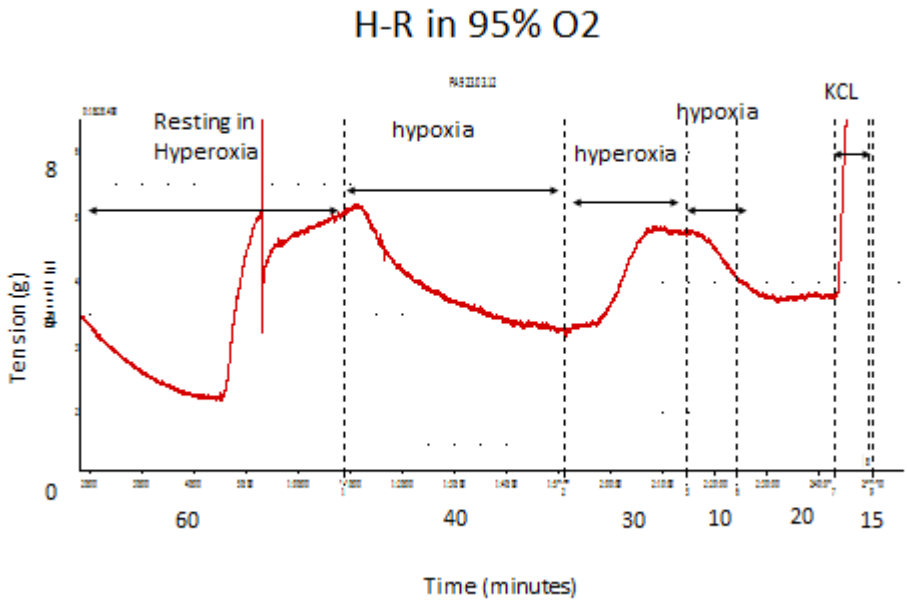

Figure 9: Acute H-R in 95% oxygen

Effect of H-R on vessels maintained in normoxia

In vessels maintained in normoxia, there was no significant vasodilatory effect to hypoxia ($p>0.05$). However, vessels constricted in response to subsequent hyperoxia with a net mean vasoconstriction of 11.5% (+/-1.6, $p<0.005$) above RT (Table 4 & Figures 10 & 11).

Table 4: Response to H-R in arteries maintained in normoxia

Ring	Resting tension (grams) in 21% O2	Tension to hypoxia (grams)	% dilation to hypoxia	tension to hyperoxia (grams)	% constriction to hyperoxia
O12	2.10	2.04	2.93	2.31	13.10
O13	1.70	1.64	3.75	1.73	5.80
O14	1.90	1.84	3.11	2.12	15.39
O15	1.90	1.80	5.41	1.99	10.50
O16	2.10	2.10	0.00	2.21	5.30
O17	2.00	2.00	0.00	2.38	19.17

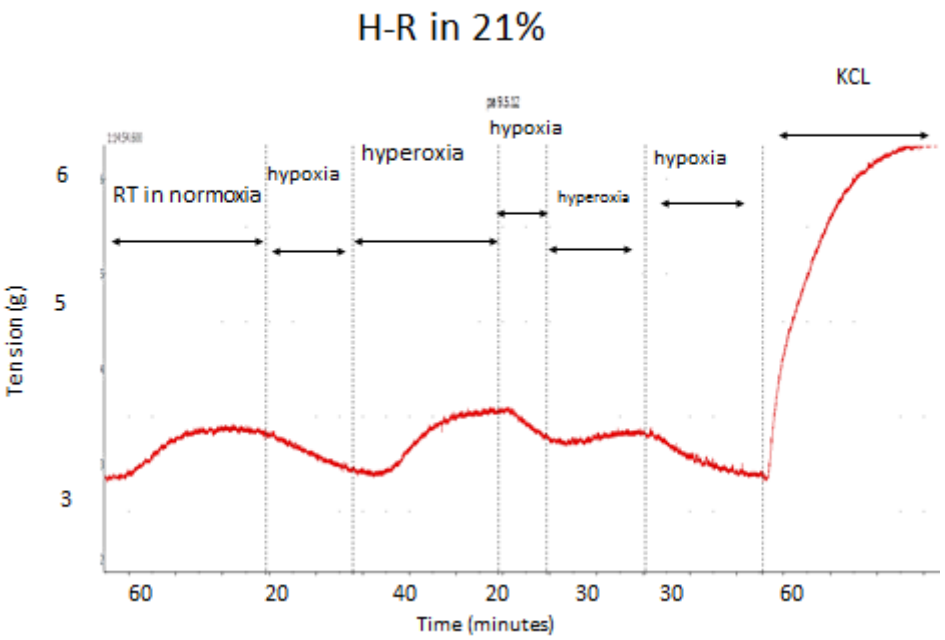

Figure 10: Acute H-R in 21% Oxygen

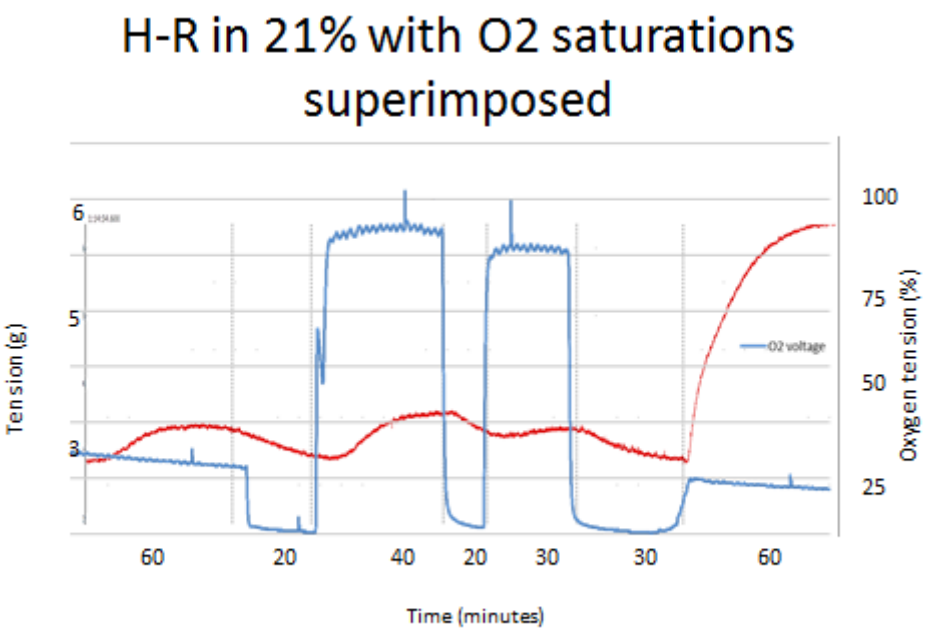

Figure 11: Acute H-R in 21% Oxygen with oxygen saturations superimposed

Effect of Nifedipine on the H-R response in isolated vessels

All vessels dilated in response to hypoxia. The mean vasodilatory response was 18.1% +/-3.4 ($p<0.05$). Upon re-oxygenation vessels responded with a mean vasoconstriction of 15.1% +/-2.5 ($p<0.05$). Nifedipine blocked the vasoconstrictive effects of hyperoxia ($p<0.05$) but did not alter the hypoxic response (Table 5 & Figure 12).

Table 5: Effect of Nifedipine on hyperoxic tensions

Pulmonary artery ring	Resting tension (grams)	hypoxic tension (grams)	% hypoxic vasodilation	hyperoxic tension (grams)	% hyperoxic vasoconstriction	hypoxic tension 2nd (grams)	hyperoxic tension to 5µM Nifedipine (grams)	% hyperoxic vasoconstriction to 5µM nifedipine
O18	2.30	1.44	37.24	1.62	11.98	1.40	1.40	0.00
O19	2.20	1.63	26.02	1.82	12.03	1.50	1.50	0.00
O20	2.30	2.03	11.76	2.62	28.94	2.20	2.20	0.00
O21	2.10	1.93	8.06	2.10	8.77	2.00	2.00	0.00
O22	2.10	1.78	15.20	1.92	7.81	1.80	1.80	0.00
O23	1.90	1.70	10.42	2.07	21.59	1.78	1.77	0.00

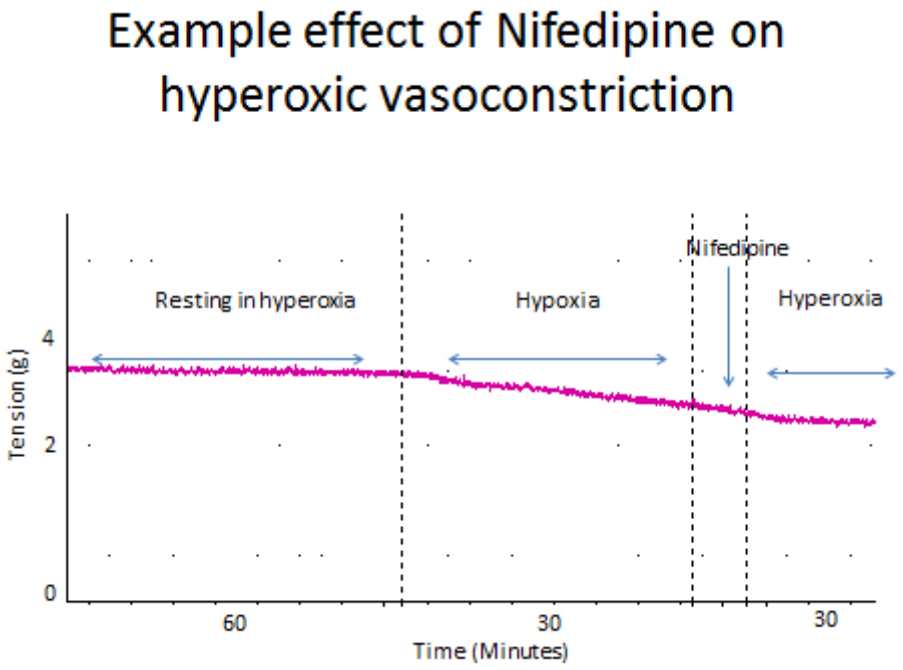

Figure 12: Effect of Nifedipine on hyperoxic tension

Effect of Dantrolene sodium on the H-R response

All vessels dilated in response to hypoxia. The mean vasodilatory response was 13.1% +/-1.0 ($p<0.05$). Upon re-oxygenation vessels responded with a mean vasoconstriction of 11.06% +/-0.69 ($p<0.05$). Dantrolene sodium blocked the vasoconstrictive effects of hyperoxia ($p<0.05$) in a dose-dependent manner (see Table 6 & Figure 13).

Table 6: Effect of dantrolene on hyperoxic tensions

Pulmonary Ring	Resting tension (grams)	hypoxic tension without dantrolene(grams)	% hypoxic dilation without dantrolene	hyperoxic tension without dantrolene (grams)	% hyperoxic vasoconstriction without dantrolene	hypoxic tension with 100 µM dantrolene (grams)	% hypoxic tension with 100µM dantrolene	hyperoxic tension with 100 µM dantrolene (grams)	hyperoxic % with 100 µM dantrolene	% inhibition of hyperoxic vasoconstriction with 100 µM dantrolene	hyperoxic tension (grams) with 200 µM dantrolene	hyperoxic % with 200 µM dantrolene	% inhibition of hyperoxic vasoconstriction with 200 µM dantrolene
O24	1.60	1.40	12.43	1.53	8.85	1.37	10.30	1.46	7.02	38.88	1.41	2.79	75.72
O25	2.30	1.88	18.43	2.05	9.24	1.76	14.15	1.90	7.91	52.01	1.79	1.78	89.19
O26	2.00	1.69	15.33	1.95	15.06	1.70	12.93	1.84	8.64	41.80	1.71	1.07	92.77
O27	2.20	1.97	10.58	2.16	9.98	1.93	11.02	2.04	5.93	52.13	1.94	0.52	95.81
O28	2.10	1.91	9.14	2.12	11.23	1.91	10.01	2.03	6.09	45.25	1.92	0.45	95.95
O29	2.20	1.91	13.21	2.14	12.03	1.88	12.34	2.02	7.64	45.75	1.91	1.82	87.06

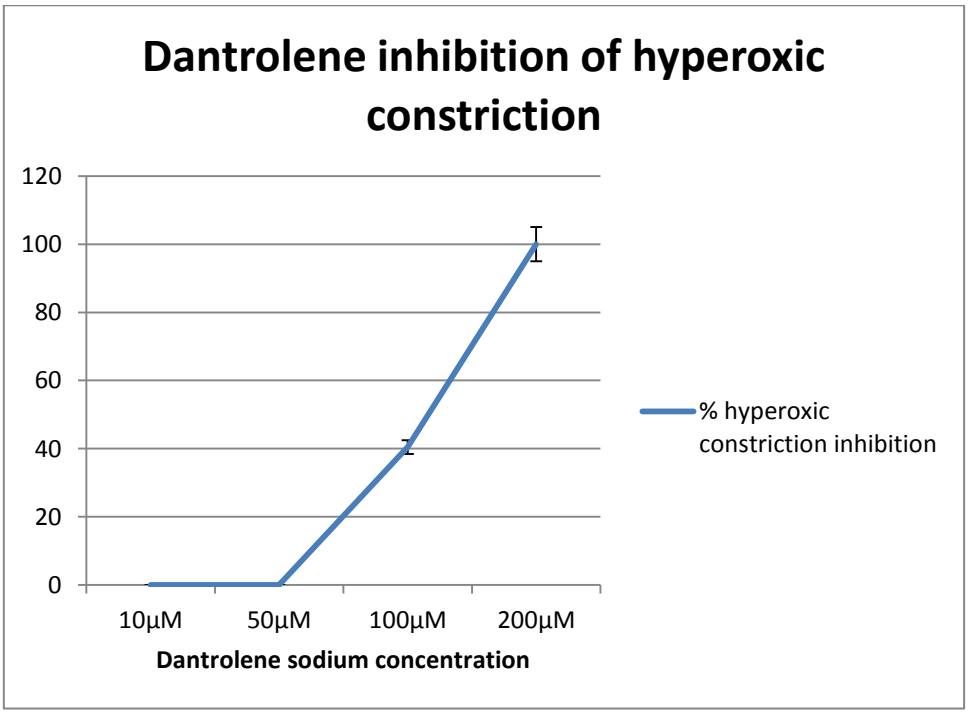

Figure 13: Effect of Dantrolene on hyperoxic tension

Effect of 2-APB on the H-R response

All vessels dilated in response to hypoxia. The mean vasodilatory response was 15.4% +/-1.18 ($p<0.05$). Upon re-oxygenation vessels responded with a mean vasoconstriction of 14.1% +/-1.49 ($p<0.05$). 500µM 2-APB inhibited the hyperoxic vasoconstrictive response ($p<0.05$) (See Table 7 & Figure 14).

Table 7: Effect of 2-APB on hyperoxic tensions

Pulmonary Ring	Resting tension (grams)	hypoxic tension	% hypoxic vasodilation	hyperoxic tension (grams)	% hyperoxic constriction	hypoxic tension with 500 µM 2-APB (grams)	% hypoxic vasodilation with 500 µM 2-APB	hyperoxic tension with500 µM 2-APB (grams)	% hyperoxic vasoconstriction with 500 µM 2-APB
O30	2.10	1.83	12.88	2.20	20.22	1.96	11.03	1.96	0.00
O31	2.20	1.76	20.04	2.03	15.30	1.69	16.76	1.69	0.00
O32	2.00	1.69	15.62	1.81	7.16	1.57	12.93	1.57	0.00
O33	1.80	1.51	16.36	1.79	18.72	1.47	17.80	1.47	0.00
O34	2.00	1.82	9.16	2.01	10.89	1.79	11.12	1.79	0.00
O35	2.00	1.63	18.32	1.83	12.26	1.56	15.10	1.56	0.00

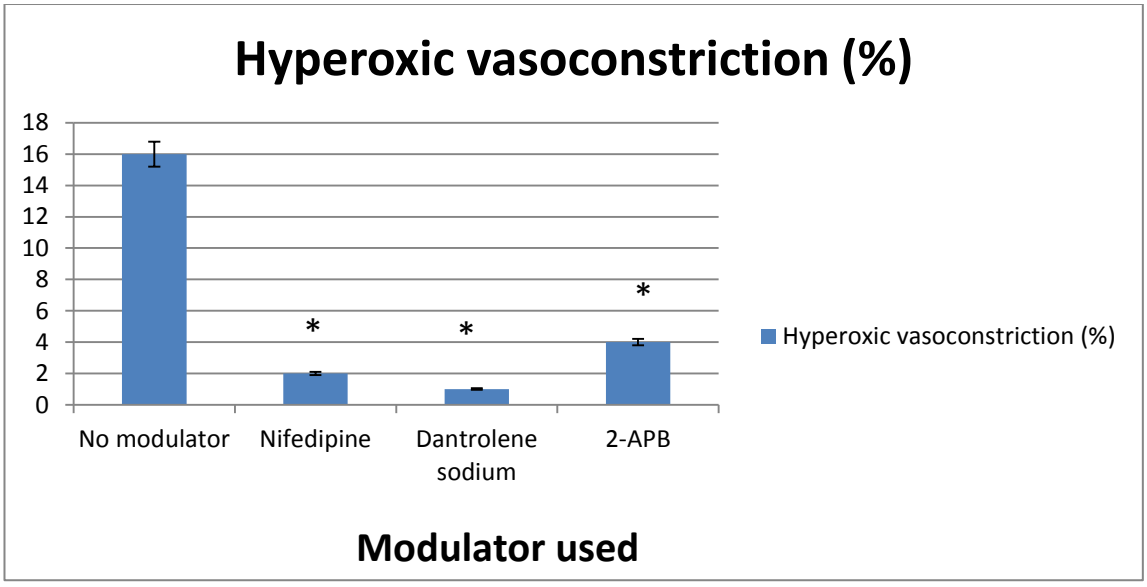

Figure 14: Effect of various calcium antagonists on hyperoxic tension

Effect of L-NAME on responses to hypoxia

Arteries dilated significantly ($p<0.05$) to hypoxia. However, there was no significant difference ($p>0.05$) in dilation in response to hypoxia in the absence or presence of L-NAME (18.5% +/- 3.3 vs 20.1 +/- 2.14 respectively) (Table 8 & Figure 15).

Table 8: Effect of L-NAME on hypoxic vasodilation

Pulmonary ring	Resting Tension (grams)	hypoxic tension without L-NAME (grams)	% hypoxic dilation without L-NAME	hyperoxic tension (grams)	% hyperoxic constriction	hypoxic tension with 1mM L-NAME (grams)	% hypoxic dilation with 1mM L-NAME
O36	1.90	1.79	5.79	1.88	4.80	1.55	17.15
O37	2.10	1.52	27.82	1.82	20.32	1.38	24.08
O38	1.90	1.52	20.22	1.85	21.80	1.65	10.57
O39	2.20	1.45	34.16	1.85	27.50	1.27	31.50
O40	2.10	1.91	9.05	2.10	10.10	1.73	17.91
O41	2.20	1.89	14.08	2.12	12.05	1.71	19.13

Figure 12 Example of the lack of effect of L-NAME on hypoxic vasodilation

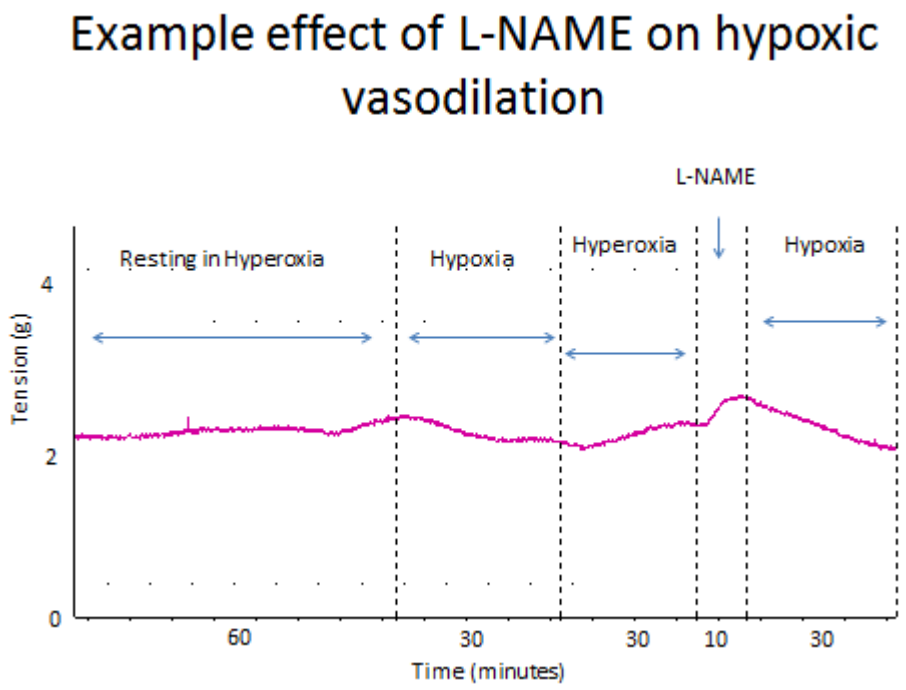

Figure 15: Effect of L-NAME on hypoxic tension

Effect of DPI on responses to hyperoxia and KCl-mediated contractions

DPI group vessels dilated very little in response to hypoxia 2.62%+/-0.13. Upon re-oxygenation, control vessels significantly constricted with a mean response of 10.76% +/-0.67 ($p<0.005$). No vasoconstrictive effect of re-oxygenation was seen in the DPI group (see Table 9 & Figure 16). DPI had no significant effect on KCl max ($p>0.05$).

Table 9: Effect of DPI on H-R

Pulmonary artery ring	Resting tension in 21% O2 (grams)	hypoxic tension (grams)	% hypoxic vasodilation	hyperoxic tension without DPI (grams)	% hyperoxic vasoconstriction without DPI	resting tension in 21% O2(grams)	hypoxic tension with 100 nM DPI (grams)	% hypoxic dilation with 100 nM DPI	hyperoxic tension with100 nM DPI (grams)	% hyperoxic vasoconstriction with 100 nM DPI dilation
O42	1.80	1.74	3.15	1.96	12.67	1.90	1.87	1.50	1.92	2.51
O43	2.30	2.26	1.93	2.50	10.97	2.20	2.17	1.50	2.17	0.00
O44	2.10	2.05	2.59	2.33	13.78	2.10	2.07	1.50	2.12	2.44
O45	2.20	2.14	2.95	2.35	9.95	2.00	1.97	1.50	2.05	4.19
O46	1.90	1.85	2.41	2.00	7.61	2.20	2.17	1.40	2.17	0.00
O47	2.00	1.95	2.66	2.13	9.59	2.10	2.07	1.60	2.07	0.00

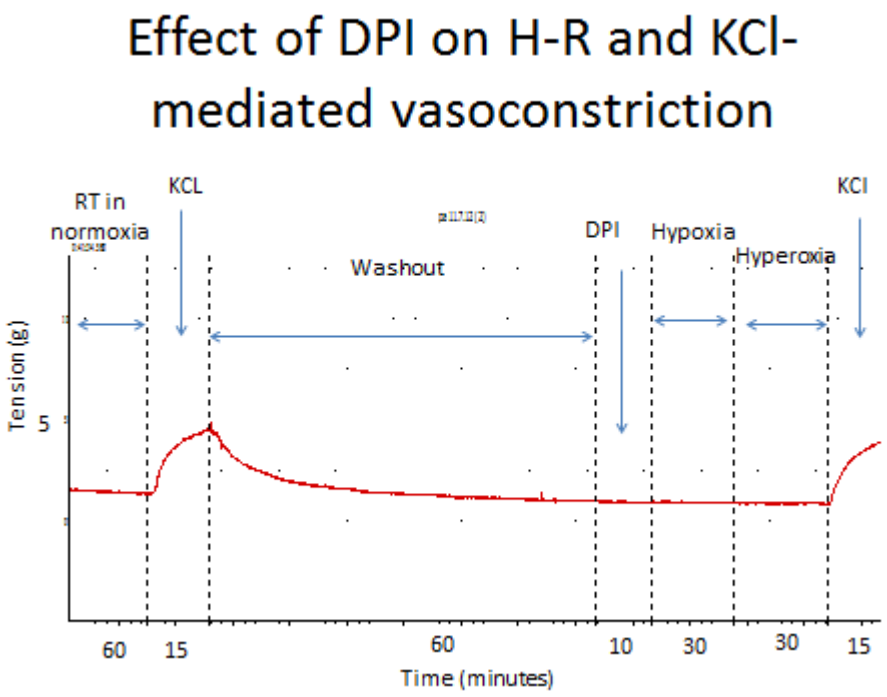

Figure 16: Effect of DPI on H-R

Effect of vessel size on hypoxia-reoxygenation

There was no significant difference ($p>0.05$) in any of the protocols regarding the effect of vessel size on the response to hypoxia-reoxygenation. Denuding the endothelium did not affect the reactivity of pulmonary arteries.

Effect of H-R on vessels resting in 17C

No response was seen in response to H-R in vessels resting in 17C. In contrast, upon warming to 37C, vessels dilated by a mean of 17 % +/-1.6

($p<0.05$) to hypoxia whilst hyperoxia induced a mean constriction of 13.7% (+/-1.8%) from the hypoxic tension (see Table 10)

Table 10: Effect of hypothermia and rewarming on H-R

Pulmonary artery ring	resting tension (grams) at 37 C	hypoxic tension (grams)	% hypoxic vasodilation at 37 ˚ C	hyperoxic tension (grams) at 37 ˚ C	% hyperoxic vasoconstriction at 37 ˚ C	resting tension 17 ˚ C (grams)	hypoxic tension at 17 ˚ C (grams)	% hypoxic vasodilation at 17 ˚ C	hyperoxic tension at 17 ˚ C (grams)	% hyperoxic vasoconstriction at 17 ˚ C
O48	1.80	1.45	19.39	1.62	11.50	1.20	1.20	0.00	1.20	0.00
O49	2.30	2.05	10.90	2.39	16.51	1.53	1.53	0.00	1.53	0.00
O50	2.10	1.59	24.50	1.84	16.11	1.48	1.48	0.00	1.48	0.00
O51	2.20	1.75	20.28	2.14	21.85	1.60	1.60	0.00	1.60	0.00
O52	1.90	1.64	13.92	1.83	11.85	1.30	1.30	0.00	1.30	0.00
O53	2.00	1.75	12.62	1.83	4.45	1.58	1.58	0.00	1.58	0.00

Effect of H-R on the isolated lobes maintained in 37C

There was no change in pulmonary artery pressures ($p>0.05$) between those hypoxic, normoxic and hyperoxic conditions either in the ventilator or perfusate (Table 11).

Table 11: Effect of H-R on isolated perfused lungs

Isolated Lung	Weight before experiment (grams)	PAP at 37 C in normoxia (mmHg)	PAP at 37 C in hyperoxia (mmHg)	PAP at 37 C in hypoxia (mmHg)	Weight after experiment (grams)
OL1	145	16.10	16.00	16.30	255
OL2	185	16.80	16.20	16.40	312
OL3	174	16.40	16.60	16.20	293

Unified model

A unified model based on calcium signalling is given below in Figure 17.

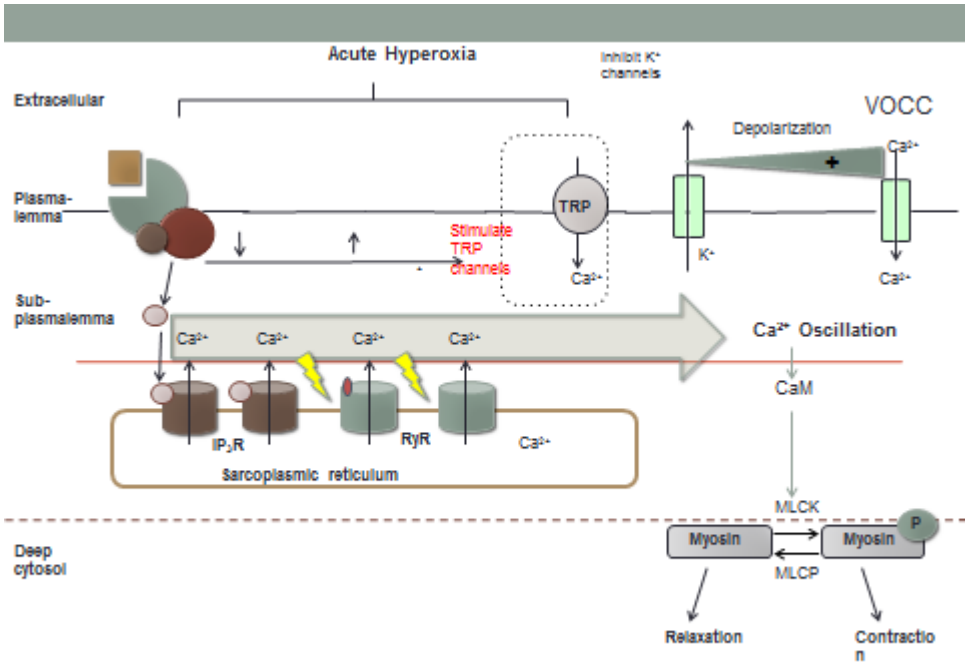

Figure 17: Proposed model for acute hyperoxic vasoconstriction from my experiments

In conclusion, vasoconstriction of human pulmonary arteries occurs naturally in isolation to hyperoxic reoxygenation. However, at the organ level, the lung counteracts these changes to maintain pulmonary artery

pressures. Hence, it is unlikely that hyperoxic reoxygenation following cardiopulmonary bypass causes acute rises in pulmonary artery pressures.

Disclosures

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Chapter 2: Effect of hypothermia on isolated human pulmonary arteries and isolated perfused lung models

Abstract to Chapter

Objective: Acute rises in pulmonary artery pressures following complex cardiac surgery are associated with a high morbidity and mortality. We hypothesised that periods of deep hypothermia predispose to elevated pulmonary pressures upon rewarming. We investigated the effect of this hypothermic preconditioning on isolated human pulmonary arteries and isolated perfused lungs.

Methods: Isometric tension was measured in human pulmonary artery rings (n=24). We assessed the constriction and dilation of these arteries at 37C and 17C. Isolated perfused human lung models consisted of lobes ventilated via a bronchial cannula and perfused with Krebs via a pulmonary artery cannula. Bronchial and pulmonary artery pressures were recorded. We investigated the effect of temperature using a heat exchanger.

Results: Rewarming from 17C to 37 C caused a 1.3 fold increase in resting tension ($p<0.05$). Arteries constricted 8.6 times greater to 30nM KCl, constricted 17 times greater to 1nM Endothelin-1 and dilated 30.3 times as much to 100 μM SNP at 37C than at 17C ($p<0.005$). No difference was observed in the responses of arteries originally maintained in 37C compared to those arteries maintained in 17C and rewarmed to 37C. Hypothermia blunted the increase in pulmonary artery pressures to stimulants such as potassium chloride as well as to H-R but did not precondition arteries to higher pulmonary artery pressures upon re-warming.

Conclusions: Deep hypothermia reduces the responsiveness of human pulmonary arteries but does not, however, precondition to an exaggerated response to vasoactive agents upon re-warming.

Introduction

We investigated the effects of constrictors and dilators of pulmonary arteries in a dose-dependent manner at deep hypothermia temperatures and at normothermic conditions separately and in combination. Potassium chloride was used to assess constriction dependent on membrane potential mechanisms whilst Endothelin-1 was used to assess constriction dependent on receptor-ligand mediated mechanisms. We used sodium nitroprusside as it is one of the most potent vasodilators known and is routinely used in cardiac surgery to manage systemic and pulmonary artery hypertension.

Complex cardiac surgery, adult or congenital, may be associated with acute pulmonary artery hypertension post-operatively. Such cases are often associated with a high morbidity [115].

Deep hypothermia employed during such operations has many beneficial effects, not least the protection from ischaemic damage it conveys to the brain and peripheral organs whilst circulatory arrest can safely be utilised to undertake complex surgical anastomoses. However, its use has been known to cause complications upon rewarming [213, 113, 214].

Little is known of the effects of deep hypothermia directly on human pulmonary arteries. We wanted to see if deep hypothermia may precondition pulmonary arteries to heightened tones upon return to normothermia.

Results

Effect of Endothelin-1, KCl and SNP at 17C warmed to 37C

Arteries constricted 17 times greater to 1nM Endothelin-1 at 37C than at 17C as a percentage of KCl contraction at 37C (156.32% +/-5.9 vs 4.98% +/-1.09) ($p<0.05$). Arteries constricted 8.6 times greater to 30nM KCl at 37 C than at 17C (11.67% +/- 1.97 vs 100%) ($p<0.005$) as a percentage of KCl contraction at 37C. Arteries dilated 30.3 times as much to 100 μM SNP at 37C than at 17C as a percentage of SNP dilation at 37C (100% vs 3.38% +/- 0.18) ($p<0.005$). (See Tables 12-14)

The dose response curves for the temperature conditions of each Endothelin-1, KCl and SNP are given in Figures 18 and 19.

Table 12: Effect of hypothermia on responses of pulmonary arteries to Endothelin-1

PA ring	resting tension (grams) at 17C	Tension to 100pM ET-1 at 17 C (grams)	% constriction to 100pM ET-1 at 17C	% of Constriction to 30mM KCl at 37C	Tension to 500pM ET-1 at 17 C (grams)	% constriction to 500pM ET-1 at 17C	% of constriction to 30mM KCl at 37C	Tension to 1nM ET-1 at 17 C (grams)	% constriction to ET-1 at 17C	% of constriction to 30mM KCl at 37C	resting tension (grams) 37C	tension to 30mM KCl at 37C (grams)	% constriction to 30mM KCl at 37C	Tension to 100pM ET-1 at 37 C (grams)	% constriction to 100 pM ET-1 at 37C	% of constriction to 30mM KCl at 37C contraction	Tension to 500pM ET-1 at 37 C (grams)	% constriction to 500pM ET-1 at 37C	% of constriction to 30mM KCl at 37C	Tension to 1nM ET-1 at 37 C (grams)	% constriction to 1nM ET-1 at 37C	% of constriction to 30mM KCl at 37C
T1	1.38	1.39	1.14	2.40	1.42	3.33	7.00	1.41	2.55	5.35	1.90	2.56	34.50	2.15	13.05	37.82	2.30	20.90	60.57	2.98	56.99	165.20
T2	1.77	1.78	0.91	1.80	1.84	3.91	7.70	1.86	5.16	10.16	2.20	3.10	40.80	2.66	20.71	50.77	2.69	22.35	54.78	3.66	66.48	162.93
T3	1.54	1.56	1.24	2.30	1.60	4.00	7.40	1.60	4.43	8.21	2.00	2.83	41.50	2.35	17.47	42.09	2.47	23.70	57.12	3.15	57.75	139.15
T4	1.83	1.85	1.12	1.90	1.90	3.82	6.50	1.97	7.80	13.29	2.20	3.27	48.70	2.66	20.86	42.84	2.86	29.81	61.21	3.89	77.00	158.11

Table 13: Effect of hypothermia on the responses of pulmonary arteries to KCl

PA ring	resting tension (grams) 17C	tension to 300µM KCl (grams)	% Constriction to 300 µM KCl at 17 C	% of 30mM KCl at 37C	tension to 3 mM KCl at 17C (grams)	% Constriction to 30m KCl at 17 C	% of 30mM KCl at 37C2	tension to 30mM KCl at 17 C	% constriction to 30mM KCl at 17C	% of 30mM KCl at 37C	resting tension (grams) 37C	tension to 300 µM KCl at 37C (grams)	% constriction to 300 µM KCl at 37C	% of Constriction to 30mM KCl at 37 C	tension to 3mM KCl at 37C (grams)	% constriction to 3mM KCl at 37C	% of 30mM KCl at 37 C	tension to 30mM KCl at 37C (grams)	% constriction to 30mM KCl at 37C	% of 30mM KCl at 37 C
T5	1.59	1.62	2.27	3.00	1.68	5.89	7.80	1.74	9.48	12.54	2.20	2.37	7.68	14.10	2.79	26.85	49.27	3.40	54.50	100.00
T6	1.48	1.50	1.36	3.40	1.53	3.36	8.40	1.52	2.40	6.00	1.90	1.98	4.44	14.23	2.10	10.35	33.16	2.49	31.20	100.00
T7	1.56	1.61	2.88	3.50	1.67	7.07	8.60	1.76	12.44	15.12	2.10	2.34	11.28	18.43	2.61	24.43	39.92	3.39	61.20	100.00
T8	1.53	1.56	2.24	3.30	1.60	4.48	6.60	1.66	8.83	13.02	2.00	2.11	5.41	10.44	2.29	14.74	28.46	3.04	51.80	100.00

Table 14: Effect of hypothermia on the responses of pulmonary arteries to SNP

PA ring	resting tension (grams) at 17C	tension to 1 µM SNP at 17C (grams)	% dilation to 1 µM SNP at 17 C	% of dilation to 100 µM SNP at 37C	tension to 10 µM SNP at 17C (grams)	% dilation to 10 µM SNP at 17 C	% of dilation to 100 µM SNP at 37C2	tension to 100µM SNP at 17C (grams)	% dilation to 100µM SNP at 17 C	% of dilation to SNP at 100 µM 37C3	resting tension (grams) 37C	tension to 1 µM SNP at 37C (grams)	% dilation to 1 µM SNP at 37 C	% of dilation to 100 µM SNP at 37C4	tension to 10 µM SNP at 37C (grams)	% dilation to 10 µM SNP at 37 C	% of dilation to 100 µM SNP at 37C	tension to 100µM SNP at 17C (grams)	% dilation to 100µM SNP at 17 C	% of dilation to 100 µM SNP at 37C
T9	1.55	1.55	0.00	0.00	1.59	2.15	3.70	1.63	4.86	2.90	2.10	2.36	12.30	28.54	2.70	28.55	66.25	3.01	43.10	100.00
T10	1.63	1.63	0.00	0.00	1.66	1.60	3.10	1.67	2.09	3.40	2.10	2.23	6.14	15.28	2.67	26.96	67.06	2.94	40.20	100.00
T11	1.72	1.72	0.00	0.00	1.76	1.87	3.00	1.80	4.10	3.40	2.20	2.35	6.87	14.08	3.05	38.65	79.21	3.27	48.80	100.00
T12	1.46	1.46	0.00	0.00	1.48	1.16	2.70	1.49	1.99	3.80	1.80	1.91	6.37	18.36	2.12	17.83	51.37	2.42	34.70	100.00

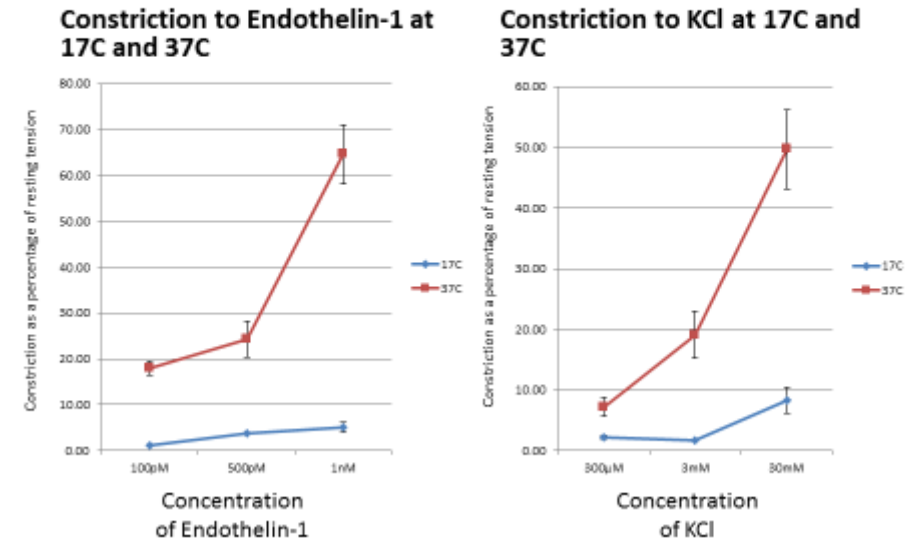

Figure 18: Effect of KCl & ET-1 at 17 C and 37 C

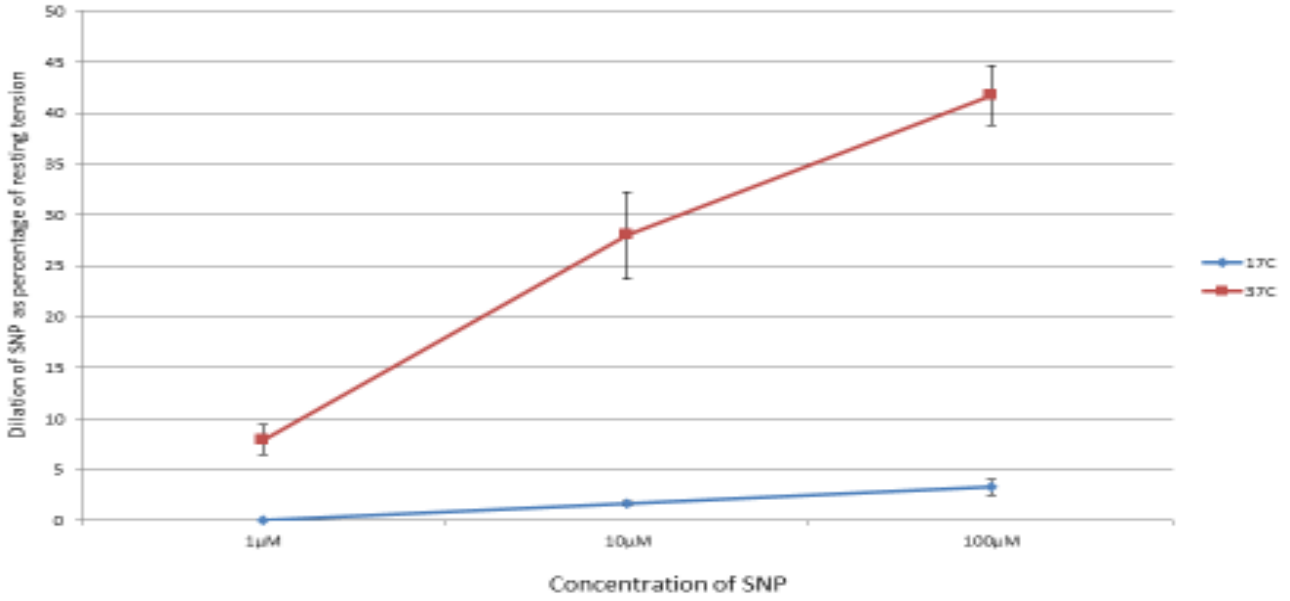

Figure 19: Effect of SNP at 17 C and 37 C

There was no significant difference between the responsiveness of arteries originally maintained in 37C to KCl and SNP compared to those arteries warmed to 37C from 17C (see Tables 15-17).

Table 15: Responses of pulmonary arteries to Endothelin-1 without hypothermia

PA Ring	Resting tension at 37C (grams)	Response to 1nM ET-1 (grams)	% change
T13	1.90	2.70	42.11
T14	1.70	2.45	44.12
T15	2.20	3.20	45.45
T16	1.70	2.65	55.88

Table 16: Responses of pulmonary arteries to KCl without hypothermia

PA ring	**Resting tension (grams)**	**Response to 30mM KCl (grams)**	**% change**
T17	2.30	3.10	34.78
T18	1.70	2.50	47.06
T19	1.90	2.80	47.37
T20	2.00	3.40	70.00

Table 17: Responses of pulmonary arteries to SNP without hypothermia

PA ring	**Resting tension (grams)**	**Response to 100** μM SNP (grams)	**% change**
T21	1.80	1.10	-38.89

T22	2.40	1.30	-45.83
T23	1.70	1.10	-35.29
T24	2.20	1.00	-54.55

Effect of rewarming on artery tension

Rewarming from 17C to 37 C caused a 1.3 (1.59g +/- 0.04 vs 2.06g +/-0.04) fold increase in resting tension ($p<0.05$) (See Table 18). There was no significant difference in the tensions of arteries originally maintained in 37C compared to those warmed to 37C from 17C.

Table 18: Effect of rewarming on pulmonary artery tension

PA ring	resting tension at 17C (grams)	resting tension at 37C (grams)	% Change in tension from rewarming
T1	1.38	1.90	37.98
T2	1.77	2.20	24.43
T3	1.54	2.00	30.17
T4	1.83	2.20	20.52
T5	1.59	2.20	38.59
T6	1.48	1.90	28.20
T7	1.56	2.10	34.36
T8	1.53	2.00	30.90

T9	1.55	2.10	35.09
T10	1.63	2.10	28.67
T11	1.72	2.20	27.54
T12	1.46	1.80	23.35

Effect of hypothermia and rewarming on isolated lobes

Lobes were unresponsive to KCl at 17C with regard to pulmonary artery pressures. Upon rewarming to 37C, there was an increase in pulmonary artery pressures by a mean of 28.1% (+/- 2.5) ($p<0.05$) and an increase in bronchial pressures by a mean of 9.3% (+/- 1.6) (see Table 19). There was an increase in pulmonary artery pressures in response to KCl by a mean of 44.18% (+/- 10.02) ($p<0.05$) at 37C (see Table 20). However, there was no difference in resting or stimulated pulmonary artery pressures between those lobes maintained in 37C and those rewarmed from 17C to 37C (Table 21).

Table 19: Effect of rewarming on pulmonary artery and bronchial pressures

Isolated lung	Resting PAP (mm Hg) at 37C	PAP pressure at 17C (mm Hg)	PAP rewarmed to at 37C (mm Hg)	% Change in PAP from rewarming	Resting at 37C Bronchial Pressure (mm Hg)	Bronchial Pressure at 17C (mm Hg)	Bronchial Pressure rewarmed to 37C (mm Hg)	% Change in Bronchial Pressure from rewarming
TL1	15.80	12.80	15.50	23.40	14.90	13.28	15.40	12.22
TL2	15.90	12.03	16.90	32.12	15.40	14.44	15.90	6.66
TL3	16.80	13.14	17.30	27.89	15.80	14.48	16.40	9.13

Table 20: Effect of rewarming on pulmonary artery pressures in response to KCl

Isolated lung	PAP at 17C (mm Hg)	PAP at 17C + 30mM KCl (mm Hg)	PAP at 37C (mm Hg)	PAP at 37C + 30mM KCl (mm Hg)	% Change in PAP from 30mM KCl at 37C
TL1	12.80	12.80	15.50	24.91	60.72
TL2	12.03	12.03	16.90	24.63	45.72
TL3	13.14	13.14	17.30	21.82	26.11

Table 21: Effect of KCl on pulmonary artery pressures without hypothermia & rewarming

Isolated Lung	PAP at 37C (mm Hg)	PAP at 37C + 30mM KCl (mm Hg)	% Change in PAP from 30mM KCl at 37C
TL4	15.70	23.29	48.32
TL5	16.10	24.50	52.16
TL6	15.20	19.89	30.83

Discussion

Conclusions

Deep hypothermia, whilst relaxing pulmonary arteries and decreasing their responsiveness to stimulants and relaxants, does not, however, precondition such arteries to an exaggerated response to vasoactive agents upon rewarming. Hence, deep hypothermia employed during complex cardiac

surgery does not appear to directly contribute to the raised pulmonary tones that can occur during such operations.

Abbreviations: KCl: Potassium Chloride

SNP: Sodium Nitroprusside

Disclosures

Conflict of Interests: None declared

Funding: Funding for this project was obtained from charitable funds made to the Cardiothoracic Department at Castle Hill Hospital.

Chapter 3: Effect of Hydrogen Sulphide on isolated human pulmonary arteries and isolated perfused lung models

Abstract for chapter

Introduction: Acute rises in pulmonary artery pressures are associated with a significant mortality and morbidity due to the significant strain on the right ventricle. Although Hydrogen Sulphide (H2S) has been studied for its potential role in the systemic circulation, little is known of its effects on the pulmonary circulation in humans. We studied the effect of H2S at both the human isolated pulmonary arterial level as well as the human isolated perfused lung level.

Methods: Human lobar pulmonary artery rings (*n*=12) and lobes (*n*=6) were obtained from resections for patients with bronchial carcinoma. Pre-constricted fresh rings were mounted in organ baths containing normoxic Krebs solution and subsequently exposed to hydrogen sulphide whilst tension was recorded. Isolated perfused human lung models consisted of lobes ventilated via a bronchial cannula and perfused with Krebs via a pulmonary artery cannula; Hydrogen Sulphide was added to the perfusate and the resulting pulmonary artery and bronchial pressures were recorded.

Results: We found that 500µM H2S caused a mean dilation of 42.3% (+/- 5.4) from the pre-constricted tension ($P<0.005$) in isolated arterial rings. In addition, 500µM H2S caused a 17.73% (3.52) reduction in pulmonary artery pressures ($p<0.05$). Furthermore, we found that 500µM H2S caused a 14.9% (6.01) reduction in bronchial airway pressures ($p<0.05$).

Conclusions: We have shown that H2S is a potent vasodilator of human pulmonary arteries and is a significant anti-hypertensive for pulmonary

artery pressures. Our results indicate that this therapeutic potential should be further evaluated in clinical trials.

Introduction

We have, therefore, looked at the effect of H2S at these non-detrimental doses within the human pulmonary circulation. We used isolated pulmonary artery rings as well as whole isolated perfused lung models (IPLM) in humans.

Pulmonary artery hypertension remains an important cause of right ventricular dysfunction and is a significant predictor of morbidity and mortality particularly in the acute setting following cardiac surgery [202].

Acute right heart dysfunction is a difficult entity to manage as the right ventricle is not as adapted as the thicker left ventricle when it comes to acute changes in afterload [203]. Medically untreatable acute right heart failure is often an indication for ventricular assist devices and possible heart transplantation [215].

It would therefore, seem prudent to aggressively manage pulmonary artery hypertension in the early stages. Various vasodilatory compounds have been explored in the past to accomplish this such as the phoshodiesterase-inhibitors Milrinone and Sildenafil [216], as well as prostacyclins [217]. However, the results are limited and there is still considerable scope for alternatives.

One compound that has undergone an ebb and flow in recent years concerning its therapeutic potential is hydrogen sulphide (H2S). H2S has long been considered a noxious gasotransmitter: as well as having an unpleasant odour associated with it, it also has serious detrimental effects on the lung in high doses associated with adverse industrial exposure [218].

Endogenous concentrations of H2S in humans vary between studies and the tissue studied; for example in the human placenta, the H2S production rate is 200 +/- 102 nM/min/g compared to the human lymphocyte production rate of 11.64 +/- 6.36 microM/min/mg [219, 220]

However exogenous hydrogen sulphide, at smaller doses, has remarkable therapeutic potential. For example, within the cardiovascular system, animal studies have shown that H2S acts as a cardioprotective drug against ischaemia-reperfusion injury [221] and as a vasodilator of systemic arteries, both larger conduit ones as well as smaller peripheral resistance vessels [222].

In contrast, human studies have shown that at these smaller exogenous doses, whilst not causing adverse effects, have negligible clinical effects on the systemic circulation [258].

Despite this, the potential within the pulmonary circulation has hitherto been widely ignored.

Results

Isolated human pulmonary arteries

Endothelial integrity was confirmed in 7 arteries whilst 5 arteries appeared to have the endothelium denuded upon harvesting.

We found that 50µM of H2S did not cause a significant dilation form the pre-constricted tension whereas 500µM caused a mean dilation of 41.7% (+/- 1.58) from the pre-constricted tension ($P<0.005$) (Table 22)... There was no significant difference in the vasodilatory phenomenon between those arteries responding to acetylcholine and those in which the endothelium was lacking. The dose-response curve is given in Figure 20.

Table 22: Effect of H2S on pulmonary artery tensions

PA Ring	**Resting tension at 37C (grams)**	Tension to **50** µM H2S (grams)	**50** µM % dilation	Tension to **100** µM H2S (grams)	**100 µM % dilation**	Tension to **250** µM H2S (grams)	**250 µM % dilation**	Tension to **500** µM H2S (grams)	**500 µM % dilation**
H1	2.20	2.15	2.16	1.69	23.03	1.40	36.51	1.23	44.18
H2	1.70	1.66	2.08	1.40	17.83	1.07	37.30	0.80	52.72
H3	1.90	1.85	2.62	1.35	29.15	1.24	34.87	1.17	38.67
H4	1.80	1.77	1.77	1.43	20.57	1.19	33.78	1.08	39.75
H5	2.30	2.26	1.81	1.77	22.93	1.67	27.48	1.32	42.56
H6	1.60	1.56	2.27	1.30	18.50	1.19	25.61	1.03	35.93
H7	2.10	2.08	0.83	1.53	27.21	1.37	34.61	1.05	50.10
H8	2.30	2.25	2.28	1.72	25.36	1.44	37.35	1.39	39.76
H9	2.30	2.25	2.25	1.73	24.77	1.58	31.49	1.50	34.94
H10	2.90	2.84	2.17	2.24	22.78	1.90	34.64	1.80	37.97
H11	2.00	1.96	2.10	1.52	24.20	1.51	24.73	1.22	38.76
H12	2.50	2.45	1.90	1.83	26.91	1.79	28.47	1.37	45.17

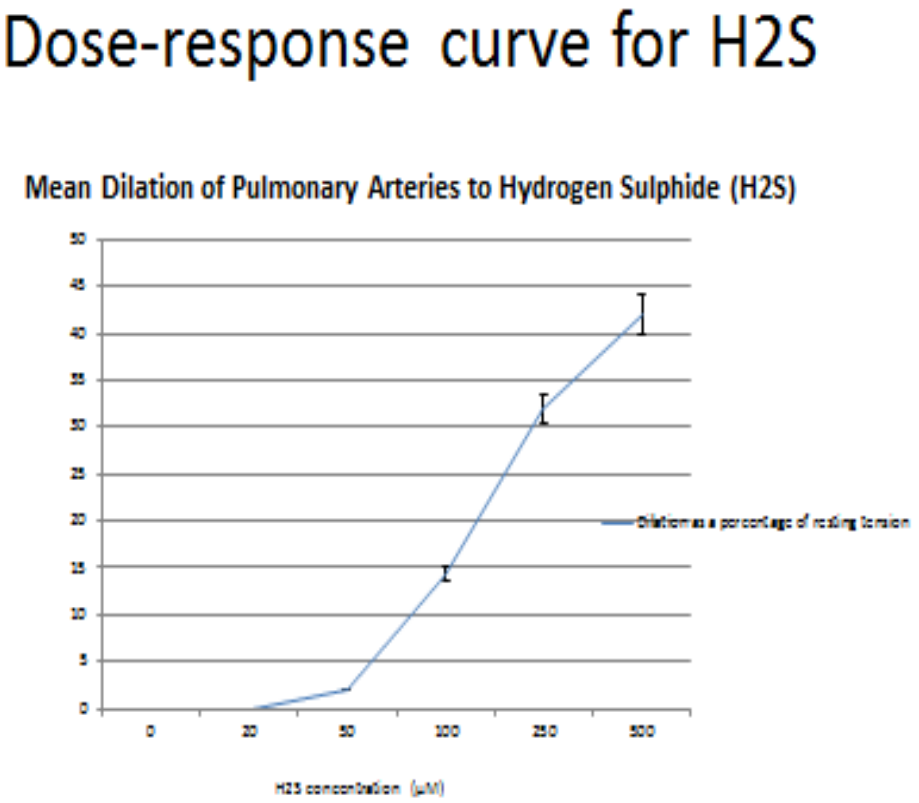

Figure 20: Effect of H2S on isolated pulmonary arteries

Isolated human perfused lungs

We found that 50µM Hydrogen Sulphide caused a 3.20% (+/- 0.92) reduction in pulmonary artery pressures whereas 500µM Hydrogen Sulphide caused a 17.7% (+/-2.03) reduction in pulmonary artery pressures ($p<0.05$) (See Table 23 & Figure 21).

Table 23: Effect of H2S on pulmonary artery pressures

Isolated lung	PAP pressure at 37C (mm Hg)	PAP to 50 µM H2S (mm Hg)	% change 50 µM H2S	PAP to 200 µM H2S (mm Hg)	% change to 200 µM H2S	PAP to 500 µM (mm Hg)	% change to 500 µM H2S
HL1	16.90	16.56	2.00	14.88	11.98	13.27	21.48
HL2	17.10	16.66	2.58	15.44	9.69	14.62	14.50
HL3	16.40	15.58	5.02	13.90	15.25	13.56	17.32

Figure 18: Effect of H2S on the pulmonary artery pressures in human isolated perfused lung models

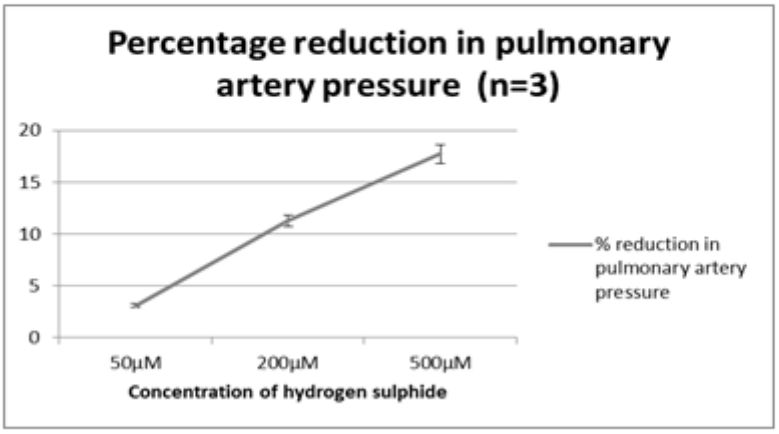

Figure 21: Effect of H2S on pulmonary artery pressures

We found that 50µM Hydrogen Sulphide caused a 4.07% (+/- 1.05) reduction in bronchial airway pressures whereas 500µM Hydrogen Sulphide caused a 14.97% (+/- 2.46) reduction in bronchial airway pressures ($p<0.05$) (See Table 24 & Figure 22).

Table 24: Effect of H2S on Bronchial artery pressures

Isolated lung	bronchial pressure at 37C (mm Hg)	Bronchial pressure to 50 µM H2S (mm Hg)	% change to 50 µM H2S	Bronchial pressure to 200 µM H2S (mm Hg)	% change 200 µM H2S	Bronchial pressure to 500 µM (mm Hg)	% change to 500 µM H2S
HL1	15.10	14.78	2.14	13.42	11.15	12.61	16.46
HL2	14.80	13.78	5.73	13.50	8.77	13.30	10.16
HL3	15.80	12.97	4.35	13.62	13.82	12.91	18.28

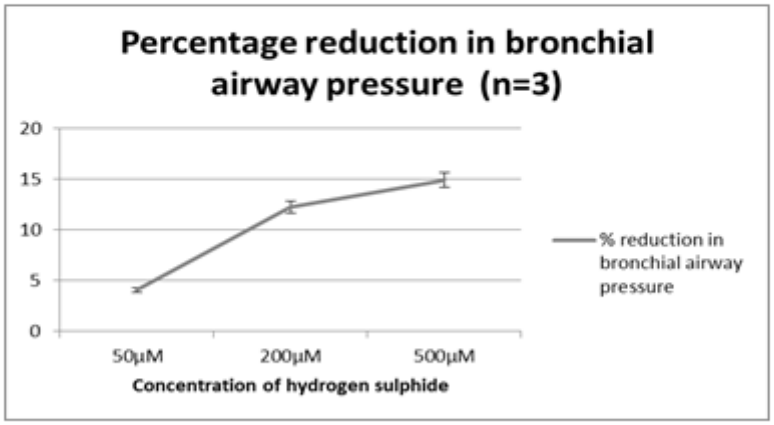

Figure 22: Effect of H2S on bronchial artery pressures

Conclusion

In conclusion, we have demonstrated that hydrogen sulphide is a potent dilator of human pulmonary arteries.

Disclosures

There are no conflicts of interests. Funding was obtained from charitable funds made to our cardiothoracic department.

Acknowledgements

We would like to thank Robert T Bennett for his invaluable help in allowing us access to the isolated perfused lung model experimental set up.

Supplemental Chapter 4: Clinical Relevance of Pulmonary Artery Hypertension in cardiac surgical patients

Abstract

Introduction: Pre-operative pulmonary artery hypertension is a significant predictor of mortality following adult cardiac surgery. We discerned factors predicting pulmonary artery hypertension in 566 cardiac surgical patients who had pre-operative right heart catheterization and evaluated post-operative outcomes.

Materials and Methods: Retrospective analysis of data entered prospectively on our cardiac surgical database between January 2000 and January 2013.

Results: We found that gender, being over 80 years of age, having peripheral vascular disease, poor left ventricular function, valvular pathology and the presence of a ventricular septal defect were all significant ($p<0.05$) predictors of raised pulmonary artery pressures (PAP). Having pulmonary artery hypertension pre-operatively (systolic PAP >55mmHg) was a significant predictor of mortality as well as renal complication ($p<0.05$).

Conclusions: Pulmonary artery hypertension pre-operatively is not only a significant predictor of peri-operative mortality but also morbidity. The identification of risk factors for developing pulmonary artery hypertension may allow for strategies to optimise patients prior to surgery.

Introduction

Pre-operative pulmonary artery hypertension (PAH) is associated with a high mortality demonstrated by its incorporation within the EUROSCORE I and II risk stratification system [226].

The mechanisms and factors influencing its pathogenesis is still the topic of much research and debate [227]. So far in this thesis, I have looked at physiological and pharmacological agents which may affect pulmonary artery tone at the tissue and organ level.

However, for this final supplemental chapter, I wanted to evaluate factors which are associated with pre-operative pulmonary artery hypertension in a cohort of our patients, namely those undergoing cardiac surgery. Hence I am moving away from acute determinants of pulmonary artery tone to more chronic determinants of pulmonary artery tone at the clinical level.

In addition, I wanted to assess not only the mortality following cardiac surgery in those patients with PAH but also whether these patients are at an increased risk of other complications.

Patients and Methods

Approval from our Audit Review Board was obtained for a retrospective analysis of data entered prospectively in our database between January 2000 and January 2013 for all patients undergoing cardiac surgery. All patients

who had right heart catheterisation prior to induction at anaesthesia were included in the study.

The following patient data were analysed: gender age; co-morbidities (including hypertension, chronic lung disease, peripheral vascular disease, renal failure); extent of coronary artery disease on preoperative coronary angiogram; previous myocardial infarction; left ventricular ejection fraction and New York Heart Association functional class before surgery; Canadian Cardiovascular Society angina score; operative priority; operation performed; 30-day mortality, post-operative complications (including arrhythmias, renal failure, neurological events, gastrointestinal and wound complications). Gastro-intestinal complications included gastro-intestinal bleeding, ileus for more than 3 days, ischaemic bowel and an exploratory laparotomy. Neurological complications included stroke, transient ischaemic attacks, and prolonged confusion (>5 days). Wound complications included deep or superficial sternal or harvest site wounds.

The definitions of co-morbidities and PAH (systolic PAP>55mm Hg) were obtained from the EUROSCORE I criteria.

Patient characteristics are expressed as means ± SD or simple frequencies and percentages. Multivariate linear regression was used to identify predictors of elevated pulmonary artery pressures. Logistic regression was used to evaluate the effect of pulmonary hypertension on post-operative mortality and complications.

Univariate analyses were performed relating operative morbidity and mortality to the following patient characteristics: age, gender, co-

morbidities (chronic obstructive pulmonary disorder, peripheral vascular disease, pre-operative renal failure, pre-operative neurological dysfunction, diabetes mellitus), left ventricular function, recent myocardial infarction and operation performed. Unpaired Student's t tests were used to compare continuous data, Fisher's exact tests for dichotomous data, and $\chi 2$ for categorical variables. A two-tailed probability value of less than 0.05 was considered significant. The variables that were significant at a probability value less than 0.20 were entered into a multivariable logistic regression with morbidity as the dependent variable and significance set at the 0.05 level. Statistical analysis was performed using SPSS statistics 19.

Results

566 patients were identified as having pre-operative invasive pulmonary artery evaluation. Table 25 summarises the main pre-operative and intra-operative characteristics of the patients.

Of the 566 patients, 299 had pulmonary hypertension pre-operatively (PAP > 55mm Hg).

Logistic analysis revealed the following to be significant predictors of raised PAPs ($p<0.05$): being female, having a poor left ventricular function and having valvular pathology. Linear regression analysis for rising pulmonary artery pressures pre-operatively revealed post-infarction VSDs to be a significant predictor in addition to the aforementioned predictors for the logistic regression model. The results of the multivariate analysis are summarised in Table 26.

PAH was a significant ($p<0.05$) predictor of mortality and post-operative complications (acute renal failure). The Hazard Ratio (HR) of developing these complications by having PAH is given in Table 27.

Abbreviations: PAH-Pulmonary artery hypertension

PAP: Pulmonary artery pressure

VSD: Ventricular septal defect

Conflict of interests: None declared

Funding: None

Table 25: Pre-operative characteristics of patients having pulmonary artery catheterisation

Characteristic (n=566)	
Mean age (+/- 1SD)	66.98(+/- 11.180)
Mean body mass index (+/- 1SD)	26.72 (+/- 5.01)
Mean logistic Euroscore I (+/- 1SD)	12.33 (15.5)
Mean Systolic Pulmonary Artery Pressure	46.54 (17.915)
% Male	52.1
% Chronic obstructive pulmonary disorder	16.4
% Cerebrovascular accident history	12.4
% Pre-operative renal failure	4.6
% Peripheral vascular disease	8.5
% Diabetes mellitus	14.8
% Canadian Cardiovascular Society angina grade IV	4.6
% New York Heart Association Class IV	11
% Poor ejection fraction	12.9
% Urgent/emergency	21.7
% Intra-operative balloon pump	9.9
% 3 Vessel Disease	16.8
% Coronary artery bypass graft only	13.4
% Valve replacement/repair only	48.6
% Post-myocardial infarction ventricular septal defect	0.5
% 30 day post-operative mortality	11.7
% Post-operative neurological complications	3.0
% Post-operative renal failure	16.7
% Post-operative atrial fibrillation	58.7
% Post-operative ventricular tachycardia/fibrillation	1.8
% Post-operative gastrointestinal complications	6.8
% Wound infection (superficial and deep)	8.7
% Post-operative Myocardial Infarction	2.1

Table 26: Predictors of raised pulmonary artery pressures

Model	proportion with PAH	Logistic Significance (p-value)	Linear Regression (p-value)
Female	172/270	0.001	0.02
Over 80 years old	19/41	0.118	0.086
CCS IV	10/26	0.175	0.123
NYHA IV	43/62	0.176	0.07
COPD	55/91	0.966	0.665
History of myocardial infarctions	61/120	0.752	0.743
Peripheral vascular disease	25/49	0.940	0.473
Poor Ejection Fraction	49/72	0.001	0.001
Valvular pathology	272/449	0.000	0.000
Post-infarction ventricular septal defect	3/3	0.999	0.000

Table 27: Effect of PAH on post-operative outcomes

Outcome	Significance	Hazard Ratio
30-day mortality	0.009	2.233
Renal Complications	0.005	1.920

Section E: Epilogue

My thesis has brought together 3 important areas of science. Firstly it has identified a problem in cardiac surgery which is the phenomenon of pulmonary artery hypertension whether this exists pre-operatively or post-operatively and the effect this has on the post-operative course. I have demonstrated this briefly in the supplemental chapter looking at factors associated with PAH in our cardiac surgical patients pre-operatively and the effects this has on the post-operative course. Secondly, I have tried to discern the mechanisms of acute PAH using both tissue and organ models to study oxygen and temperature. Thirdly, I have tried to find a novel therapeutic target to relieve PAH in the acute phase.

This bringing together of clinical relevance, mechanistic insights and potential therapeutic strategies is the essence of what this MD is about. It is by no means extensive in each of these fields but then nothing ever is complete.

Effect of oxygen on the human pulmonary circulation

Normoxia, hypoxia and hyperoxia

We have shown that acute hypoxia causes vasodilation in pre-oxygenated large human pulmonary arteries. A subsequent return to hyperoxia causes vasoconstriction. We believe that this represents a return to a greater basal tone in hyperoxic conditions since arteries *originally maintained in normoxia* prior to hypoxic challenge demonstrated a net vasoconstriction above their basal tone. Thus, contrary to the perceived wisdom, hyperoxia appears to induce vasoconstriction in our human pulmonary arteries.

The fact that the arteries *maintained in hyperoxia* merely return to basal tone upon re-oxygenation after hypoxic challenge and do not 'overshoot' their basal tone, indicates that hypoxia does not stimulate an increased vasoconstrictive response to hyperoxia. Arteries were capable of further vasoconstriction as is evidenced by the maximum vasoconstrictive response to be around 35% vasoconstriction of KCl maximum. This suggests that arterial tone is dependent on O2 tension alone without evidence, in this system, of 'hypoxic pre-conditioning'.

Our consistent finding of hyperoxic pulmonary vasoconstriction may seem to conflict with the generally agreed physiological response of hypoxic pulmonary vasoconstriction. However, we suggest that by studying large

and medium sized pulmonary arteries, we are not investigating the origin of factors controlling overall pulmonary vascular flow. It is possible that larger arteries may relax to hypoxia and that the physiological change limiting the flow to the alveoli occurs in the smaller "resistance" arteries.

Hypoxic vasodilation appears to occur independently of Nitric Oxide mediated pathways.

Role of Calcium in hyperoxic vasoconstriction

Dantrolene works by inhibiting calcium-induced calcium release from the SR [232] and its inhibition of the hyperoxic vasoconstrictive response implies the involvement of calcium-induced, calcium release from the SR in response to hyperoxia. Nifedipine works by blocking extra-cellular calcium channels and its blockade of hyperoxic vasoconstriction implies extracellular calcium channels are involved. Finally, 2-APB is a TRP channel antagonist and the blockage of TRP channels causes a blockage of the hyperoxic vasoconstriction implying that TRP channels are also involved. Figure 14 illustrates a possible mechanistic approach to calcium involvement in hyperoxic pulmonary vasoconstriction.

Role of membrane potential changes on hyperoxic vasoconstriction

In, this present study, we consistently show that this vasoconstriction is independent of changes in membrane potential as demonstrated by a lack of

effect on KCl-mediated contraction in response to DPI which blocked hyperoxic vasoconstriction.

Role of oxygen-free radicals on hyperoxic vasoconstriction

DPI has a plethora of speculated functions from animal studies varying from acting as a cholinesterase inhibitor in bronchial smooth muscle to blockade of reactive species via enzymes such as NADPH oxidase [233]. In our study, the effect on oxygen-induced vasoconstriction may point to the role of reactive species in the effecter arm of the response to hyperoxia in humans.

Role of hypothermia on hypoxia and hyperoxia

Our results consistently show that deep hypothermia abolishes the hypoxia-reoxygenation response. The fact that it also abolishes the vasoconstrictive response to potassium chloride demonstrates that hypothermia reduces the activity of pulmonary arteries in general. This is confirmed in the systemic vasculature in numerous animal and human studies where hypothermia conveys a protective role during ischaemia [234, 235]. However, our results also indicate that whilst rewarming increases the resting tension of pulmonary arteries in isolation as well as within the whole lobe, reflected in the rise in pulmonary artery pressures, the resultant vasoactivity in response to H-R or KCl at 37C is no different than had they not been cooled in the

first place. This demonstrates that deep hypothermia and rewarming does not predispose arteries to increased vasoactivity in response to stimulants.

Hypoxia and hyperoxia on isolated lungs

Our study is the first to demonstrate in isolated human perfused lung models that hypoxia-re-oxygenation either via the ventilator system or via the perfusate does not cause a net vasoconstriction. Given that our results show that at the higher order arterial level, re-oxygenation with hyperoxia causes a vasoconstriction, there appears to be either a nullification of this effect when arteries are placed in the context of an organ environment or that there are vasodilatory mechanisms downstream perhaps in the smaller pulmonary arteriole level in response to hyperoxia.

The idea that smaller resistance arterioles react to changes in oxygen tension differently to the large conductance arteries in the pulmonary circulation is not new in animal studies [236]. Why this should be, remains elusive especially as no such phenomenon exists in the systemic circulation.

Animal studies on isolated resistance arteries in the pulmonary circulation demonstrate a vasoconstrictive response to hypoxia [39]. However, there has always been a flaw in the logical process underlying this entity of hypoxic pulmonary vasoconstriction in that the blood in the pulmonary circulation is already hypoxic.

A way around this conundrum is to postulate that there is some central control within the pulmonary circulation in response to oxygen changes in the alveolar-capillary bed. Indeed, recent evidence may confirm that the oxygen sensor resides in the alveolar capillary bed and that the constrictive/dilatory response is conducted to the resistance arterioles and the conductance arteries [157].

An alternative explanation from our finding of no HPV in isolated lungs may point to the fact that the raised pulmonary pressures highlighted from clinical studies into HPV may be as a result of either pulmonary vasoconstrictors derived from outside the pulmonary system or changes in pulmonary flow that may be dependent on systemic factors.

Clinical implications in cardiopulmonary bypass

The clinical implications of our study pertain to different areas. Firstly, our results clearly show that whilst H-R affects human pulmonary artery tones, this effect is diminished or even nullified at the organ level. Hence acute rises in pulmonary arterial pressures following cardiopulmonary bypass (CPB) are unlikely to be due to the changes in oxygen tension that occur during reperfusion and re-oxygenation in the lung.

This contrasts to animal models of pulmonary artery hypertension following CPB. For example, Pearl et al have shown in pigs undergoing hypoxia-re-oxygenation during CPB, pulmonary vascular resistance is increased during

hypoxia and remains elevated during re-oxygenation. In addition, they found that endothelin-1 levels become elevated during re-oxygenation [237]. It is important to realise that their model accounted for systemic effects of hypoxia-reoxygenation such as leucocyte-mediated injury and the release of catelcholamines in the circulation. Our isolated vessels and isolated lungs, demonstrate that the pulmonary circulation in isolation acts to compensate for any local effects of hypoxia-reoxygenation on the pulmonary circulation.

The effect of shutting off the pulmonary arterial supply to the lungs is intriguing. As I mentioned in the introduction, the lung has a dual blood supply from the pulmonary artery and the bronchial circulation. The bronchial artery is believed to supply the nutrient supply. However, this is not completely fool-proof as lung transplants very rarely involve re-anastomosing the bronchial supply and so the pulmonary circulation must be providing the nutrient and oxygen supply in these patients [238]. In addition, many experiments to study ischaemia-reperfusion in the lung utilise clamping and unclamping of the pulmonary artery hence demonstrating that these authors consider the nutrient and energy supply to be derivative of the pulmonary artery and not necessarily the bronchial circulation [239].

Our results may also be relevant in the area of selective pulmonary artery perfusion during cardiopulmonary bypass. Studies have shown that lung injury is reduced during perfusion of the pulmonary artery with oxygenated

perfusate. Our results demonstrate that there are no adverse effects with regard to pulmonary artery hypertension with this method at least from the isolated lung level [240, 241].

Clinical implications in Ex-Vivo Lung Perfusion

Secondly, our results may impact on the employment of ex-vivo lung perfusion (EVLP). EVLP is now routinely used to optimise substandard donor lungs prior to implantation in the recipient during lung transplantation [242]. This optimisation process relies on a careful balance of electrolytes, temperature and flow [243]. Our results indicate that hyperoxia, cooling and rewarming may not adversely affect pulmonary artery pressures at least not in the short term.

This is particularly relevant in the assessment of lungs for optimisation. Many studies accept an arterial-venous oxygen difference of 300 mmHg upon challenging the lungs with 100% for short periods of time [244]. Our results from the isolated lung models indicate that such brief periods do not adversely affect pulmonary pressures.

Our lungs developed significant oedema regardless of whether they underwent hypoxia-reoxygenation or not. This may be due to the fact that we used an isotonic buffer Krebs which may over-time decrease the oncotic pressure in the pulmonary circulation causing an extravasion of fluid into

the lung parenchyma. This contrasts to Ex-Vivo Models which use an acellular Steen solution which is hypertonic and tends to draw fluid from the tissue of the lung into to perfusate thus reducing oedema [245]. Perhaps, in future, we can assess hypoxia-reoxygenation in isolated lungs with solutions such as Steen solution to discern any changes in pulmonary or bronchial pressures.

Limitations

There are limitations to our study. Firstly we are using lungs from patients with lung cancer and tumours are known to affect the responsiveness of arteries locally and systemically.

Secondly, we have not looked at smaller peripheral pulmonary arterioles in isolation. This is difficult in practice as the lung tumour is often peripheral and dissection of smaller resistance arterioles is cumbersome as well as running the risk of inadvertently affecting subsequent tumour staging.

Thirdly for the isolated lung models we used variable ventilator and pulmonary perfusion flows in order to achieve a constant bronchial and pulmonary artery pressure respectively. This may impact on outcomes such as the degree of reactivity of airways and puilmonary arteries to stimulants as well as the rate of oedema formation.

Finally, the time intervals used for observations were chosen from preliminary experiments which guided the length of time needed for maximum constriction and dilation of arteries. We acknowledge that these

time intervals are shorter than those experienced commonly in cardiac surgery.

However, despite these limitations, human studies on the pulmonary circulation are rare especially isolated perfused lung models. We feel, therefore, that this study provides an important insight into the descriptive phenomenon that may occur following the end of cardiopulmonary bypass in the human lung.

Effect of hypothermia on the human pulmonary circulation

Although previous animal experiments have shown the effects of hypothermia on blood vessels, particularly the systemic vasculature [246], we have shown for the first time the effect of deep hypothermia on the human pulmonary vasculature. This is important as we know that the pulmonary circulation does not always conform to the same principles underlying systemic vasoactivity.

Hypothermic preconditioning has been studied in ischaemia-reperfusion models in animals to demonstrate that bursts of hypothermia can be exploited in protecting against organ damage during prolonged periods of ischaemia [247, 248].

Our results demonstrate that re-warming from hypothermia causes a vasoconstriction in isolated pulmonary arteries. However this final tension is no different to that of arteries had they not originally been subjected to

hypothermic conditions. This implies that hypothermia does not precondition the arteries to a greater tension upon rewarming.

We consistently show that hypothermia blunts or abolishes the responsiveness of arteries to vasoconstriction that is dependent on both membrane potentials and ligand receptor-mediated activation. We also show that any vasodilatory effect is abolished at deep hypothermic conditions.

However, we demonstrate that there actually is no difference in the vasoconstrictive and vasodilatory responses of arteries had they originally be maintained in 37 C, compared to those warmed to 37C from 17C. This illustrates that arteries are not preconditioned to an exaggerated response to vasoactive agents upon re-warming.

The fact that a moderate reduction of temperature causes an inhibitory effect in the smooth muscle machinery is not new with regard to the systemic vasculature. It is likely that the temperature reduction is sufficient to compromise either the influx of extracellular Ca2+ through voltage-operated Ca2+ channels or other downstream enzyme elements of the contractile apparatus [249]. The mechanisms by which hypothermia attenuates contractions and tone may also be related to the activation of endothelium-dependent mechanisms that are associated with the NO–cGMP and PGI2–cAMP pathways as demonstrated in animal models of systemic arteries [250].

We used Endothelin-1 as this has been associated with the development of pulmonary artery hypertension [251].The concentrations of Endothelin-1 were used as these have been shown by our group in the past to be the most

effective in human pulmonary arteries [212]. However, our result does not preclude on a potential preconditioning for another agonist dependent response for example, alpha adrenoceptor agonist, as temperature-dependent alteration have been reported in different vascular beds.

We evaluated the effect of hypothermia on endothelium-independent relaxation using SNP. The concentrations of SNP used reflect those used in clinical practice.

One experimental concept of pre-conditioning is to expose cells to a stress, in this case 17 C, and then return to a metabolically active state in order to respond for example by making stress proteins and even repeat this cyclically. One limitation of the present study is that we have not allowed sufficient time for the cells to respond to the stress before re-testing as it may take some few hours to respond to stress by making heat shock proteins. Hence, it may be argued that our experiments may not discern potential pre-conditioning in the longer term. However, we argue that we are primarily concerned about the immediate impact of pulmonary artery hypertension that occurs very rapidly after the termination of cardiopulmonary bypass. From our experiment, it appears that this phenomenon cannot be demonstrated in the short-term.

We used high order pulmonary arteries as these have been shown to be the main determinants in developing strain on the right ventricle [252]. However, we acknowledge that further studies on the smaller resistance arteries may need to be undertaken to discern whether our observed findings

can be generalised across the pulmonary tree. In addition, further studies should also try to evaluate the effect on isolated perfused lungs.

Our results may impact on the employment of ex-vivo lung perfusion (EVLP). EVLP is now routinely used to optimise substandard donor lungs prior to implantation in the recipient during lung transplantation [253]. This optimisation process relies on a careful balance of electrolytes, temperature and flow [254]. Our results indicate that cooling and rewarming may not adversely affect pulmonary artery pressures at least not in the short term.

Effect of Hydrogen Sulphide on the human pulmonary circulation

In this report, we have shown for the first time that hydrogen sulphide is a potent vasodilator in human pulmonary arteries in the pre-constricted state and this vasodilation appears to be in an endothelium-independent manner.

Within the systemic vasculature, the mechanisms by which H2S seems to exert its vasodilatory effects remain unclear. However certain channels such as the K_{ATP} and TRPA1 have been implicated in animal models [255, 256]. This would need to be evaluated in more complex patch-clamp and pharmacological studies on human pulmonary arteries.

Our IPLM consistently demonstrates that as well as at the isolated arterial level, H2S exerts vasodilatory effects at the organ level indicated by a reduction in pulmonary artery pressures in a dose-dependent manner.

Interestingly, we also show that H2S added to the pulmonary circulation, causes a significant dilation in the bronchial airway pressures demonstrating that this may be due to diffusion of H2S into the bronchial tissue. This finding of bronchial dilation has been shown in previous animal studies on isolated bronchial tissue [257]. The finding that in hydrogen sulphide injected into the circulation diffuses to the bronchial tissue is confirmed in clinical studies [258].

The limitations of our study include the pre-constriction of isolated arteries and lungs using Potassium Chloride rather than receptor-mediated contraction agonists such as Endothelin-1, which is implicated in pulmonary artery hypertension in the more chronic stages [259]. In addition we used lung tissue from patients with lung cancer and this may influence the results and tumours are known to produce vasoactive substances.

The doses we used are at the concentrations used in clinical trials that would not cause significant systemic side-effects. Hence, there is a potential scope based on our human in-vitro experiments demonstrating H2S's vasodilatory potential, to take it to clinical trials.

Predictors of PAH

We found that being female, being over 80 years old, having a poor left ventricular function, having peripheral vascular disease, having a CCS class 4, having valvular pathology and the presence of a ventricular septal defect were all factors associated with a high pre-operative pulmonary artery pressure.

With regard to valvular heart disease, studies have shown that certain pathologies such as moderate-severe mitral regurgitation and aortic stenosis/regurgitation are all predictors of raised pulmonary artery pressures [228]. The mechanism for this is uncertain but may relate to the chronic left atrial volume overload/pressures. This may cause back pressure into the pulmonary circulation which leads to chronic remodelling of the pulmonary vasculature [229]. Valvular pathologies on the right side of the heart such as tricuspid regurgitation may actually be as a result of pulmonary artery hypertension causing remodelling of the right ventricle rather than as a cause of the initial raised pulmonary artery pressures.

Contrary to some models, we did not find that chronic obstructive pulmonary disorders were important in predicting raised PAPs [230]. Ventricular Septal Defects (VSDs) are rare and anaesthetic clinicians are not always eager to place a pulmonary artery catheter in these patients due to the possibility of the catheter passing through the defect. VSDs are known to create PAH in chronic states such as Eisenmenger's syndrome but the evidence in acute cases is not so convincing. The factors may involve an increased flow through the VSD to the pulmonary artery but may also be due to the vasoconstrictive effect of the high oxygen content into the pulmonary artery [177].

Other predictors are more in keeping with chronic factors and remodelling seen in animal models of pulmonary artery hypertension, for instance as a consequence of an increase in post-capillary pressures (in the case of poor left ventricular function) and chronic arteriopathic factors (in the case of peripheral vascular disease).

Our results indicate that a raised pre-operative PAP is a predictor of mortality and morbidity.

The morbidity and mortality associated with pulmonary artery hypertension in our post-operative patients may reflect the burden such an entity has on the right ventricle which comes under further strain during cardiopulmonary bypass.

Our result that PAH is associated with acute renal failure may not be a simple associative phenomenon. Studies have demonstrated that venous congestion as a result of heart failure is linked to renal failure in the so-called “cardio-renal” syndrome [260, 261, 262] although this is thought to involve a component of left ventricular failure. Despite this, recent studies may point to an isolated right heart failure (secondary to PAH) as a contributing factor to worsening renal failure [263, 264]. The mechanism is unknown but would be a hot-topic for future studies.

We used right heart catheterisation studies as these are proven to be more accurate than other methods of discerning pulmonary artery pressures such as echocardiography which is reliant on factors such as tricuspid regurgitation [231].

The limitations of our study are firstly the weaknesses and inconsistencies inherent in a retrospective analysis. The decision to perform a retrospective analysis of all patients undergoing cardiac surgery (a heterogeneous group) allows for greater numbers in analysis, however it also creates a situation in which there are many variables that it may be more difficult to find risk factors in groups of patients who are likely to have a different aetiology for

PAH than other groups, for example mitral valve disease compared to aortic valve disease. Secondly, not all patients have invasive pulmonary artery studies pre-operatively and so there is a selection bias likely reflected in the high logistic Euroscore in this group of patients. Finally, our study only concerns peri-operative mortality and morbidity and does not explore longer-term outcomes and survival.

Despite this, I feel our study detailing the factors contributing to raised pre-operative pulmonary artery pressures and the subsequent outcomes in cardiac surgery, confirms a link between factors which are known to increase mortality and morbidity (age, poor LV, peripheral vascular disease, VSD) and both PAH and poorer outcome. Pulmonary artery hypertension, although a local phenomenon, has widespread implications in the cardiac surgical patient and therefore, continues to be a real problem to manage.

Afterthoughts

From the first part of my Thesis, I have shown that the mechanisms governing the role of oxygen in the determination of pulmonary artery tone and pressures in humans is actually in its infancy despite detailed evidence in animals relating to the effects of acute hypoxia on the pulmonary circulation. In the following section, I have attempted to discern the effects of acute hypoxia and hyperoxia on the human pulmonary circulation at both the tissue level and the organ level. Following this, I have explored the role of hypothermia and rewarming on the human pulmonary circulation.

Although one can look at these experiments in isolation as separate avenues concerning the question “What determines pulmonary tone and pressures in humans”, I would like to think they can be amalgamated to address 2 important issues in my field of cardiothoracic surgery.

Firstly, “what causes the rises in pulmonary artery pressures following cardiopulmonary bypass?” The safest answer is that it is multifactorial but this is in many ways a cop out. Of course, many things can determine such a phenomenon such as anatomical considerations of the valvular and sub-valvular apparatus as well as physiological consequence of poor ventricular function but these are speculative at worst and casually associated at best. I have shown that re-oxygenation and rewarming employed during cardiac surgery, although causing acute rises in pulmonary artery tones, does not directly affect pulmonary artery pressures as a whole. This either implies that hypoxia-reoxygenation and cooling-rewarming does not cause pulmonary artery hypertension or that if it does, it must occur as a systemic mechanism rather than a phenomenon attributed to the pulmonary circulation.

Secondly, “Do the conditions of the lungs in ex-vivo optimisation affect pulmonary artery pressures?”. The drastic reduction of the donor lung pool in the West opens the door to explore the role of lung optimisation of substandard lungs for transplantation. It is apparent now that successful lung transplantation not only relies on the characteristics of the recipient but the quality of the donor lung. Ex-Vivo lung perfusion is in its infancy and has

yet to fully blossom. Most research has focussed on animal models which unfortunately do not always provide the groundwork for clinical application: one only has to look at the example of the Fontan operation developed back in the 1970s to correct babies born with a single ventricle: all the animals died during experimentation but the people they tried it on succeeded. My experiments allow for the possibility of using resected lungs from lung cancer patients to study optimum conditions for Ex-Vivo Lung Perfusion.

We can study pulmonary artery hypertension all day long but we need to bite the bullet and find ways to alleviate it. Although numerous pulmonary vasodilators exist, there is room for more. I have shown that Hydrogen Sulphide has that potential and has laid the foundations to explore its use in clinical practice.

So in conclusion, this thesis brings together many aspects concerning understanding the pulmonary circulation in humans from the tissue level and organ level as well as exploring therapeutic strategies for a very complex disease pattern.

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Appendix I: Tests of normality

Oxygen Experiments

(1) Tensions of arteries undergoing H-R in 95% O2

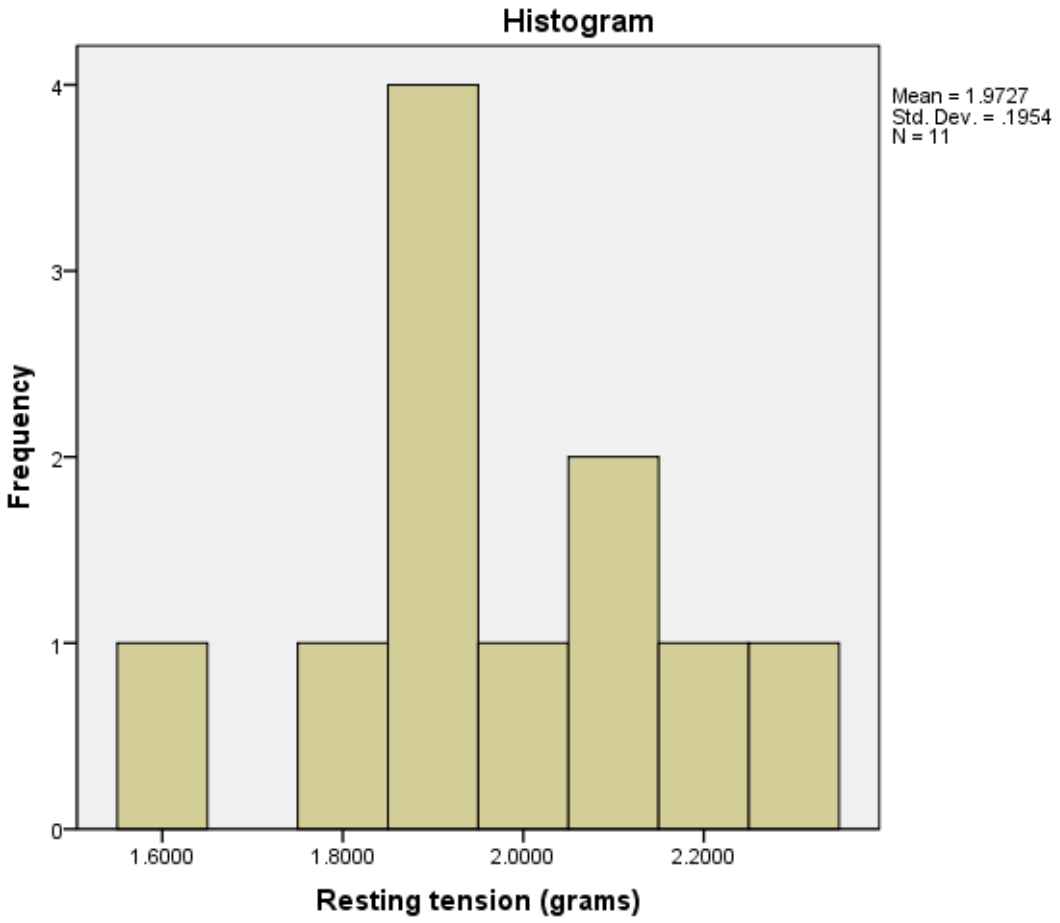
Tests of Normality

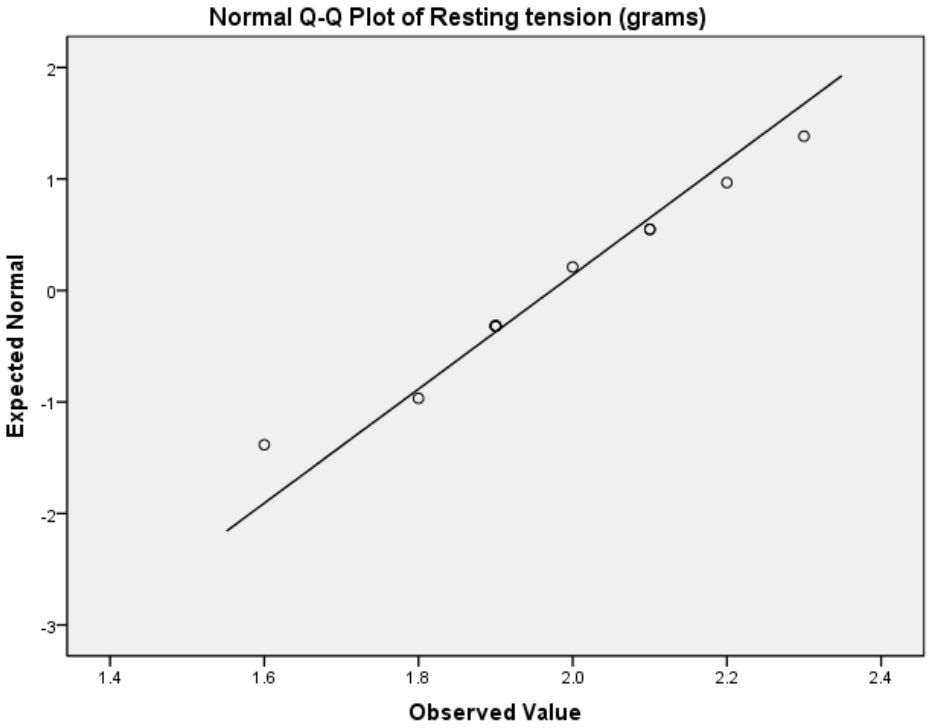
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.191	11	.200*	.960	11	.773

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension (grams)


Histogram
Mean = 1.9727
Std. Dev. = .1954
N = 11
Frequency
0
1
2
3
4
1.6000
1.8000
2.0000
2.2000
Resting tension (grams)



(2) Tensions in arteries undergoing H-R in 21% Oxygen

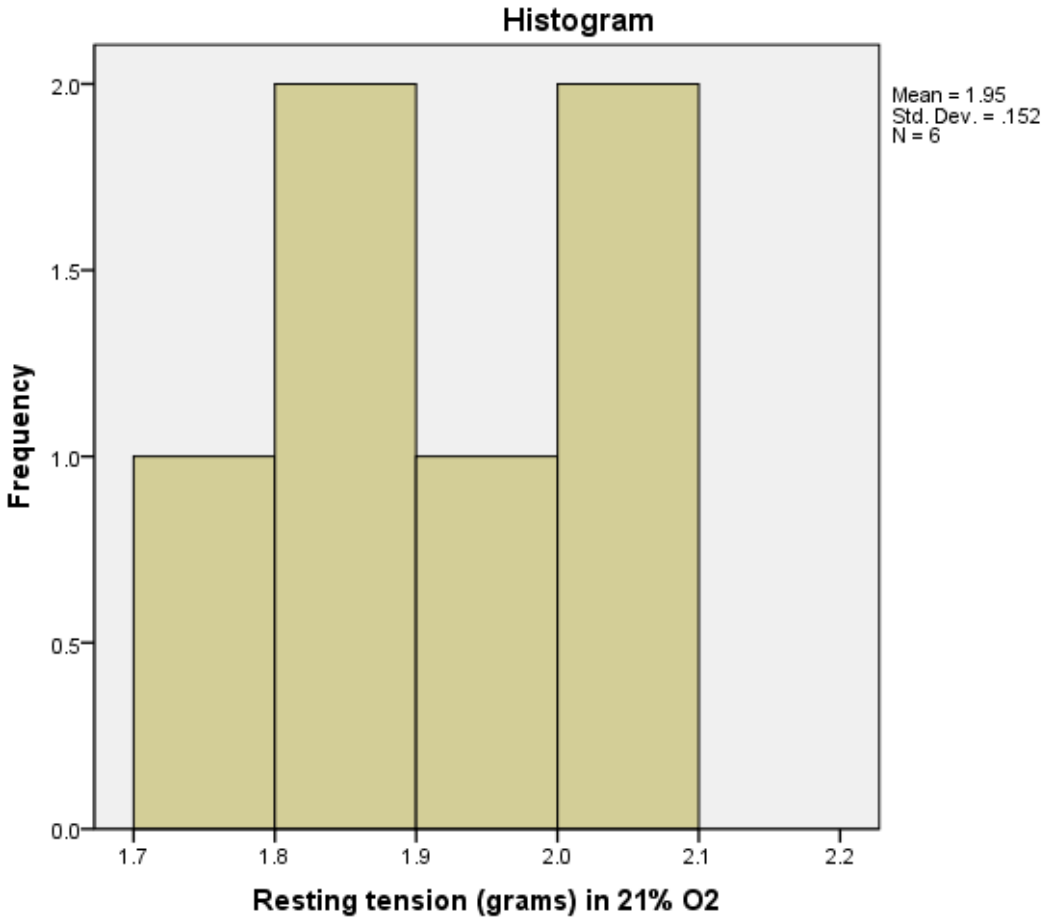
	Kolmogorov-Smirnov[a]	Shapiro-Wilk

	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams) in 21% O2	.204	6	.200*	.902	6	.389

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension (grams) in 21% O2



(3) Tensions for Nifedipine experiments

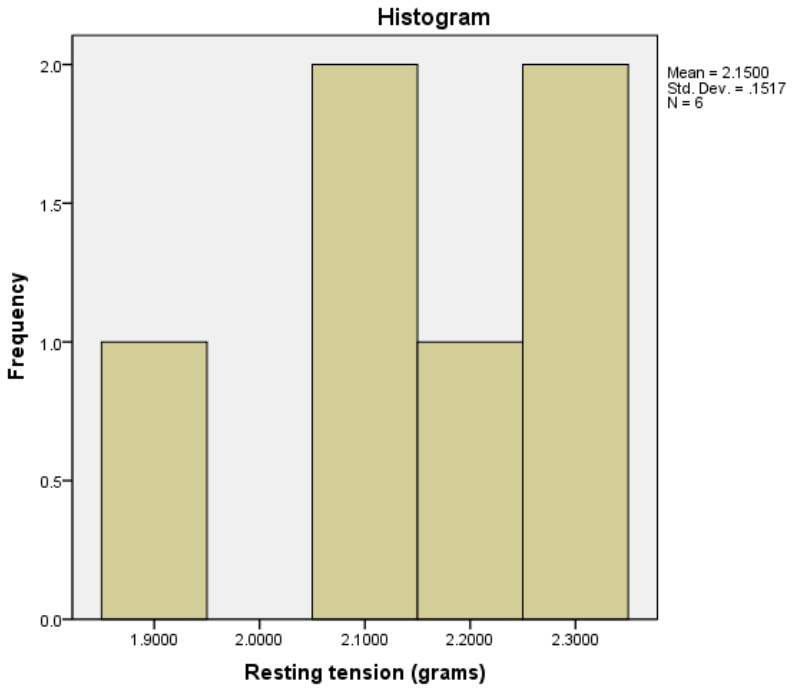
Tests of Normality

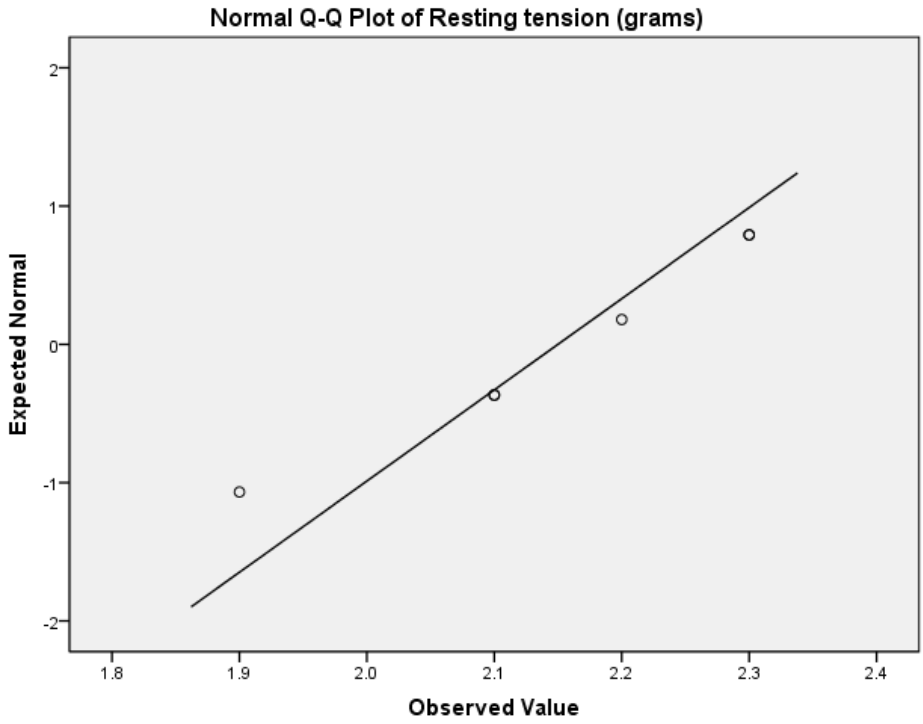
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.204	6	.200*	.902	6	.389

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension (grams)


Histogram
Mean = 2.1500
Std. Dev. = .1517
N = 6
Frequency
2.0
1.5
1.0
0.5
0.0
1.9000
2.0000
2.1000
2.2000
2.3000
Resting tension (grams)


Normal Q-Q Plot of Resting tension (grams)
Expected Normal
2
1
0
-1
-2
1.8
1.9
2.0
2.1
2.2
2.3
2.4
Observed Value

(4) Tensions for dantrolene experiments

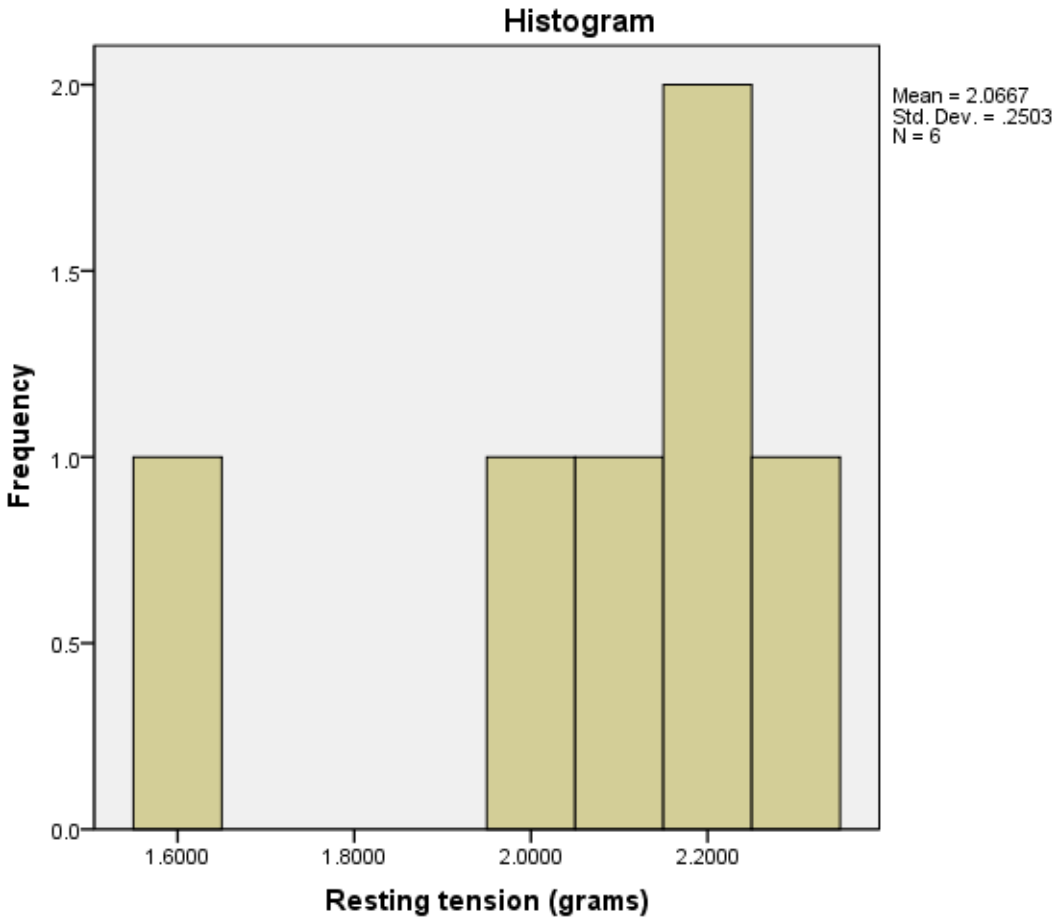
Tests of Normality

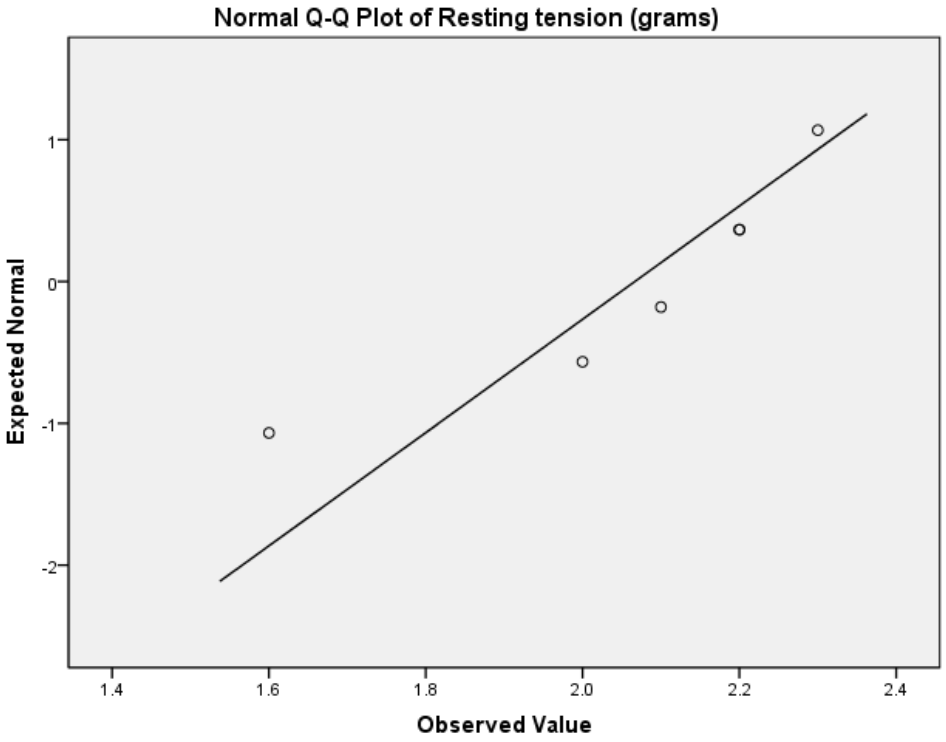
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.228	6	.200*	.847	6	.148

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension (grams)


Histogram
Mean = 2.0667
Std. Dev. = .2503
N = 6
Frequency
2.0
1.5
1.0
0.5
0.0
1.6000
1.8000
2.0000
2.2000
Resting tension (grams)



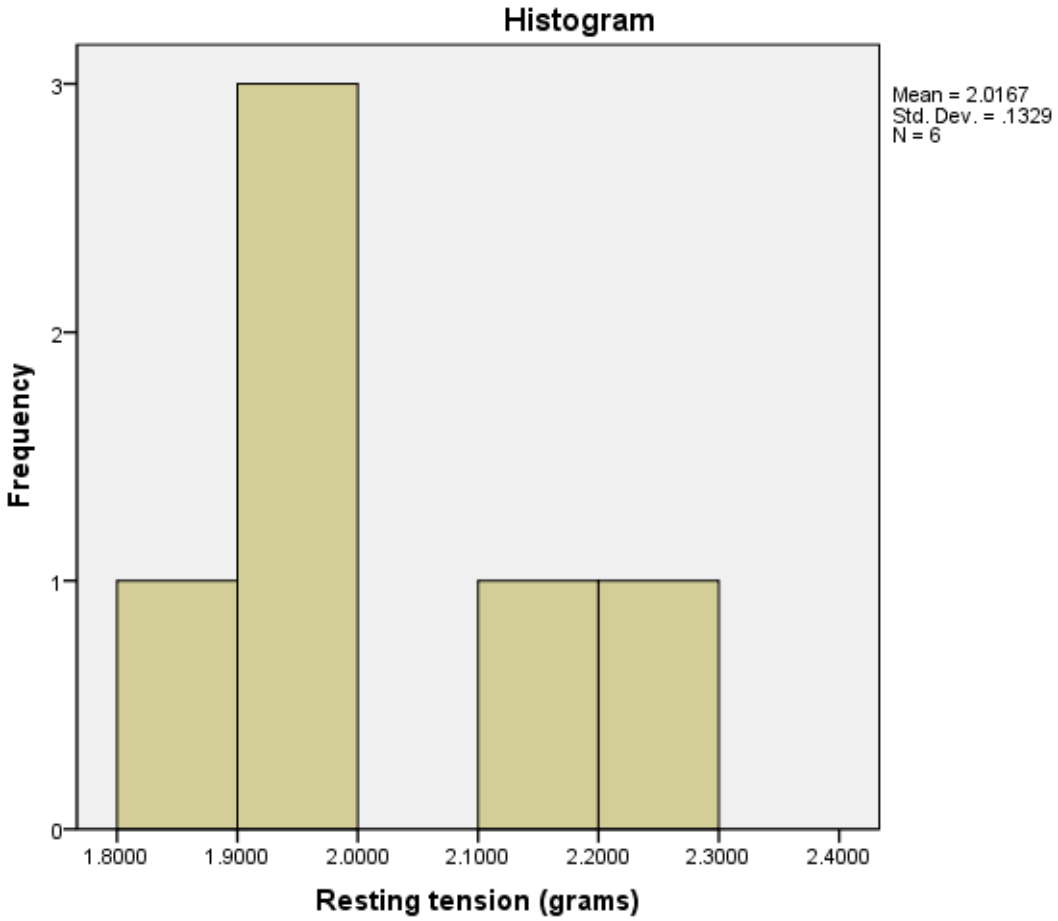
(5) Tensions for 2-APB experiments

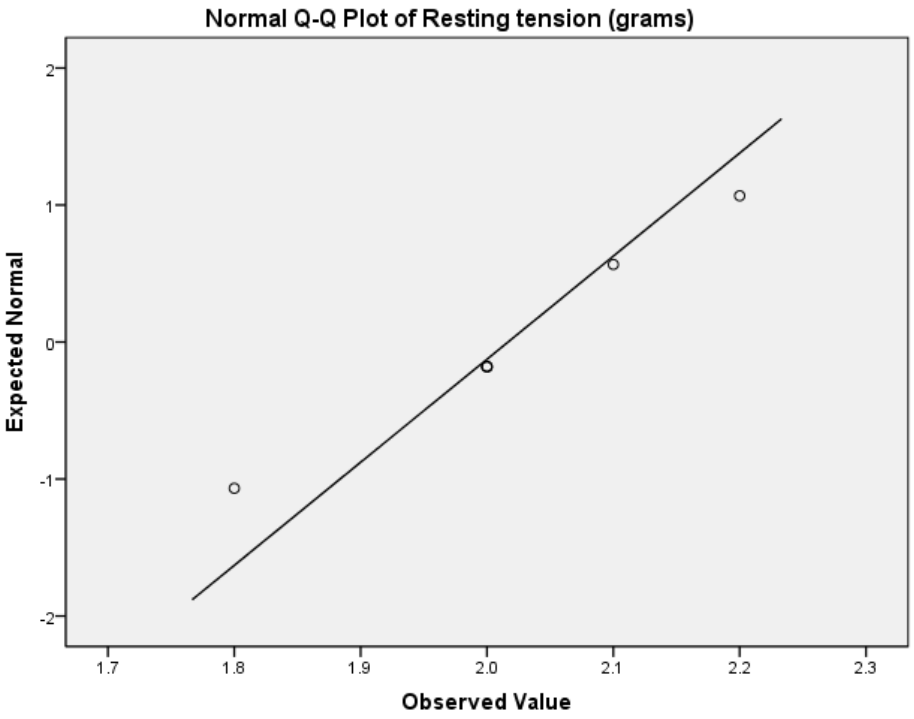
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.283	6	.143	.921	6	.514

a. Lilliefors Significance Correction

Resting tension (grams)





(6) Tensions for L-NAME experiments

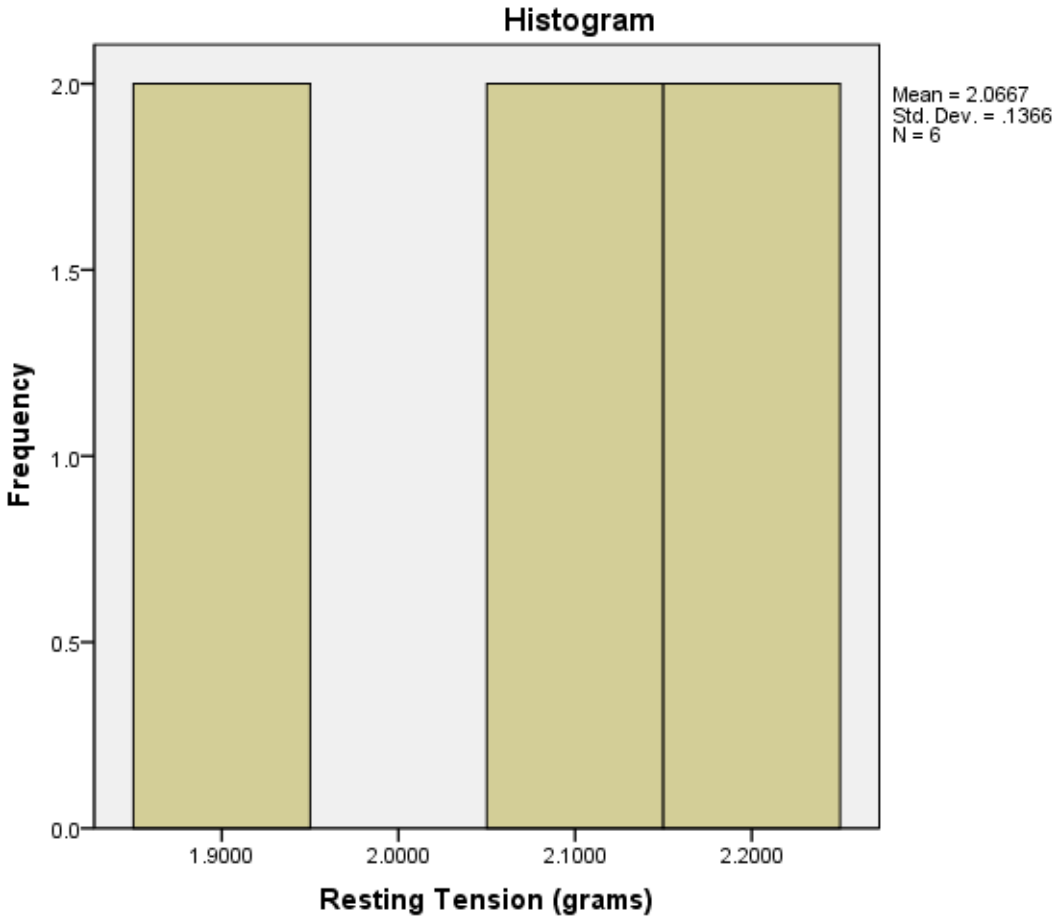
Tests of Normality

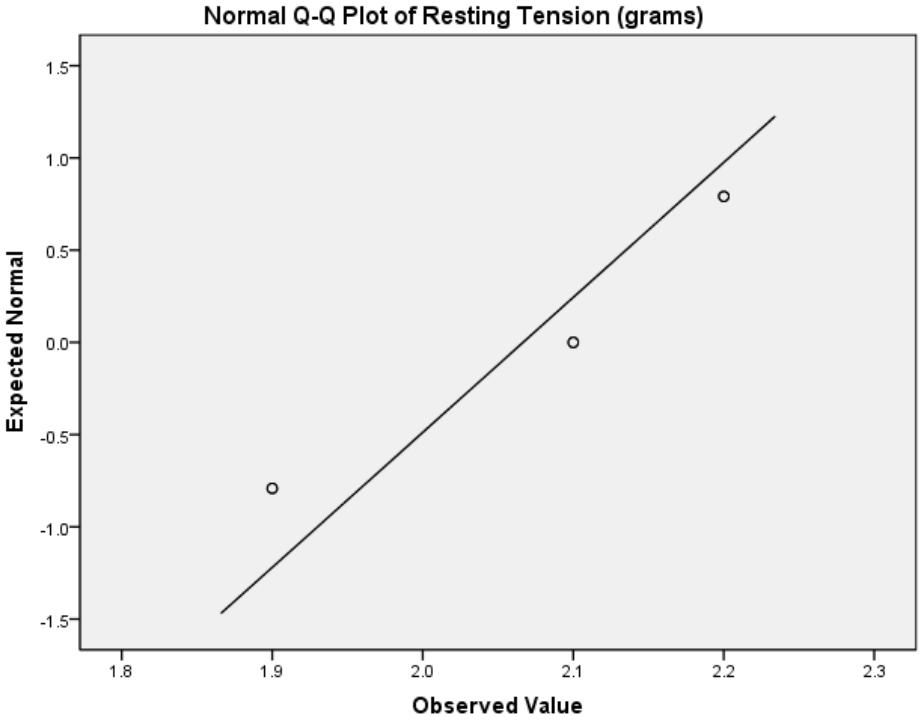
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting Tension (grams)	.263	6	.200*	.823	6	.093

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting Tension (grams)





(7) Tensions for DPI experiments

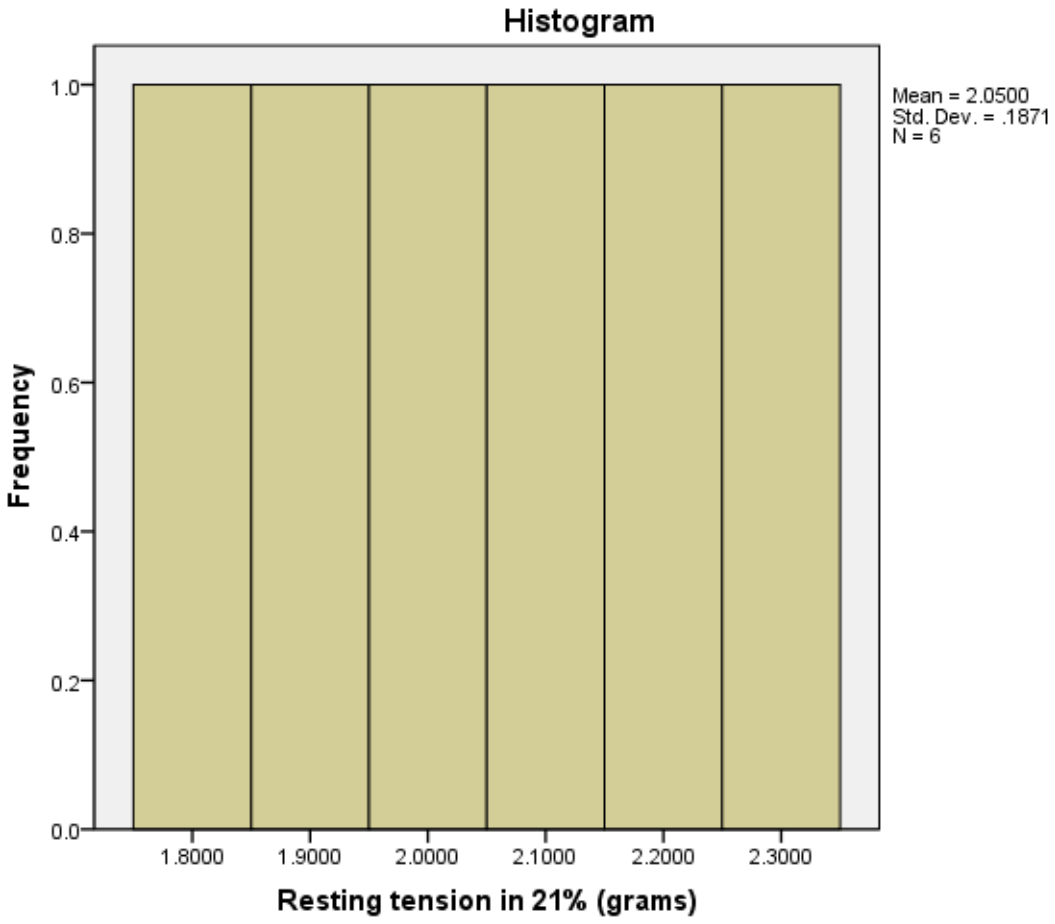
Tests of Normality

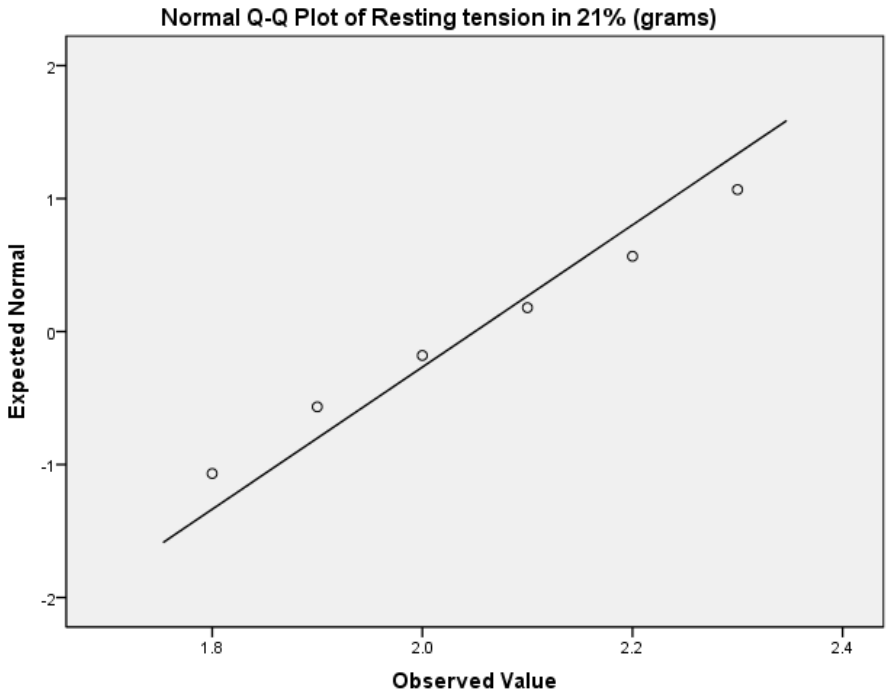
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension in 21% (grams)	.122	6	.200*	.982	6	.961

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension in 21% (grams)





(8) Tensions for H-R in hypothermia experiments

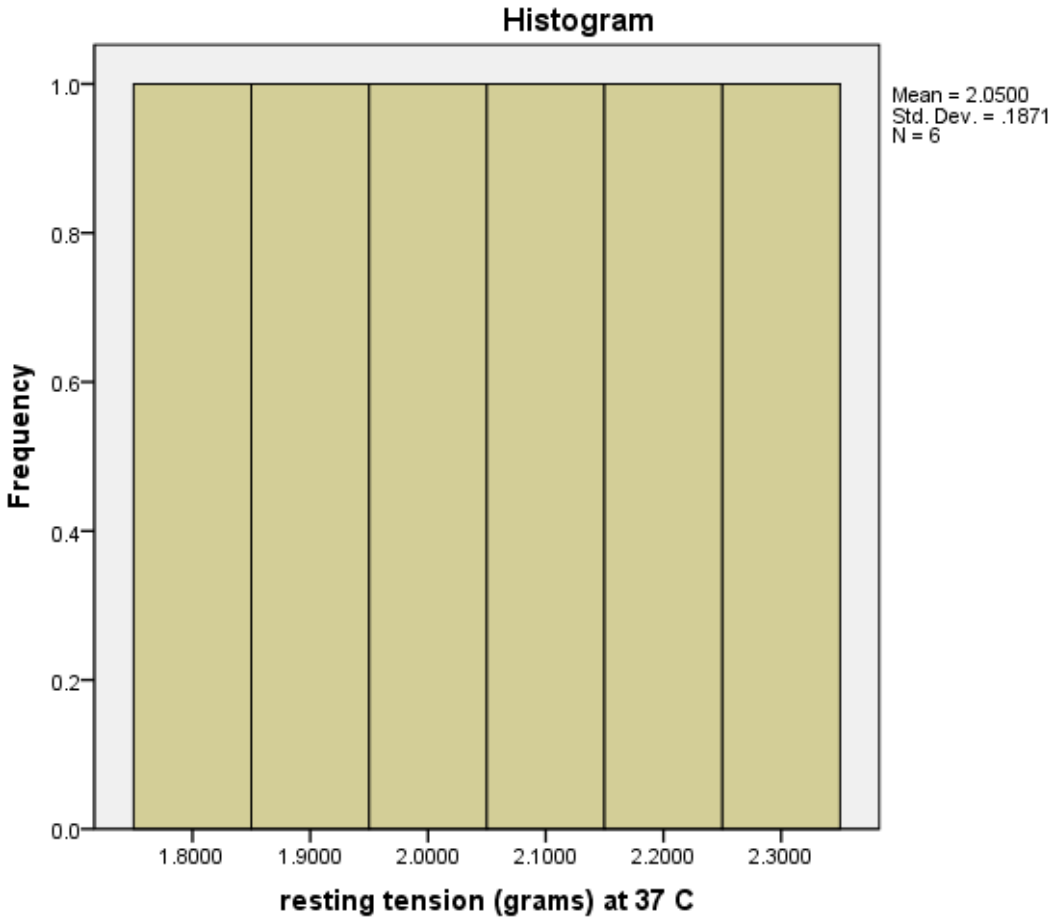
Tests of Normality

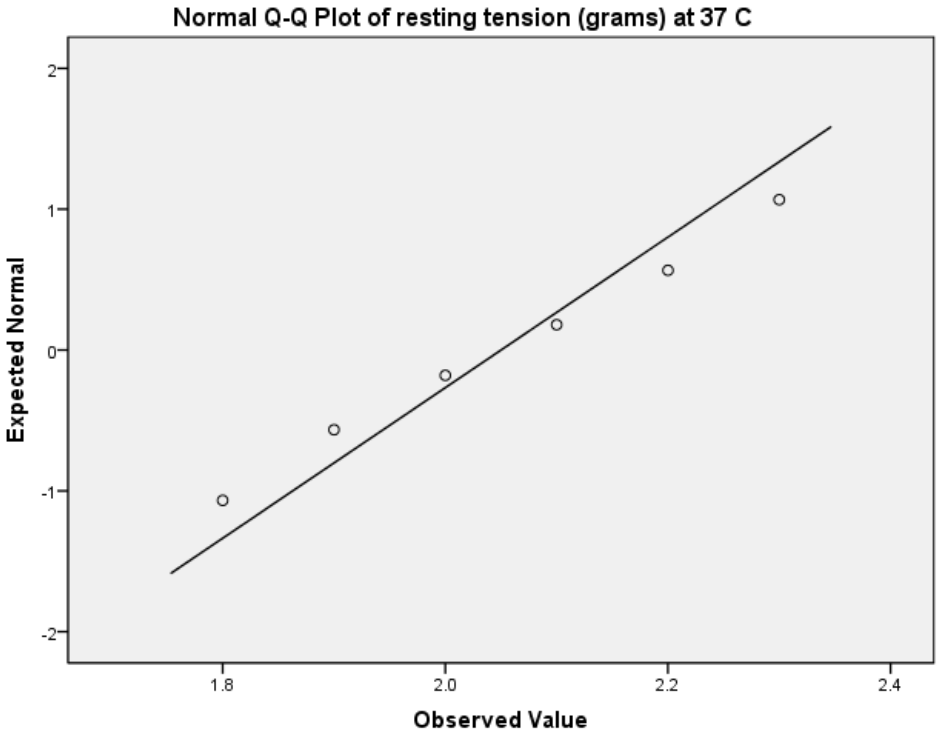
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
resting tension (grams) at 37 C	.122	6	.200*	.982	6	.961

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

resting tension (grams) at 37 C





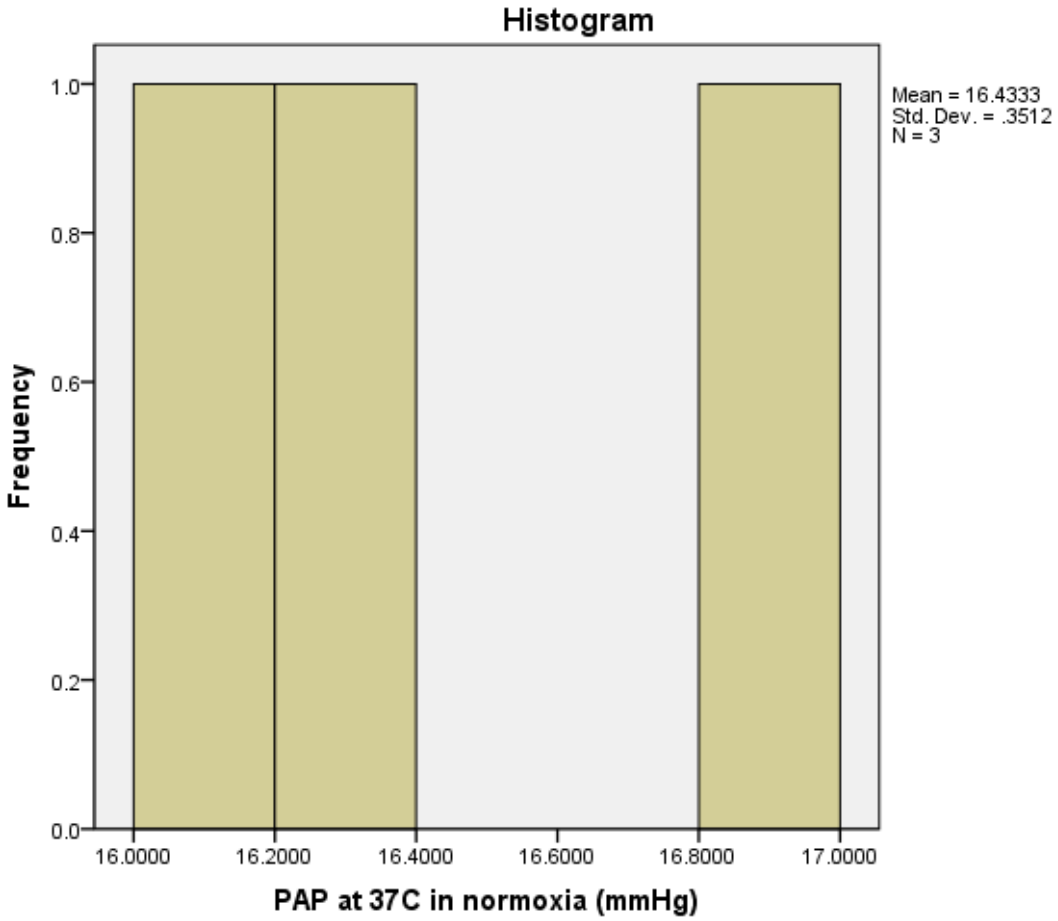
(9) Pulmonary artery pressures in H-R experiments

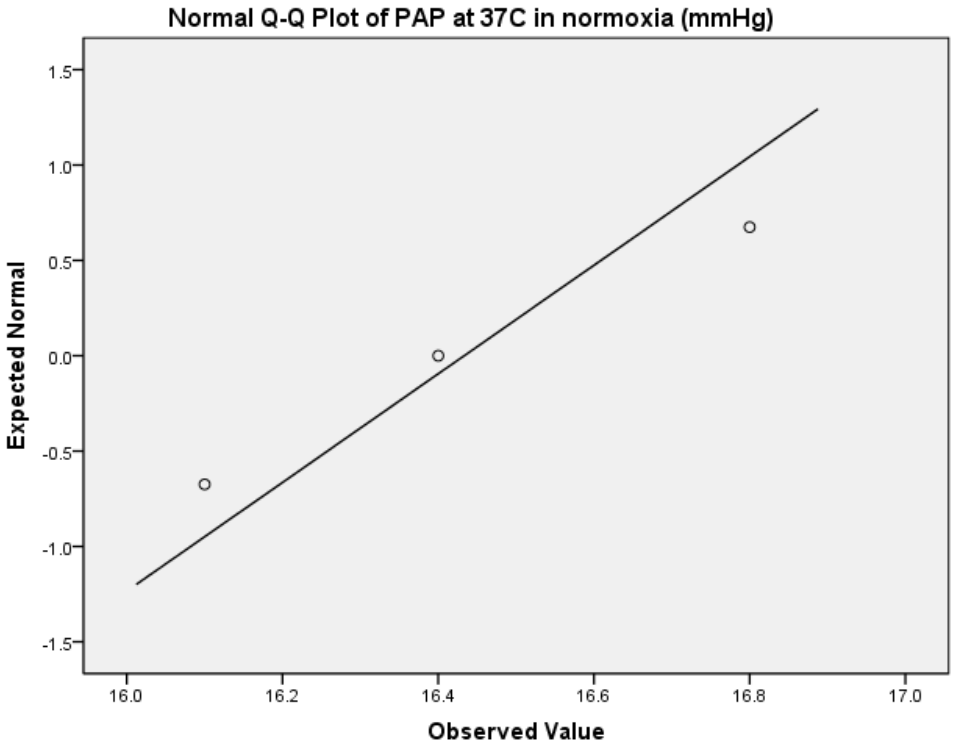
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PAP at 37C in normoxia (mmHg)	.204	3	.	.993	3	.843

a. Lilliefors Significance Correction

PAP at 37C in normoxia (mmHg)




Normal Q-Q Plot of PAP at 37C in normoxia (mmHg)
Expected Normal
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
16.0
16.2
16.4
16.6
16.8
17.0
Observed Value

(B) Temperature Experiments

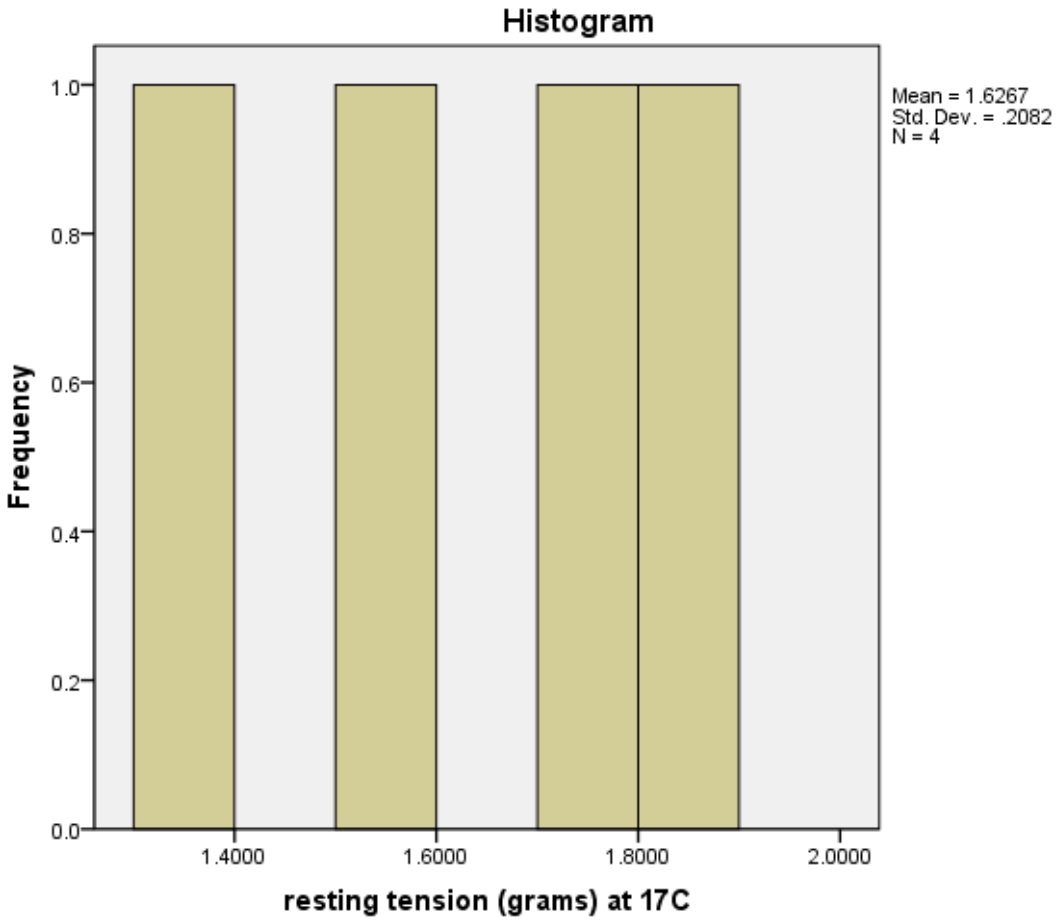
(1) Endothelin-1 Experiments

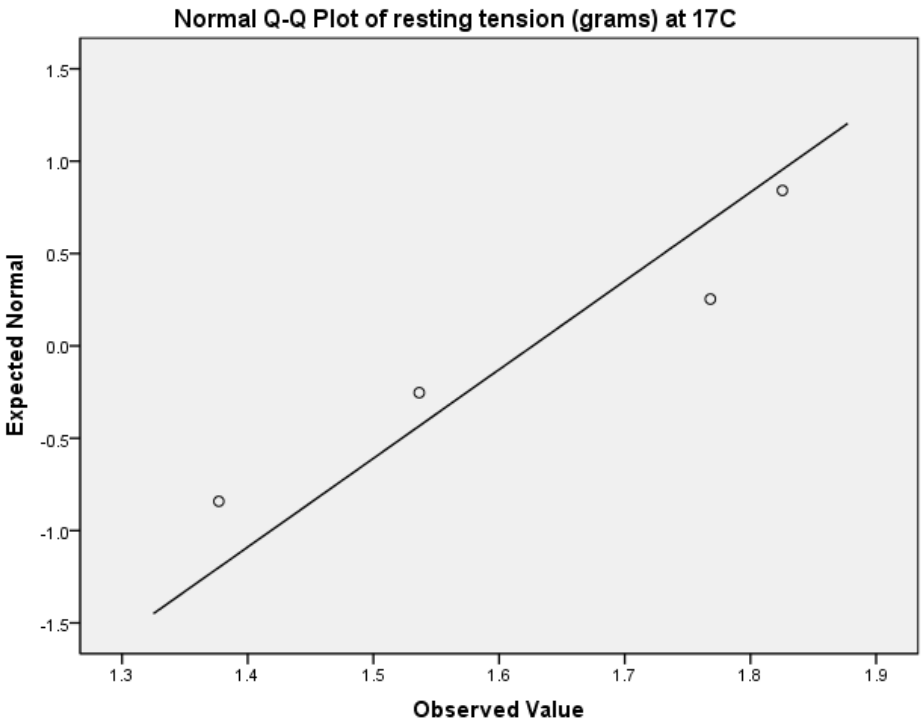
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
resting tension (grams) at 17C	.251	4	.	.925	4	.565

a. Lilliefors Significance Correction

resting tension (grams) at 17C





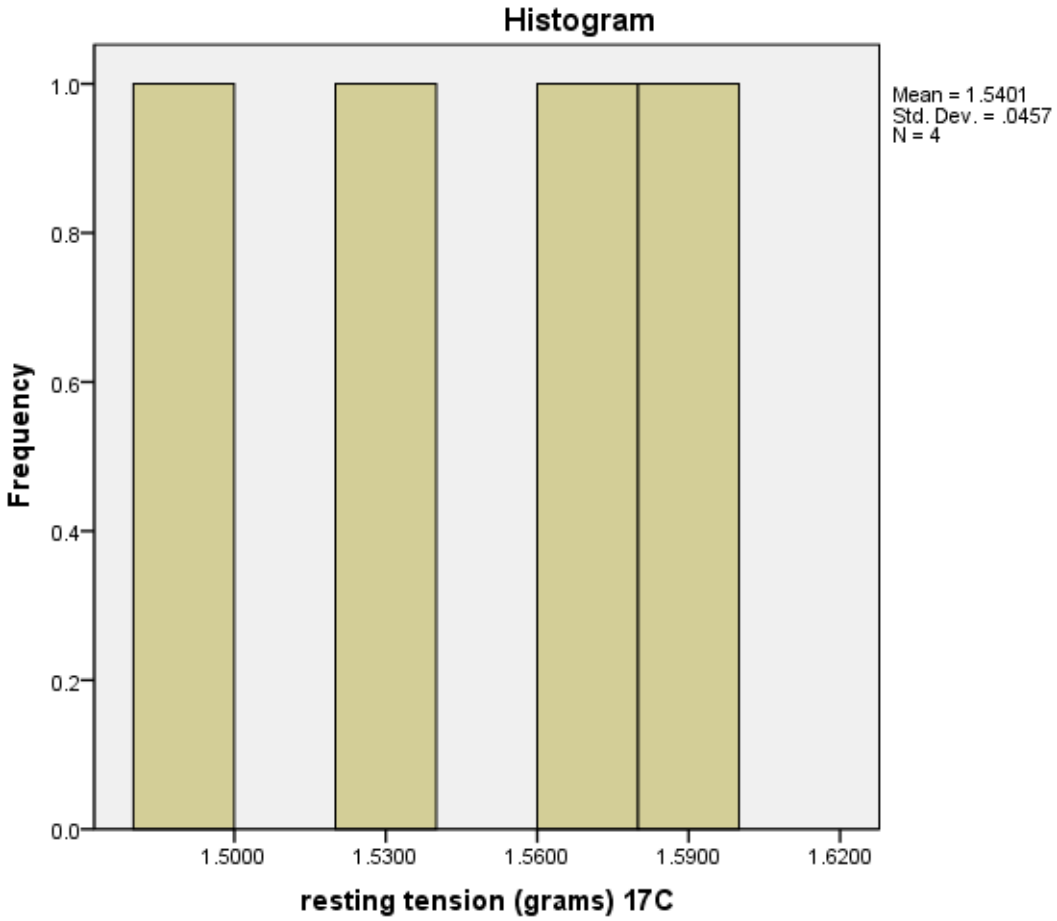
(2) Tensions for KCl experiments hypothermia-rewarming

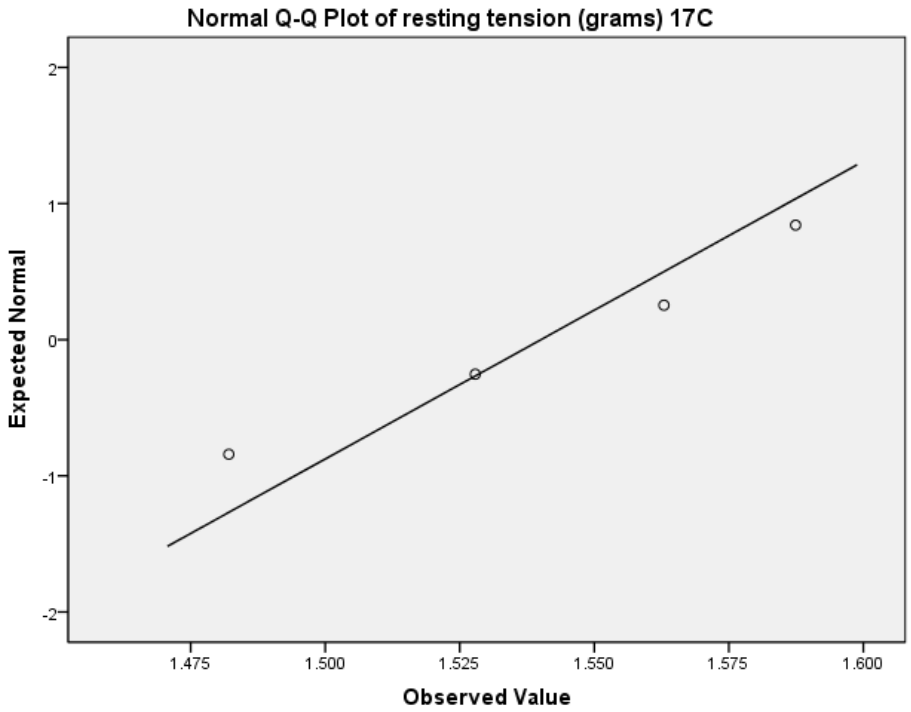
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
resting tension (grams) 17C	.191	4	.	.975	4	.872

a. Lilliefors Significance Correction

resting tension (grams) 17C





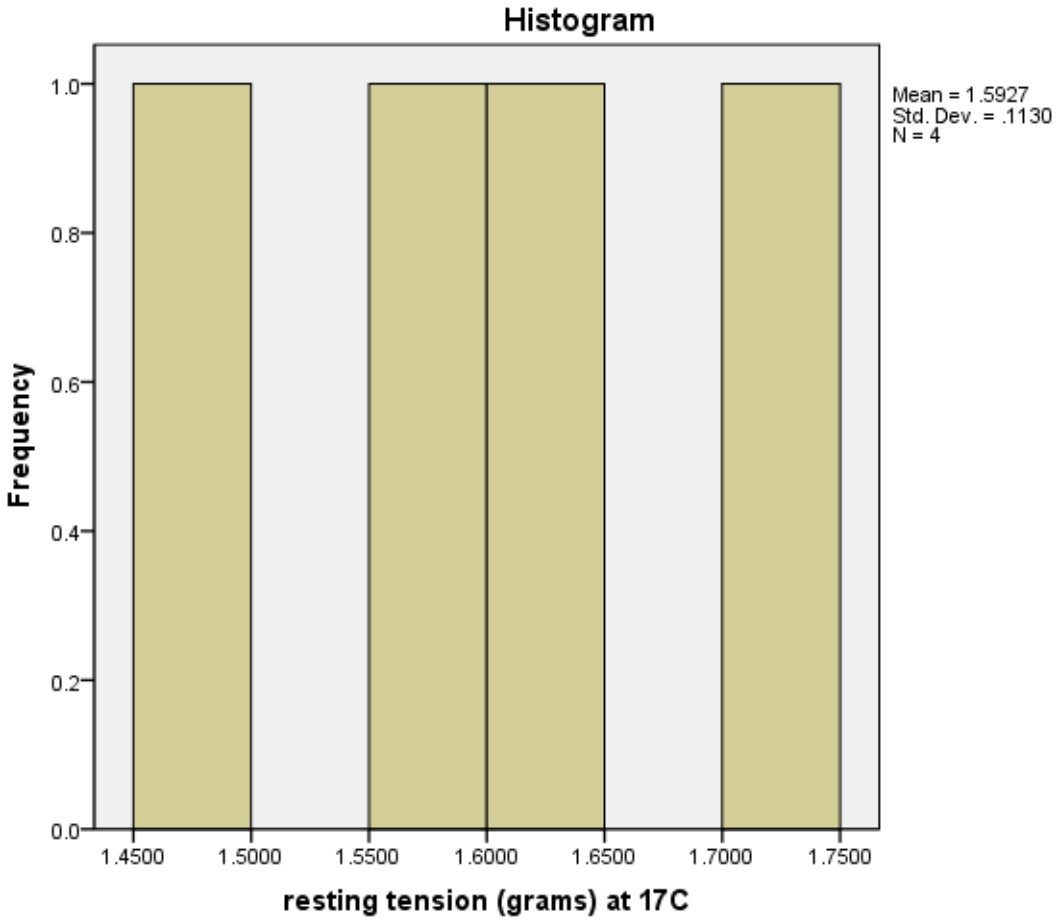
(3) Tensions for SNP experiments for hypothermia-rewarming

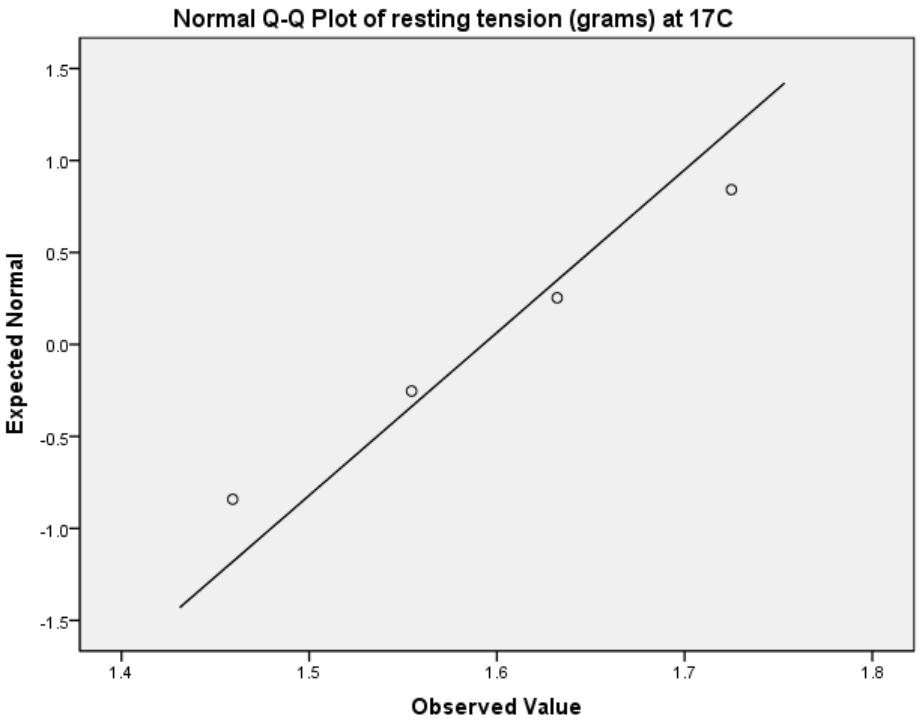
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
resting tension (grams) at 17C	.136	4	.	.998	4	.993

a. Lilliefors Significance Correction

resting tension (grams) at 17C





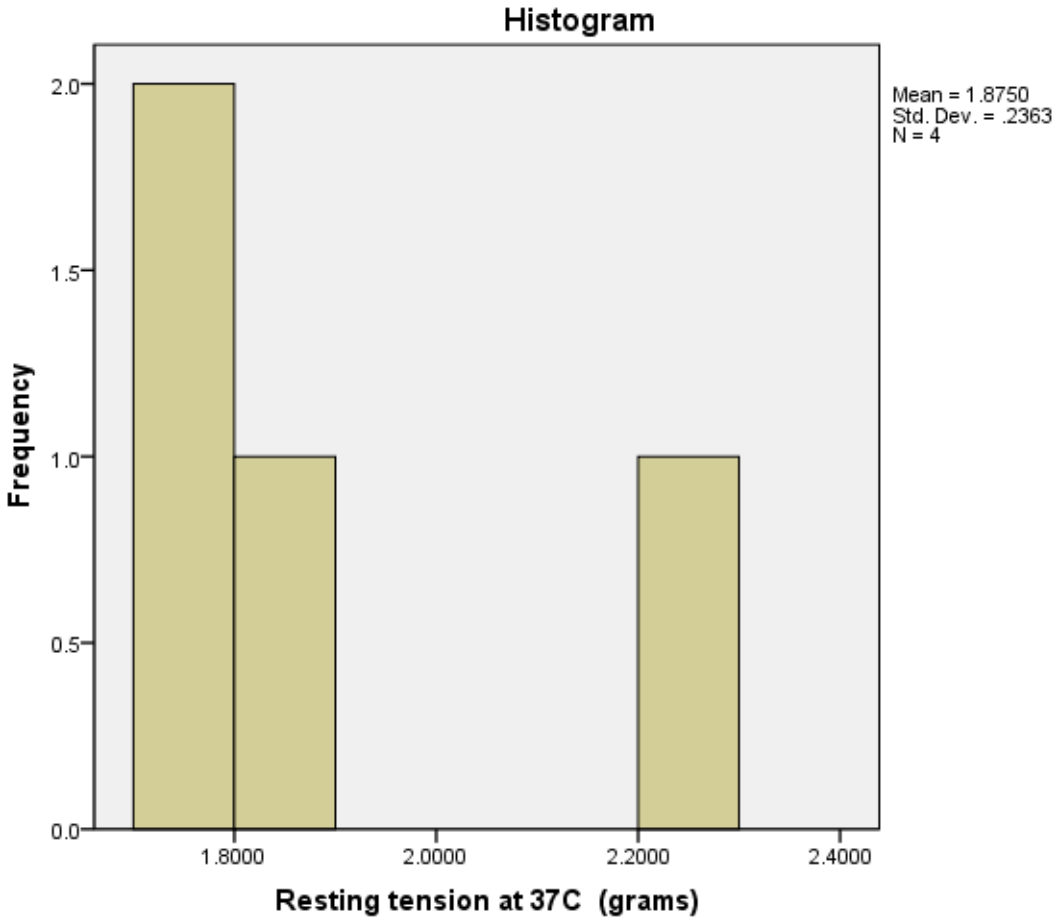
(4) Tensions for arteries maintained at 37C exposed to endothelin-1

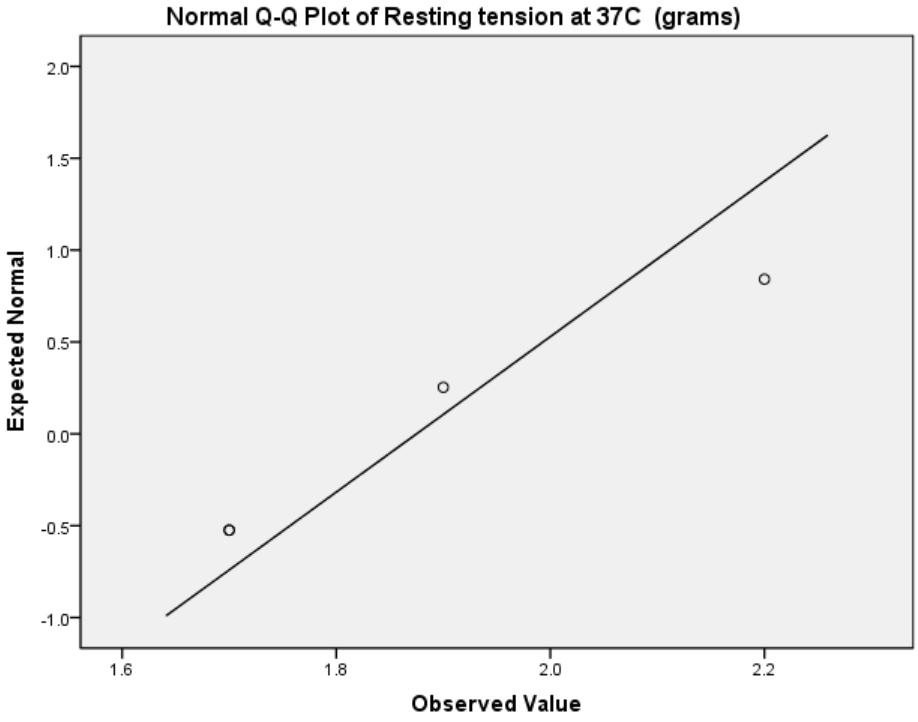
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension at 37C (grams)	.271	4	.	.848	4	.220

a. Lilliefors Significance Correction

Resting tension at 37C (grams)





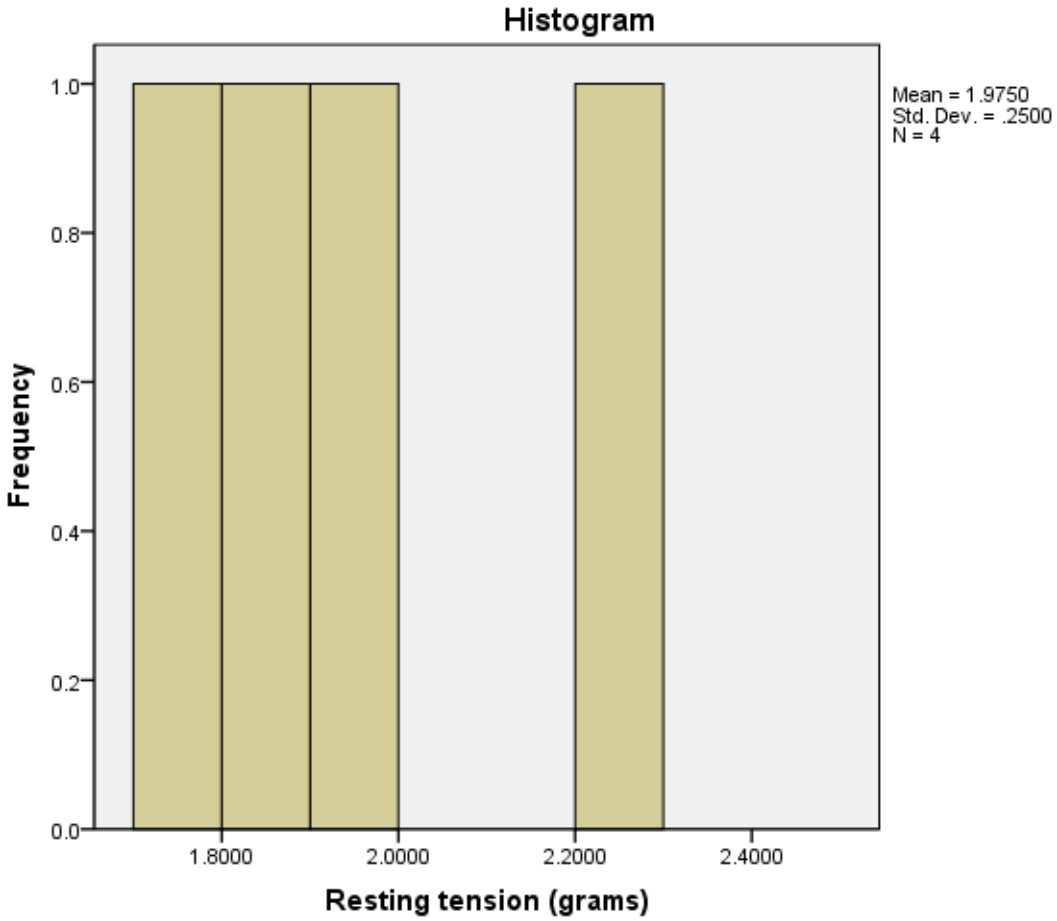
(5) Tensions for arteries maintained in 37C exposed to KCl

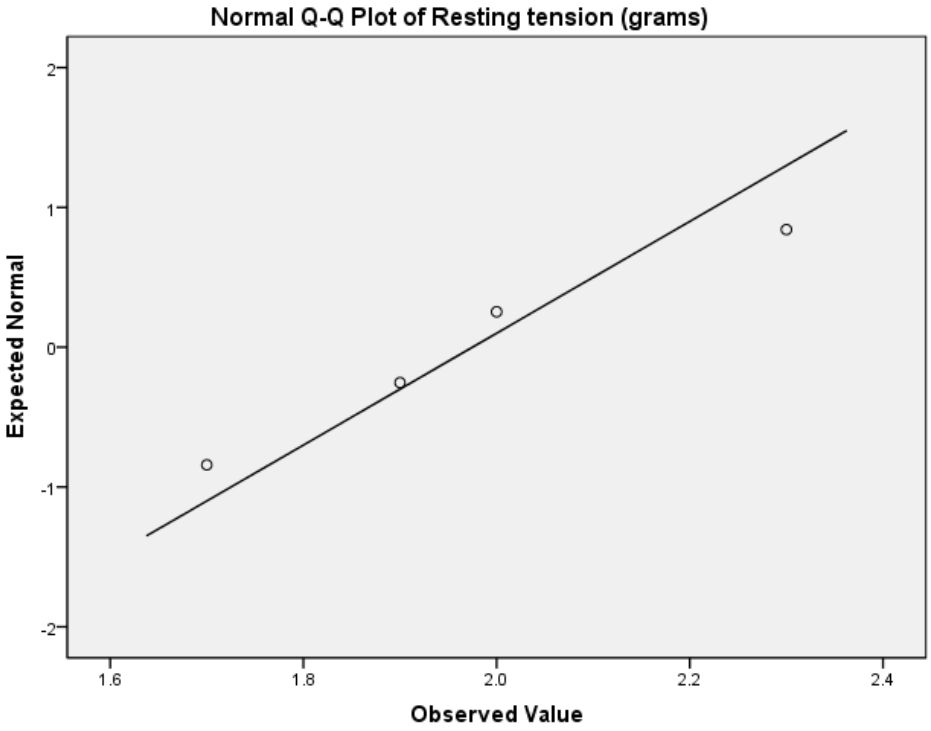
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.210	4	.	.982	4	.911

a. Lilliefors Significance Correction

Resting tension (grams)





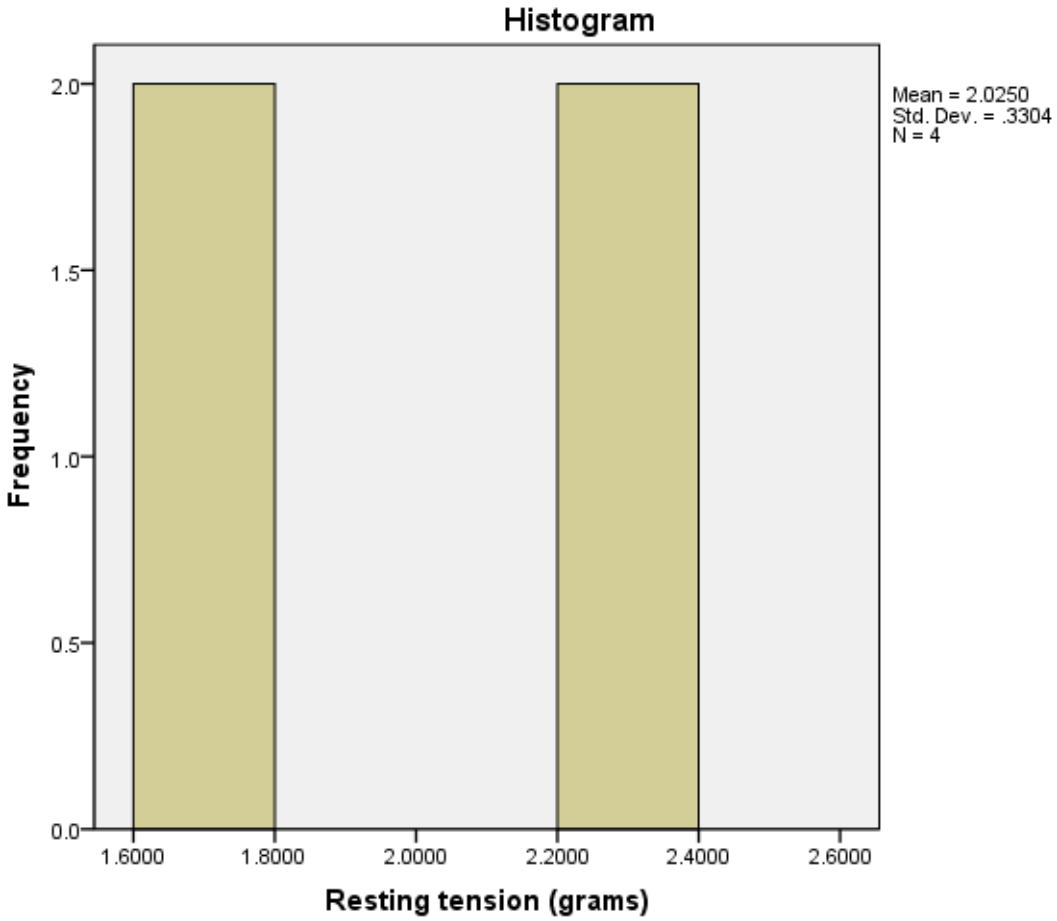
(6) Tensions of arteries maintained at 37C exposed to SNP

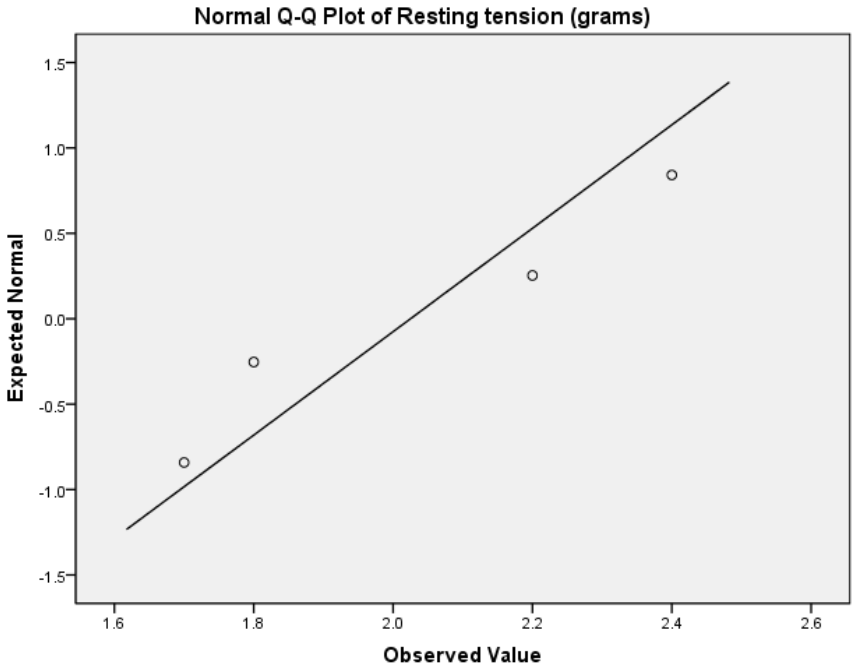
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension (grams)	.252	4	.	.916	4	.513

a. Lilliefors Significance Correction

Resting tension (grams)





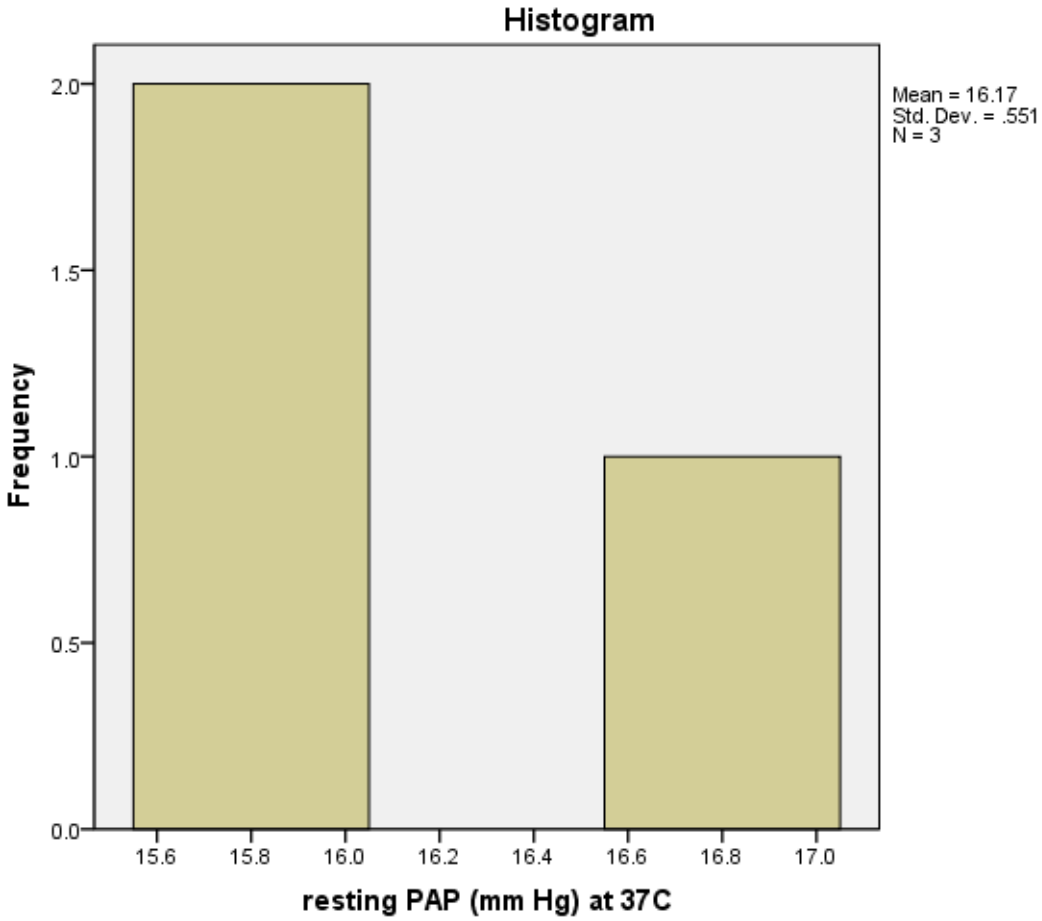
(7) Pulmonary artery pressures

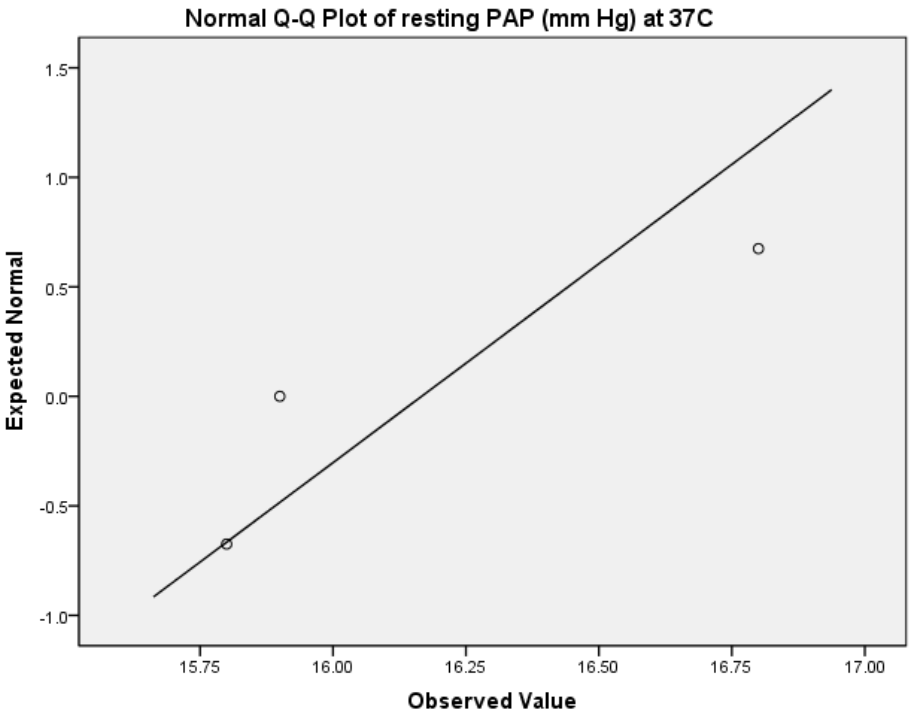
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
resting PAP (mm Hg) at 37C	.353	3	.	.824	3	.174

a. Lilliefors Significance Correction

resting PAP (mm Hg) at 37C





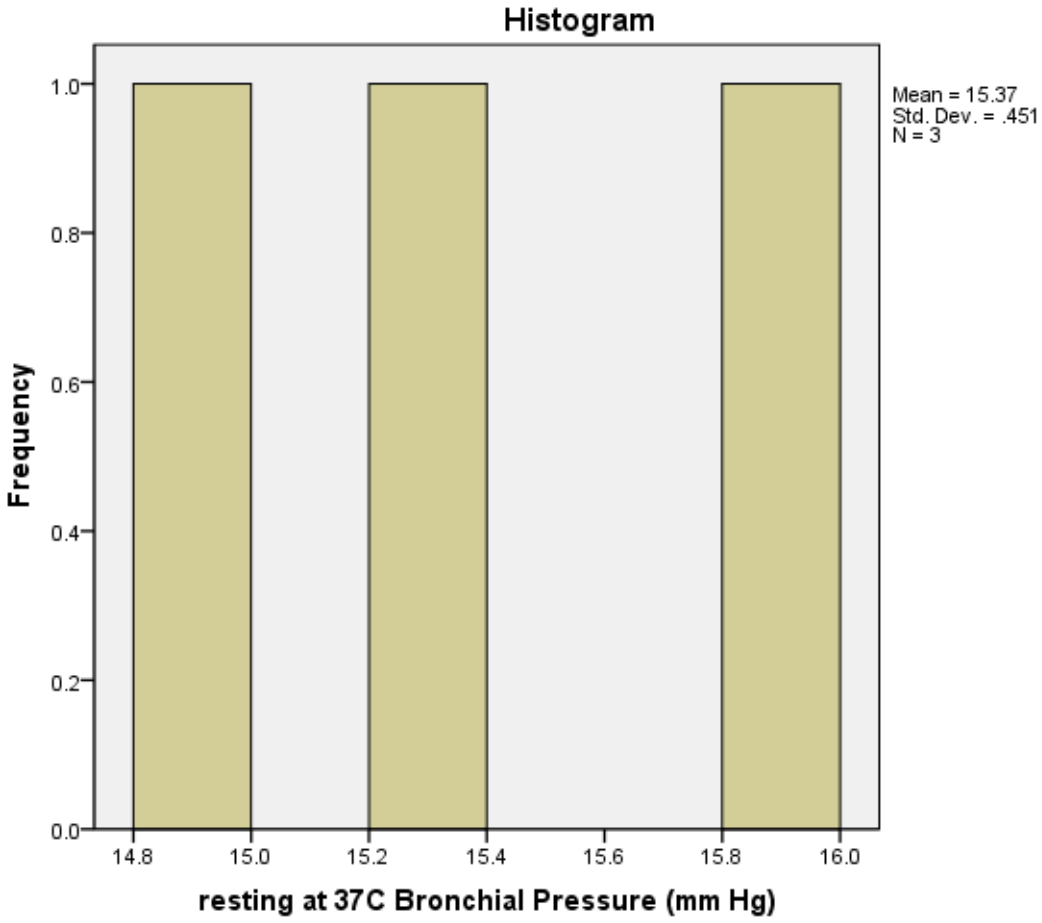
(8) Bronchial pressures upon rewarming

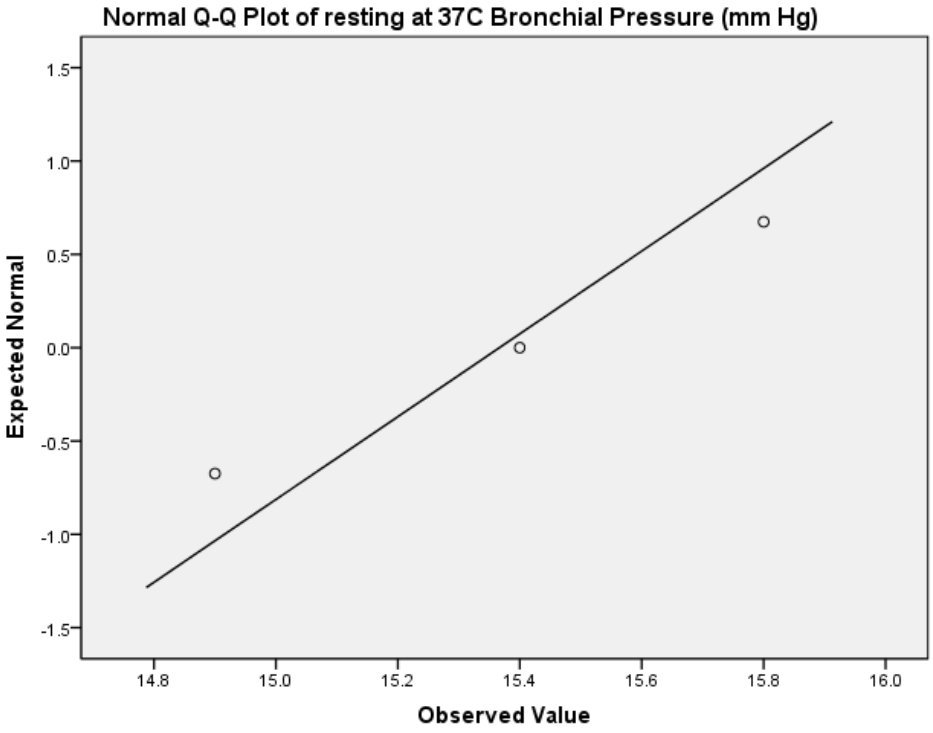
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
resting at 37C Bronchial Pressure (mm Hg)	.196	3	.	.996	3	.878

a. Lilliefors Significance Correction

resting at 37C Bronchial Pressure (mm Hg)





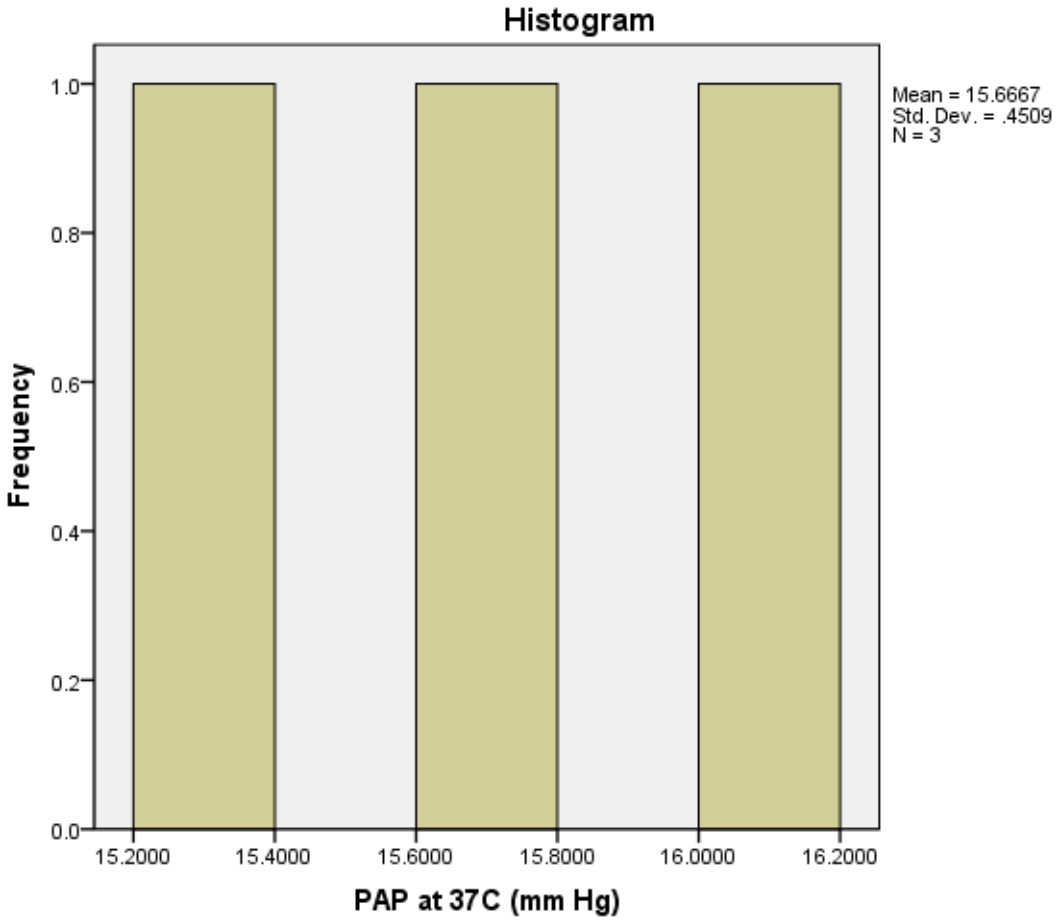
(9) Pulmonary artery pressures to KCl at 37C

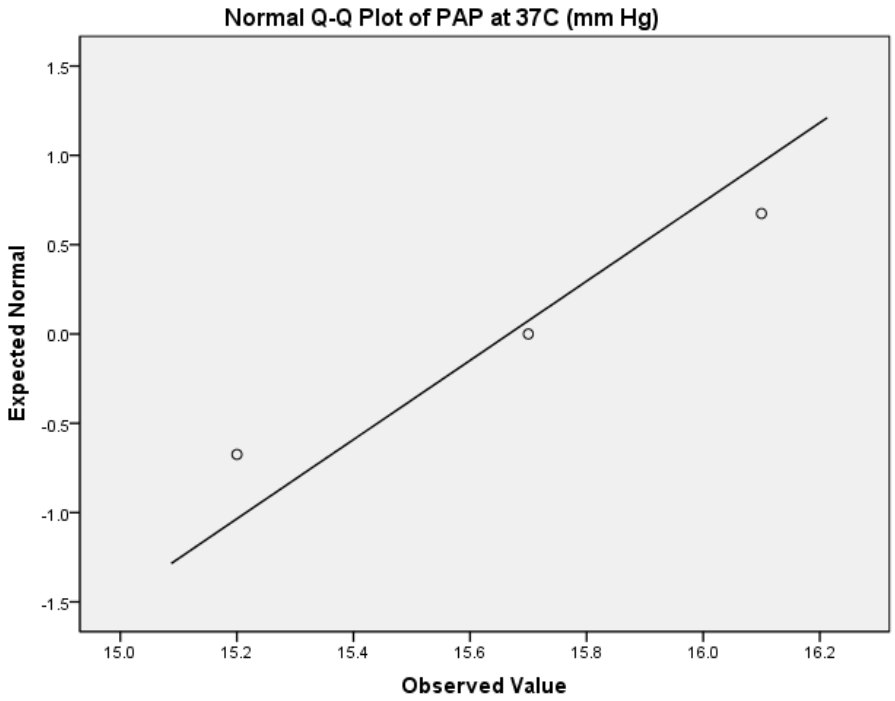
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PAP at 37C (mm Hg)	.196	3	.	.996	3	.878

a. Lilliefors Significance Correction

PAP at 37C (mm Hg)





(C) Hydrogen Sulphide Experiments

(1) Tensions of pulmonary artery rings exposed to Hydrogen Sulphide

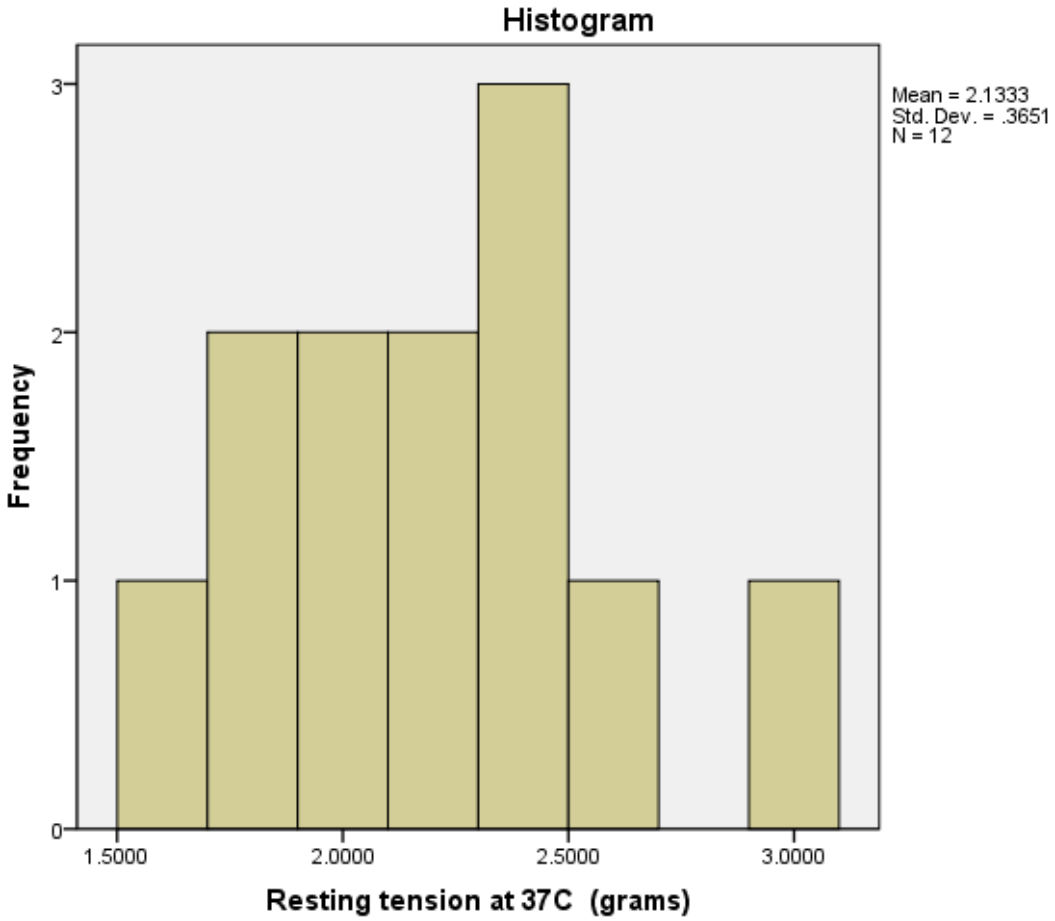
Tests of Normality

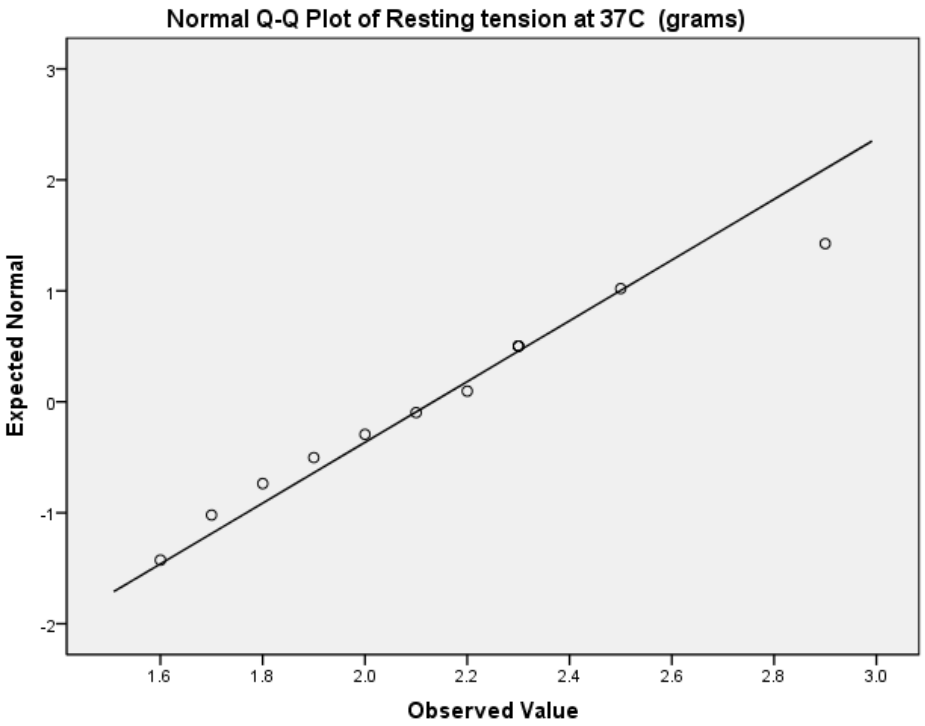
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Resting tension at 37C (grams)	.157	12	.200*	.964	12	.844

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Resting tension at 37C (grams)





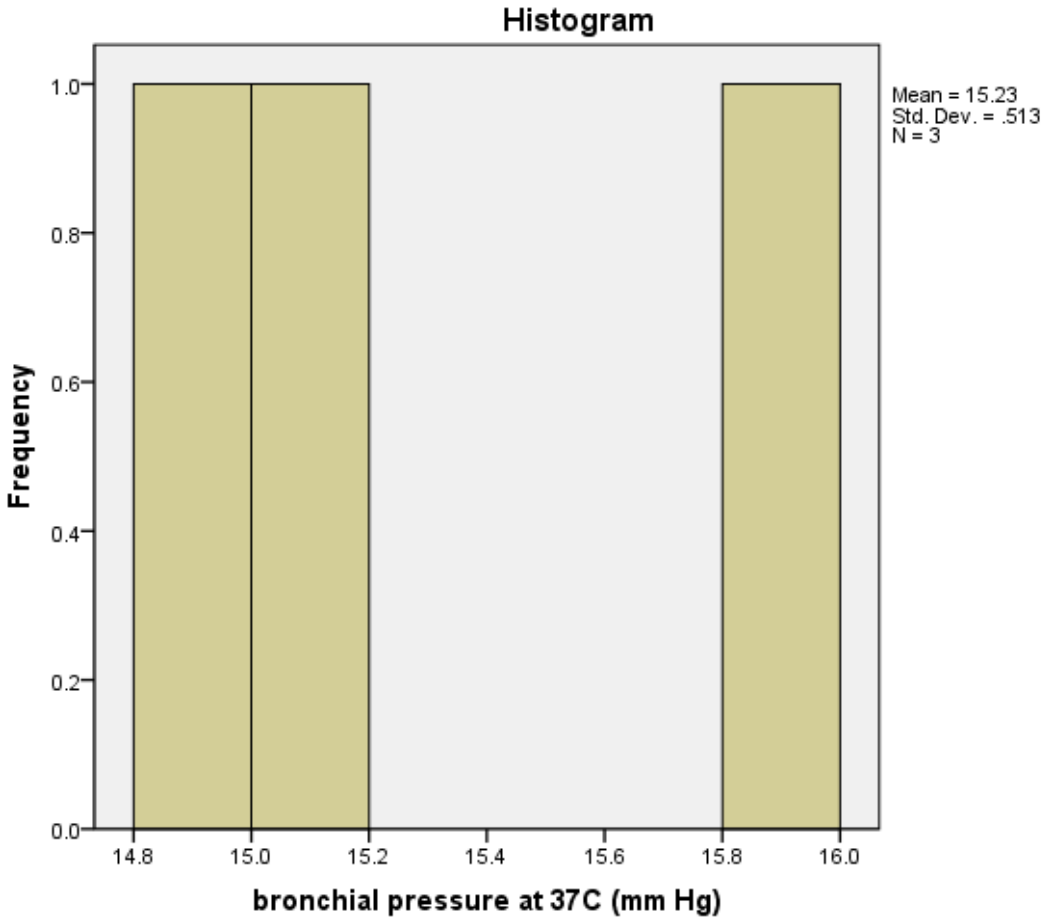
(2) Bronchial pressures exposed to hydrogen sulphide

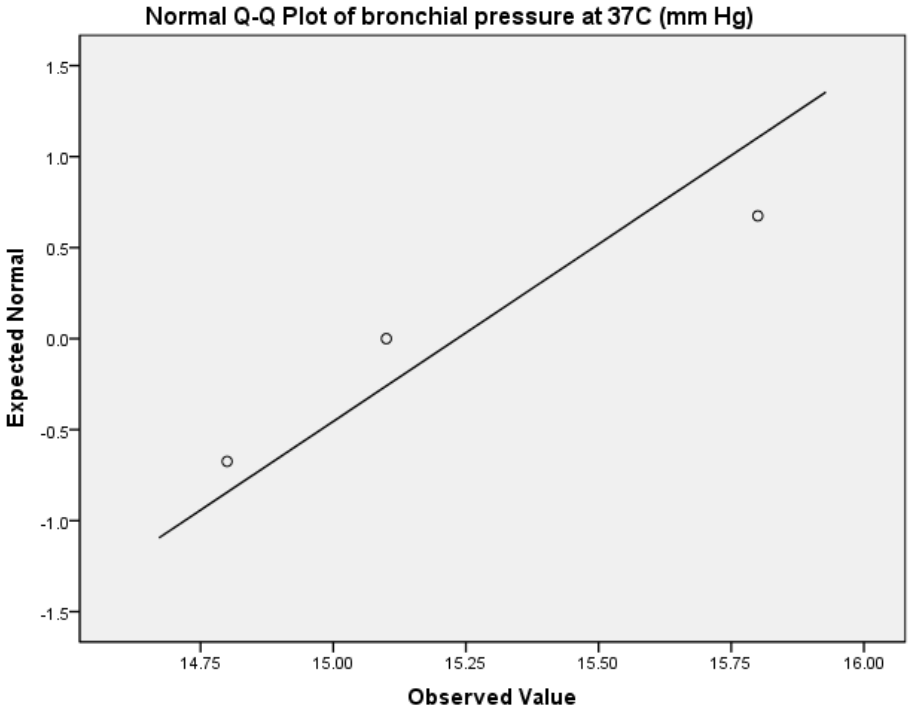
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
bronchial pressure at 37C (mm Hg)	.269	3	.	.949	3	.567

a. Lilliefors Significance Correction

bronchial pressure at 37C (mm Hg)





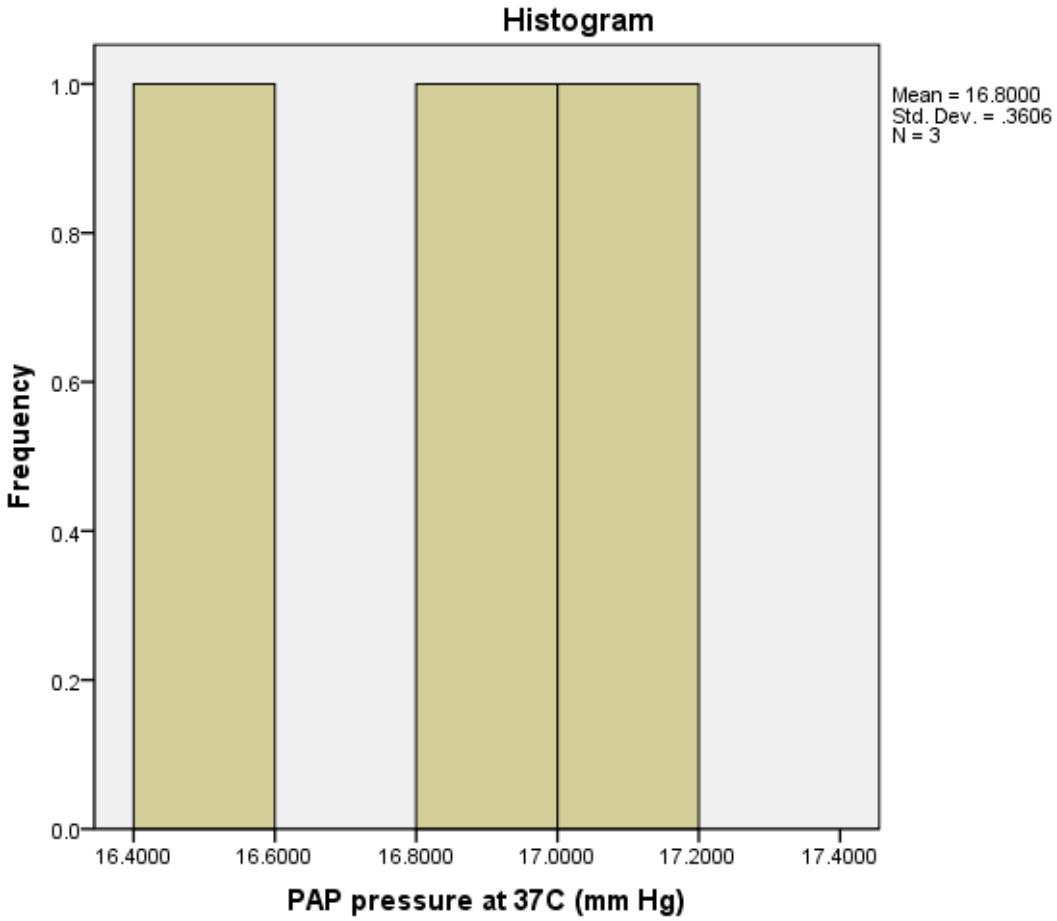
(3) Bronchial pressures exposed to hydrogen sulphide

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PAP pressure at 37C (mm Hg)	.276	3	.	.942	3	.537

a. Lilliefors Significance Correction

PAP pressure at 37C (mm Hg)



Appendix II : Statistical analysis of data

Section A: Oxygen Experiments

(1) H-R in pulmonary arteries maintained in hyperoxia

Within-Subjects Factors

Measure: tension

oxygen	Dependent Variable
1	Restingtensiongrams95
2	tensiongramstohypoxiaround1
3	tensiontohyperoxiaround1grams
4	tensiontohypoxiaround2grams
5	tensiontohyperoxiaround2grams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
oxygen	Pillai's Trace	.984	106.911[b]	4.000	7.000	.000	427.643	1.000
	Wilks' Lambda	.016	106.911[b]	4.000	7.000	.000	427.643	1.000
	Hotelling's Trace	61.092	106.911[b]	4.000	7.000	.000	427.643	1.000
	Roy's Largest Root	61.092	106.911[b]	4.000	7.000	.000	427.643	1.000

a. Design: Intercept

Within Subjects Design: oxygen

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) oxygen	(J) oxygen	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.361*	.032	.000	.290	.432
	3	.150*	.026	.000	.092	.208
	4	.466*	.025	.000	.411	.521
	5	.245*	.033	.000	.171	.319
2	1	-.361*	.032	.000	-.432	-.290
	3	-.211*	.019	.000	-.254	-.169
	4	.105*	.035	.014	.027	.183
	5	-.117*	.045	.028	-.218	-.015
3	1	-.150*	.026	.000	-.208	-.092
	2	.211*	.019	.000	.169	.254
	4	.316*	.028	.000	.254	.378
	5	.095*	.035	.021	.017	.172
4	1	-.466*	.025	.000	-.521	-.411
	2	-.105*	.035	.014	-.183	-.027
	3	-.316*	.028	.000	-.378	-.254
	5	-.221*	.019	.000	-.264	-.179
5	1	-.245*	.033	.000	-.319	-.171
	2	.117*	.045	.028	.015	.218
	3	-.095*	.035	.021	-.172	-.017
	4	.221*	.019	.000	.179	.264

(2) H-R in arteries maintained in normoxia

Within-Subjects Factors

Measure: tension

normoxia	Dependent Variable
1	Restingtensiongramsin21O2
2	tensiontohypoxiagrams
3	tensiontohyperoxiagrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
normoxia	Pillai's Trace	.891	16.298[b]	2.000	4.000	.012	32.597	.918
	Wilks' Lambda	.109	16.298[b]	2.000	4.000	.012	32.597	.918
	Hotelling's Trace	8.149	16.298[b]	2.000	4.000	.012	32.597	.918
	Roy's Largest Root	8.149	16.298[b]	2.000	4.000	.012	32.597	.918

a. Design: Intercept

Within Subjects Design: normoxia

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) normoxia	(J) normoxia	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.048*	.016	.034	.005	.090
	3	-.174*	.051	.020	-.306	-.041
2	1	-.048*	.016	.034	-.090	-.005
	3	-.221*	.045	.004	-.338	-.105
3	1	.174*	.051	.020	.041	.306
	2	.221*	.045	.004	.105	.338

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(3) Effect of Nifedipine on hyperoxic tension in pulmonary artery rings

Within-Subjects Factors

Measure: tension

nifedipine	Dependent Variable
1	Restingtensiongrams_Nifedipine
2	hypoxictensiongrams
3	hyperoxictensiongrams
4	hypoxictension2ndgrams
5	hyperoxictensiontoNifedipinegrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
nifedipine	Pillai's Trace	.905	4.789[b]	4.000	2.000	.180	19.155	.255
	Wilks' Lambda	.095	4.789[b]	4.000	2.000	.180	19.155	.255
	Hotelling's Trace	9.577	4.789[b]	4.000	2.000	.180	19.155	.255
	Roy's Largest Root	9.577	4.789[b]	4.000	2.000	.180	19.155	.255

a. Design: Intercept

Within Subjects Design: nifedipine

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) nifedipine	(J) nifedipine	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.398*	.109	.015	.118	.677
	3	.126	.150	.441	-.260	.512
	4	.370*	.142	.048	.006	.734
	5	.372*	.141	.046	.009	.735
2	1	-.398*	.109	.015	-.677	-.118
	3	-.272*	.071	.012	-.455	-.089

	4	-.028	.042	.543	-.137	.081
	5	-.026	.042	.563	-.134	.082
	1	-.126	.150	.441	-.512	.260
	2	.272*	.071	.012	.089	.455
3	4	.244*	.050	.005	.116	.373
	5	.246*	.050	.005	.117	.375
	1	-.370*	.142	.048	-.734	-.006
	2	.028	.042	.543	-.081	.137
4	3	-.244*	.050	.005	-.373	-.116
	5	.002	.002	.363	-.003	.006
	1	-.372*	.141	.046	-.735	-.009
	2	.026	.042	.563	-.082	.134
5	3	-.246*	.050	.005	-.375	-.117
	4	-.002	.002	.363	-.006	.003

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(4) Effect of Dantrolene on hyperoxic tension in pulmonary artery rings

Within-Subjects Factors

Measure: tension

dantrolene	Dependent Variable
1	Restingtensiongrams_Dantrolene
2	hypoxictensionwithoutdantrolenegrams
3	hyperoxictensionwithoutdantrolenegrams
4	hypoxictensionwithdantrolenegrams
5	hyperoxictensionwithdantrolenegrams
6	hyperoxictensiongramswithdantrolene200

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
dantrolene	Pillai's Trace	.998	113.351[b]	5.000	1.000	.071	566.753	.519
	Wilks' Lambda	.002	113.351[b]	5.000	1.000	.071	566.753	.519
	Hotelling's Trace	566.753	113.351[b]	5.000	1.000	.071	566.753	.519
	Roy's Largest Root	566.753	113.351[b]	5.000	1.000	.071	566.753	.519

a. Design: Intercept

Within Subjects Design: dantrolene

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) dantrolene	(J) dantrolene	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	.274*	.036	.001	.183	.365
	3	.075	.038	.102	-.021	.172
1	4	.311*	.050	.002	.182	.440
	5	.185*	.046	.010	.067	.303
	6	.288*	.048	.002	.164	.411
	1	-.274*	.036	.001	-.365	-.183
	3	-.199*	.019	.000	-.247	-.150
2	4	.037	.018	.093	-.009	.083
	5	-.089*	.019	.005	-.137	-.041
	6	.013	.016	.442	-.028	.055
	1	-.075	.038	.102	-.172	.021
	2	.199*	.019	.000	.150	.247
3	4	.236*	.019	.000	.187	.284
	5	.110*	.012	.000	.078	.142
	6	.212*	.020	.000	.161	.264
	1	-.311*	.050	.002	-.440	-.182
	2	-.037	.018	.093	-.083	.009
4	3	-.236*	.019	.000	-.284	-.187
	5	-.126*	.008	.000	-.147	-.105
	6	-.023*	.005	.007	-.037	-.010
	1	-.185*	.046	.010	-.303	-.067
	2	.089*	.019	.005	.041	.137
5	3	-.110*	.012	.000	-.142	-.078
	4	.126*	.008	.000	.105	.147
	6	.102*	.010	.000	.078	.127
	1	-.288*	.048	.002	-.411	-.164
	2	-.013	.016	.442	-.055	.028
6	3	-.212*	.020	.000	-.264	-.161
	4	.023*	.005	.007	.010	.037
	5	-.102*	.010	.000	-.127	-.078

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(5) Effect of 2-APB on hyperoxic tension in pulmonary artery rings

Within-Subjects Factors

Measure: tension

a2pb	Dependent Variable
1	Restingtensiongrams_APB
2	hypoxictension
3	hyperoxictensiongrams_A
4	hypoxictensionwith2APBgrams
5	hyperoxictensionwith2APBgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
a2pb	Pillai's Trace	.976	41.164[b]	3.000	3.000	.006	123.493	.994
	Wilks' Lambda	.024	41.164[b]	3.000	3.000	.006	123.493	.994
	Hotelling's Trace	41.164	41.164[b]	3.000	3.000	.006	123.493	.994
	Roy's Largest Root	41.164	41.164[b]	3.000	3.000	.006	123.493	.994

a. Design: Intercept

Within Subjects Design: a2pb

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) a2pb	(J) a2pb	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.311*	.036	.000	.220	.403
	3	.071	.050	.209	-.056	.199
	4	.344*	.059	.002	.193	.495
	5	.344*	.059	.002	.193	.495
2	1	-.311*	.036	.000	-.403	-.220
	3	-.240*	.035	.001	-.330	-.150
	4	.033	.034	.386	-.056	.121
	5	.033	.034	.386	-.056	.121
3	1	-.071	.050	.209	-.199	.056
	2	.240*	.035	.001	.150	.330
	4	.273*	.019	.000	.222	.323
	5	.273*	.019	.000	.222	.323
4	1	-.344*	.059	.002	-.495	-.193
	2	-.033	.034	.386	-.121	.056
	3	-.273*	.019	.000	-.323	-.222
	5	.000	.000	.	.000	.000
5	1	-.344*	.059	.002	-.495	-.193
	2	-.033	.034	.386	-.121	.056
	3	-.273*	.019	.000	-.323	-.222
	4	.000	.000	.	.000	.000

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(6) Effect of L-NAME on hypoxic tension in pulmonary artery rings

Within-Subjects Factors

Measure: tension

LNAME	Dependent Variable
1	RestingTensiongrams_LNAME
2	hypoxictensionwithoutLNAMEgrams
3	hyperoxictensiongrams_B
4	hypoxictensionwithLNAMEgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
LNAME	Pillai's Trace	.978	44.740[b]	3.000	3.000	.005	134.220	.996
	Wilks' Lambda	.022	44.740[b]	3.000	3.000	.005	134.220	.996
	Hotelling's Trace	44.740	44.740[b]	3.000	3.000	.005	134.220	.996
	Roy's Largest Root	44.740	44.740[b]	3.000	3.000	.005	134.220	.996

a. Design: Intercept

Within Subjects Design: LNAME

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) LNAME	(J) LNAME	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.388*	.099	.011	.134	.642
	3	.131	.060	.081	-.023	.285
	4	.518*	.106	.005	.245	.790
2	1	-.388*	.099	.011	-.642	-.134
	3	-.257*	.046	.002	-.374	-.140
	4	.129	.055	.064	-.011	.270
3	1	-.131	.060	.081	-.285	.023
	2	.257*	.046	.002	.140	.374
	4	.387*	.052	.001	.252	.521
4	1	-.518*	.106	.005	-.790	-.245
	2	-.129	.055	.064	-.270	.011
	3	-.387*	.052	.001	-.521	-.252

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(7) Effect of DPI on H-R in pulmonary artery rings

Within-Subjects Factors

Measure: tension

DPI	Dependent Variable
1	Restingtensionin 21grams_DPI
2	hypoxictensiongr ams_A
3	hyperoxictension withoutDPIgrams
4	restingtensionin2 1grams2
5	hypoxictensionwit hDPIgrams
6	hyperoxictension withDPIgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
DPI	Pillai's Trace	.999	257.350[b]	5.000	1.000	.047	1286.750	.710
	Wilks' Lambda	.001	257.350[b]	5.000	1.000	.047	1286.750	.710
	Hotelling's Trace	1286.750	257.350[b]	5.000	1.000	.047	1286.750	.710
	Roy's Largest Root	1286.750	257.350[b]	5.000	1.000	.047	1286.750	.710

a. Design: Intercept

Within Subjects Design: DPI

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) DPI	(J) DPI	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	.053*	.003	.000	.045	.061
	3	-.162*	.020	.000	-.212	-.112
1	4	-.033	.071	.661	-.217	.150
	5	-.002	.072	.978	-.186	.182
	6	-.032	.064	.640	-.198	.134
	1	-.053*	.003	.000	-.061	-.045
	3	-.215*	.020	.000	-.266	-.164
2	4	-.087	.070	.271	-.266	.093
	5	-.055	.070	.465	-.235	.125
	6	-.085	.063	.236	-.248	.078
	1	.162*	.020	.000	.112	.212
	2	.215*	.020	.000	.164	.266
3	4	.129	.084	.187	-.088	.345
	5	.160	.084	.116	-.057	.376
	6	.130	.077	.154	-.069	.328
	1	.033	.071	.661	-.150	.217
	2	.087	.070	.271	-.093	.266
4	3	-.129	.084	.187	-.345	.088
	5	.031*	.001	.000	.029	.033
	6	.001	.015	.937	-.037	.039
	1	.002	.072	.978	-.182	.186
	2	.055	.070	.465	-.125	.235
5	3	-.160	.084	.116	-.376	.057
	4	-.031*	.001	.000	-.033	-.029
	6	-.030	.014	.091	-.067	.007
	1	.032	.064	.640	-.134	.198
	2	.085	.063	.236	-.078	.248
6	3	-.130	.077	.154	-.328	.069
	4	-.001	.015	.937	-.039	.037
	5	.030	.014	.091	-.007	.067

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(8) Effect of temperature on H-R in pulmonary artery rings

Within-Subjects Factors

Measure: tension

temp	Dependent Variable
1	restingtensiongramsat_temp
2	hypoxictensiongrams_B
3	hyperoxictensiongrams_C
4	restingtension17Cgrams
5	hypoxictensionat17Cgrams
6	hyperoxictensionat17Cgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
temp	Pillai's Trace	.987	75.543[b]	3.000	3.000	.003	226.630	1.000
	Wilks' Lambda	.013	75.543[b]	3.000	3.000	.003	226.630	1.000
	Hotelling's Trace	75.543	75.543[b]	3.000	3.000	.003	226.630	1.000
	Roy's Largest Root	75.543	75.543[b]	3.000	3.000	.003	226.630	1.000

a. Design: Intercept

Within Subjects Design: temp

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	.346*	.046	.001	.228	.464
	3	.110	.050	.078	-.018	.238
1	4	.602*	.045	.000	.485	.718
	5	.602*	.045	.000	.485	.718
	6	.602*	.045	.000	.485	.718
	1	-.346*	.046	.001	-.464	-.228
	3	-.236*	.046	.004	-.355	-.117
2	4	.255*	.062	.009	.095	.416
	5	.255*	.062	.009	.095	.416
	6	.255*	.062	.009	.095	.416
	1	-.110	.050	.078	-.238	.018
	2	.236*	.046	.004	.117	.355
3	4	.491*	.086	.002	.271	.712
	5	.491*	.086	.002	.271	.712
	6	.491*	.086	.002	.271	.712
	1	-.602*	.045	.000	-.718	-.485
	2	-.255*	.062	.009	-.416	-.095
4	3	-.491*	.086	.002	-.712	-.271
	5	.000	.000	.	.000	.000
	6	.000	.000	.	.000	.000
	1	-.602*	.045	.000	-.718	-.485
	2	-.255*	.062	.009	-.416	-.095
5	3	-.491*	.086	.002	-.712	-.271
	4	.000	.000	.	.000	.000
	6	.000	.000	.	.000	.000
	1	-.602*	.045	.000	-.718	-.485
	2	-.255*	.062	.009	-.416	-.095
6	3	-.491*	.086	.002	-.712	-.271
	4	.000	.000	.	.000	.000
	5	.000	.000	.	.000	.000

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(9) Effect of H-R on pulmonary artery pressures in isolated lungs

Within-Subjects Factors

Measure: pressures

PAP	Dependent Variable
1	PAPat37Cinnormoxiammhg
2	PAPat37Cinhyperoxiammhg
3	PAPat37Cinhypoxiammhg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
PAP	Pillai's Trace	.271	.186[b]	2.000	1.000	.854	.371	.055
	Wilks' Lambda	.729	.186[b]	2.000	1.000	.854	.371	.055
	Hotelling's Trace	.371	.186[b]	2.000	1.000	.854	.371	.055
	Roy's Largest Root	.371	.186[b]	2.000	1.000	.854	.371	.055

a. Design: Intercept

Within Subjects Design: PAP

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: pressures

(I) PAP	(J) PAP	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	.167	.233	.549	-.837	1.171
	3	.133	.176	.529	-.626	.892
2	1	-.167	.233	.549	-1.171	.837
	3	-.033	.219	.893	-.974	.907

3	1	-.133	.176	.529	-.892	.626
	2	.033	.219	.893	-.907	.974

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Section B: Temperature experiments

(1) Effect of hypothermia on Endothelin-1 constriction of pulmonary arteries

Within-Subjects Factors

Measure: tension

endothelin	Dependent Variable
1	restingtensiongramsat17CET1
2	Tensionto100pMET1at17Cgrams
3	Tensionto500pMET1at17Cgrams
4	Tensionto1nMET1at17Cgrams
5	restingtensiongrams37C
6	Tensionto100ET1at37Cgrams
7	Tensionto500ET1at37Cgrams
8	Tensionto1nMET1at37Cgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
endothelin	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: endothelin

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) endothelin	(J) endothelin	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.018*	.001	.001	-.021	-.014
	3	-.062*	.006	.002	-.079	-.044
	4	-.084*	.023	.033	-.156	-.012
	5	-.448*	.031	.001	-.547	-.350
	6	-.826*	.024	.000	-.903	-.749
	7	-.953*	.026	.000	-1.036	-.870
	8	-1.797*	.112	.001	-2.155	-1.439
2	1	.018*	.001	.001	.014	.021
	3	-.044*	.005	.003	-.060	-.028
	4	-.066	.022	.055	-.136	.003
	5	-.430*	.032	.001	-.532	-.329
	6	-.808*	.024	.000	-.886	-.731
	7	-.935*	.025	.000	-1.015	-.855
	8	-1.779*	.112	.001	-2.135	-1.423
3	1	.062*	.006	.002	.044	.079
	2	.044*	.005	.003	.028	.060
	4	-.023	.018	.296	-.080	.035
	5	-.387*	.036	.002	-.502	-.272

	6	-.765*	.020	.000	-.827	-.702
	7	-.891*	.024	.000	-.967	-.816
	8	-1.735*	.108	.001	-2.079	-1.391
	1	.084*	.023	.033	.012	.156
	2	.066	.022	.055	-.003	.136
	3	.023	.018	.296	-.035	.080
4	5	-.364*	.053	.006	-.534	-.194
	6	-.742*	.022	.000	-.811	-.673
	7	-.869*	.013	.000	-.909	-.828
	8	-1.713*	.091	.000	-2.004	-1.422
	1	.448*	.031	.001	.350	.547
	2	.430*	.032	.001	.329	.532
	3	.387*	.036	.002	.272	.502
5	4	.364*	.053	.006	.194	.534
	6	-.378*	.050	.005	-.538	-.218
	7	-.505*	.054	.003	-.678	-.331
	8	-1.349*	.142	.002	-1.799	-.898
	1	.826*	.024	.000	.749	.903
	2	.808*	.024	.000	.731	.886
	3	.765*	.020	.000	.702	.827
6	4	.742*	.022	.000	.673	.811
	5	.378*	.050	.005	.218	.538
	7	-.127*	.034	.033	-.234	-.019
	8	-.971*	.099	.002	-1.285	-.657
	1	.953*	.026	.000	.870	1.036
	2	.935*	.025	.000	.855	1.015
	3	.891*	.024	.000	.816	.967
7	4	.869*	.013	.000	.828	.909
	5	.505*	.054	.003	.331	.678
	6	.127*	.034	.033	.019	.234
	8	-.844*	.094	.003	-1.142	-.546
	1	1.797*	.112	.001	1.439	2.155
	2	1.779*	.112	.001	1.423	2.135
	3	1.735*	.108	.001	1.391	2.079
8	4	1.713*	.091	.000	1.422	2.004
	5	1.349*	.142	.002	.898	1.799
	6	.971*	.099	.002	.657	1.285
	7	.844*	.094	.003	.546	1.142

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(2) Effect of hypothermia on Potassium Chloride constriction of pulmonary arteries

Within-Subjects Factors

Measure: tension

KCLtemp	Dependent Variable
1	restingtensiongrams17CKCL
2	tensionto300KClgrams
3	tensionto3KClat17Cgrams
4	tensionto30mMKCl
5	restingtensiongrams37C_A
6	tensionto300KClat37Cgrams
7	tensionto3KClat37Cgrams
8	tensionto30mMKClat37Cgrams_A

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
KCLtemp	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: KCLtemp

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) KCLtemp	(J) KCLtemp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	-.034*	.005	.007	-.050	-.017
	3	-.081*	.013	.009	-.123	-.038
	4	-.129*	.034	.031	-.236	-.022
1	5	-.510*	.042	.001	-.644	-.376
	6	-.660*	.070	.003	-.883	-.436
	7	-.909*	.133	.006	-1.333	-.484
	8	-1.538*	.190	.004	-2.144	-.933
	1	.034*	.005	.007	.017	.050
	3	-.047*	.009	.013	-.075	-.019
	4	-.095*	.028	.044	-.185	-.005
2	5	-.476*	.039	.001	-.599	-.353
	6	-.626*	.066	.002	-.835	-.416
	7	-.875*	.130	.007	-1.287	-.463
	8	-1.504*	.186	.004	-2.095	-.914
	1	.081*	.013	.009	.038	.123
	2	.047*	.009	.013	.019	.075
	4	-.048	.022	.111	-.117	.020
3	5	-.429*	.032	.001	-.532	-.327
	6	-.579*	.057	.002	-.762	-.396
	7	-.828*	.122	.007	-1.216	-.441
	8	-1.458*	.178	.004	-2.024	-.892
	1	.129*	.034	.031	.022	.236
	2	.095*	.028	.044	.005	.185
	3	.048	.022	.111	-.020	.117
4	5	-.381*	.029	.001	-.473	-.289
	6	-.531*	.045	.001	-.673	-.389
	7	-.780*	.109	.006	-1.127	-.433
	8	-1.409*	.158	.003	-1.913	-.905
	1	.510*	.042	.001	.376	.644
	2	.476*	.039	.001	.353	.599
	3	.429*	.032	.001	.327	.532
5	4	.381*	.029	.001	.289	.473
	6	-.150*	.034	.022	-.258	-.041
	7	-.399*	.092	.023	-.692	-.106
	8	-1.028*	.154	.007	-1.519	-.538
6	1	.660*	.070	.003	.436	.883

	2	.626*	.066	.002	.416	.835
	3	.579*	.057	.002	.396	.762
	4	.531*	.045	.001	.389	.673
	5	.150*	.034	.022	.041	.258
	7	-.249*	.067	.033	-.461	-.037
	8	-.879*	.126	.006	-1.280	-.477
	1	.909*	.133	.006	.484	1.333
	2	.875*	.130	.007	.463	1.287
	3	.828*	.122	.007	.441	1.216
7	4	.780*	.109	.006	.433	1.127
	5	.399*	.092	.023	.106	.692
	6	.249*	.067	.033	.037	.461
	8	-.629*	.085	.005	-.901	-.357
	1	1.538*	.190	.004	.933	2.144
	2	1.504*	.186	.004	.914	2.095
	3	1.458*	.178	.004	.892	2.024
8	4	1.409*	.158	.003	.905	1.913
	5	1.028*	.154	.007	.538	1.519
	6	.879*	.126	.006	.477	1.280
	7	.629*	.085	.005	.357	.901

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(3) Effect of hypothermia on Sodium Nitroprusside dilation of pulmonary arteries

Within-Subjects Factors

Measure: tension

snptemp	Dependent Variable
1	restingtensiongramsat17C_SNP
2	tensionto1SNPat17Cgrams
3	tensionto10SNPat17Cgrams
4	tensionto100µMSNPat17Cgrams
5	restingtensiongrams37C_B
6	tensionto1SNPat37Cgrams
7	tensionto10SNPat37Cgrams
8	tensionto100µMSNPat17Cgrams2

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
snptemp	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: snptemp

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) snptemp	(J) snptemp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	.000	.000	.	.000	.000
	3	-.027*	.004	.006	-.039	-.015
	4	-.052*	.012	.023	-.091	-.014
1	5	-.457*	.043	.002	-.593	-.322
	6	-.621*	.072	.003	-.848	-.393
	7	-1.042*	.140	.005	-1.488	-.595
	8	-1.319*	.128	.002	-1.725	-.913
	1	.000	.000	.	.000	.000
	3	-.027*	.004	.006	-.039	-.015
	4	-.052*	.012	.023	-.091	-.014
2	5	-.457*	.043	.002	-.593	-.322
	6	-.621*	.072	.003	-.848	-.393
	7	-1.042*	.140	.005	-1.488	-.595
	8	-1.319*	.128	.002	-1.725	-.913
	1	.027*	.004	.006	.015	.039
	2	.027*	.004	.006	.015	.039
	4	-.025	.009	.064	-.053	.003
3	5	-.430*	.039	.002	-.554	-.306
	6	-.593*	.068	.003	-.810	-.376
	7	-1.014*	.137	.005	-1.449	-.580
	8	-1.292*	.124	.002	-1.686	-.898
	1	.052*	.012	.023	.014	.091
	2	.052*	.012	.023	.014	.091
	3	.025	.009	.064	-.003	.053
4	5	-.405*	.034	.001	-.513	-.297
	6	-.568*	.062	.003	-.765	-.371
	7	-.989*	.130	.005	-1.404	-.574
	8	-1.267*	.117	.002	-1.641	-.893
	1	.457*	.043	.002	.322	.593
	2	.457*	.043	.002	.322	.593
	3	.430*	.039	.002	.306	.554
5	4	.405*	.034	.001	.297	.513
	6	-.163*	.033	.015	-.267	-.060
	7	-.584*	.108	.012	-.929	-.240
	8	-.862*	.093	.003	-1.157	-.567

	1	.621*	.072	.003	.393	.848
	2	.621*	.072	.003	.393	.848
	3	.593*	.068	.003	.376	.810
6	4	.568*	.062	.003	.371	.765
	5	.163*	.033	.015	.060	.267
	7	-.421*	.104	.027	-.752	-.090
	8	-.699*	.086	.004	-.972	-.425
	1	1.042*	.140	.005	.595	1.488
	2	1.042*	.140	.005	.595	1.488
	3	1.014*	.137	.005	.580	1.449
7	4	.989*	.130	.005	.574	1.404
	5	.584*	.108	.012	.240	.929
	6	.421*	.104	.027	.090	.752
	8	-.278*	.019	.001	-.339	-.217
	1	1.319*	.128	.002	.913	1.725
	2	1.319*	.128	.002	.913	1.725
	3	1.292*	.124	.002	.898	1.686
8	4	1.267*	.117	.002	.893	1.641
	5	.862*	.093	.003	.567	1.157
	6	.699*	.086	.004	.425	.972
	7	.278*	.019	.001	.217	.339

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(4) Effect of temperature on the resting tension of pulmonary arteries

Within-Subjects Factors

Measure: tension

resting	Dependent Variable
1	restingtensionat17Cgrams_rewarming
2	restingtensionat37Cgrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
resting	Pillai's Trace	.977	463.865[b]	1.000	11.000	.000	463.865	1.000
	Wilks' Lambda	.023	463.865[b]	1.000	11.000	.000	463.865	1.000
	Hotelling's Trace	42.170	463.865[b]	1.000	11.000	.000	463.865	1.000
	Roy's Largest Root	42.170	463.865[b]	1.000	11.000	.000	463.865	1.000

a. Design: Intercept

Within Subjects Design: resting

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) resting	(J) resting	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.472*	.022	.000	-.520	-.424
2	1	.472*	.022	.000	.424	.520

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(5) Effect of no hypothermia on Endothelin-1 constriction of pulmonary arteries

Within-Subjects Factors

Measure: tension

endothelin	Dependent Variable
1	Restingtensionat 37Cgrams_ET1
2	Responseto1nME T1tensiongrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
endothelin	Pillai's Trace	.986	216.176[b]	1.000	3.000	.001	216.176	1.000
	Wilks' Lambda	.014	216.176[b]	1.000	3.000	.001	216.176	1.000
	Hotelling's Trace	72.059	216.176[b]	1.000	3.000	.001	216.176	1.000
	Roy's Largest Root	72.059	216.176[b]	1.000	3.000	.001	216.176	1.000

a. Design: Intercept

Within Subjects Design: endothelin

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) endothelin	(J) endothelin	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.875*	.060	.001	-1.064	-.686
2	1	.875*	.060	.001	.686	1.064

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(6) Effect of no hypothermia on Potassium Chloride constriction of pulmonary arteries

Within-Subjects Factors

Measure: tension

kcl37	Dependent Variable
1	Restingtensiongrams_KCl
2	Responseto30mMKCltensiongrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
kcl37	Pillai's Trace	.939	46.091[b]	1.000	3.000	.007	46.091	.989
	Wilks' Lambda	.061	46.091[b]	1.000	3.000	.007	46.091	.989
	Hotelling's Trace	15.364	46.091[b]	1.000	3.000	.007	46.091	.989
	Roy's Largest Root	15.364	46.091[b]	1.000	3.000	.007	46.091	.989

a. Design: Intercept

Within Subjects Design: kcl37

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) kcl37	(J) kcl37	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.975*	.144	.007	-1.432	-.518
2	1	.975*	.144	.007	.518	1.432

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(7) Effect of no hypothermia on Sodium Nitroprusside dilation of pulmonary arteries

Within-Subjects Factors

Measure: tension

snp37	Dependent Variable
1	Restingtensiongrams_SNP
2	Responseto100µMSNPtensiongrams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
snp37	Pillai's Trace	.926	37.385[b]	1.000	3.000	.009	37.385	.971
	Wilks' Lambda	.074	37.385[b]	1.000	3.000	.009	37.385	.971
	Hotelling's Trace	12.462	37.385[b]	1.000	3.000	.009	37.385	.971
	Roy's Largest Root	12.462	37.385[b]	1.000	3.000	.009	37.385	.971

a. Design: Intercept

Within Subjects Design: snp37

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) snp37	(J) snp37	Mean Difference (I-	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]

		J)			Lower Bound	Upper Bound
1	2	.900*	.147	.009	.432	1.368
2	1	-.900*	.147	.009	-1.368	-.432

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(8) Effect of rewarming on PAP

Within-Subjects Factors

Measure: pap

paprewarming	Dependent Variable
1	restingPAPmmHg
2	at37C_rewarming PAPpressureat17 CmmHg
3	PAPrewarmedtoa t37CmmHg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
paprewarming	Pillai's Trace	1.000	1380.774[b]	2.000	1.000	.019	2761.548	.991
	Wilks' Lambda	.000	1380.774[b]	2.000	1.000	.019	2761.548	.991
	Hotelling's Trace	2761.548	1380.774[b]	2.000	1.000	.019	2761.548	.991
	Roy's Largest Root	2761.548	1380.774[b]	2.000	1.000	.019	2761.548	.991

a. Design: Intercept

Within Subjects Design: paprewarming

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: pap

(I) paprewarming	(J) paprewarming	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	3.509*	.263	.006	2.379	4.639
	3	-.400	.379	.401	-2.029	1.229
2	1	-3.509*	.263	.006	-4.639	-2.379
	3	-3.909*	.639	.026	-6.658	-1.159
3	1	.400	.379	.401	-1.229	2.029
	2	3.909*	.639	.026	1.159	6.658

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(9) Effect of rewarming on bronchial pressures

Within-Subjects Factors

Measure: bronchialpressures

bronchialrewarming	Dependent Variable
1	restingPAPmmHg
	at37C_rewarming
2	BronchialPressur
	eat17CmmHg
3	BronchialPressur
	erewarmedto37C
	mmHg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
bronchialrewarming	Pillai's Trace	.997	160.584[b]	2.000	1.000	.056	321.167	.631
	Wilks' Lambda	.003	160.584[b]	2.000	1.000	.056	321.167	.631
	Hotelling's Trace	321.167	160.584[b]	2.000	1.000	.056	321.167	.631
	Roy's Largest Root	321.167	160.584[b]	2.000	1.000	.056	321.167	.631

a. Design: Intercept

Within Subjects Design: bronchialrewarming

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: bronchialpressures

(I) bronchialrewarming	(J) bronchialrewarming	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	2.102*	.325	.023	.703	3.501
	3	.267	.133	.184	-.307	.840
2	1	-2.102*	.325	.023	-3.501	-.703
	3	-1.835*	.195	.011	-2.676	-.995
3	1	-.267	.133	.184	-.840	.307
	2	1.835*	.195	.011	.995	2.676

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(10)Effect of KCl at 17 C and 37C on PAP

Within-Subjects Factors

Measure: PAP

kcl17and37	Dependent Variable
1	PAPat17CmmHg
2	PAPat17C30mM KClmmHg
3	PAPat37CmmHg
4	PAPat37CKClmm Hg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
kcl17and37	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: kcl17and37

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: PAP

(I) kcl17and37	(J) kcl17and37	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.000	.000	.	.000	.000
	3	-3.909*	.639	.026	-6.658	-1.159
	4	-11.127*	1.231	.012	-16.424	-5.830
2	1	.000	.000	.	.000	.000
	3	-3.909*	.639	.026	-6.658	-1.159
	4	-11.127*	1.231	.012	-16.424	-5.830
3	1	3.909*	.639	.026	1.159	6.658
	2	3.909*	.639	.026	1.159	6.658
	4	-7.218*	1.435	.037	-13.394	-1.042
4	1	11.127*	1.231	.012	5.830	16.424
	2	11.127*	1.231	.012	5.830	16.424
	3	7.218*	1.435	.037	1.042	13.394

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(11)Effect of KCl at 37C only on PAP

Within-Subjects Factors

Measure: PAP

kcl17and37	Dependent Variable
1	PAPat17CmmHg
2	PAPat17C30mMKClmmHg
3	PAPat37CmmHg
4	PAPat37CKClmmHg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
kcl17and37	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: kcl17and37

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: PAP

(I) kcl17and37	(J) kcl17and37	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.000	.000	.	.000	.000
	3	-3.909*	.639	.026	-6.658	-1.159
	4	-11.127*	1.231	.012	-16.424	-5.830
2	1	.000	.000	.	.000	.000
	3	-3.909*	.639	.026	-6.658	-1.159
	4	-11.127*	1.231	.012	-16.424	-5.830
3	1	3.909*	.639	.026	1.159	6.658
	2	3.909*	.639	.026	1.159	6.658
	4	-7.218*	1.435	.037	-13.394	-1.042
4	1	11.127*	1.231	.012	5.830	16.424
	2	11.127*	1.231	.012	5.830	16.424
	3	7.218*	1.435	.037	1.042	13.394

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Within-Subjects Factors

Measure: PAP

kcl37only	Dependent Variable
1	PAPat37CmmHg_A_control
2	PAPat37CKClmmHg_A

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
kcl37only	Pillai's Trace	.949	37.405[b]	1.000	2.000	.026	37.405	.847
	Wilks' Lambda	.051	37.405[b]	1.000	2.000	.026	37.405	.847
	Hotelling's Trace	18.702	37.405[b]	1.000	2.000	.026	37.405	.847
	Roy's Largest Root	18.702	37.405[b]	1.000	2.000	.026	37.405	.847

a. Design: Intercept

Within Subjects Design: kcl37only

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: PAP

(I) kcl37only	(J) kcl37only	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-6.890*	1.127	.026	-11.737	-2.043
2	1	6.890*	1.127	.026	2.043	11.737

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Section C: Hydrogen Sulphide Experiments

(1) Effect of Hydrogen Sulphide on pulmonary artery tension

Within-Subjects Factors

Measure: tension

h2srings	Dependent Variable
1	Restingtensionat 37Cgrams_rings
2	@50µMtensiongr ams
3	@100µMtensiong rams
4	@250µMtensiong rams
5	@500µMtensiong rams

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
h2srings	Pillai's Trace	.976	80.975[b]	4.000	8.000	.000	323.899	1.000
	Wilks' Lambda	.024	80.975[b]	4.000	8.000	.000	323.899	1.000
	Hotelling's Trace	40.487	80.975[b]	4.000	8.000	.000	323.899	1.000
	Roy's Largest Root	40.487	80.975[b]	4.000	8.000	.000	323.899	1.000

a. Design: Intercept

Within Subjects Design: h2srings

b. Exact statistic

c. Computed using alpha = .05

Pairwise Comparisons

Measure: tension

(I) h2srings	(J) h2srings	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.043*	.003	.000	.036	.051
	3	.508*	.036	.000	.429	.588
	4	.689*	.046	.000	.589	.790
	5	.887*	.049	.000	.779	.995
2	1	-.043*	.003	.000	-.051	-.036
	3	.465*	.035	.000	.389	.541
	4	.646*	.044	.000	.550	.743
	5	.844*	.049	.000	.737	.951
3	1	-.508*	.036	.000	-.588	-.429
	2	-.465*	.035	.000	-.541	-.389
	4	.181*	.033	.000	.109	.253
	5	.379*	.035	.000	.302	.456
4	1	-.689*	.046	.000	-.790	-.589
	2	-.646*	.044	.000	-.743	-.550
	3	-.181*	.033	.000	-.253	-.109
	5	.198*	.036	.000	.120	.277
5	1	-.887*	.049	.000	-.995	-.779
	2	-.844*	.049	.000	-.951	-.737
	3	-.379*	.035	.000	-.456	-.302
	4	-.198*	.036	.000	-.277	-.120

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(2) Effect of Hydrogen Sulphide on pulmonary artery pressures

Within-Subjects Factors

Measure: PAP

H2SPAP	Dependent Variable
1	PAPpressureat37CmmHg
2	@50µMPAPmmHg
3	@200µMPAPmmHg
4	@500µMPAPmmHg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
H2SPAP	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: H2SPAP

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: PAP

(I) H2SPAP	(J) H2SPAP	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	.534	.147	.069	-.101	1.168
1	3	2.061*	.244	.014	1.009	3.112
	4	2.984*	.340	.013	1.522	4.445
	1	-.534	.147	.069	-1.168	.101
2	3	1.527*	.156	.010	.857	2.197
	4	2.450*	.421	.028	.637	4.263
	1	-2.061*	.244	.014	-3.112	-1.009
3	2	-1.527*	.156	.010	-2.197	-.857
	4	.923	.369	.129	-.663	2.509
	1	-2.984*	.340	.013	-4.445	-1.522
4	2	-2.450*	.421	.028	-4.263	-.637
	3	-.923	.369	.129	-2.509	.663

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

(3) Effect of Hydrogen Sulphide on bronchial pressures

Within-Subjects Factors

Measure: bronchial

H2Sbronchial	Dependent Variable
1	bronchialpressure at37CmmHg
2	@50µMbronchial pressuremmHg
3	@200µMbronchia lpressuremmHg
4	@500µMbronchia lpressuremmHg

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[c]
H2Sbronchial	Pillai's Trace	.[b]	.	.	.	.	.	.
	Wilks' Lambda	.[b]	.	.	.	.	.	.
	Hotelling's Trace	.[b]	.	.	.	.	.	.
	Roy's Largest Root	.[b]	.	.	.	.	.	.

a. Design: Intercept

Within Subjects Design: H2Sbronchial

b. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

c. Computed using alpha = .05

Pairwise Comparisons

Measure: bronchial

(I) H2Sbronchial	(J) H2Sbronchial	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
	2	1.390	.748	.204	-1.828	4.608
1	3	1.722*	.256	.021	.620	2.824
	4	2.293*	.411	.031	.525	4.061
	1	-1.390	.748	.204	-4.608	1.828
2	3	.332	.581	.625	-2.167	2.830
	4	.902	.643	.295	-1.862	3.667
	1	-1.722*	.256	.021	-2.824	-.620
3	2	-.332	.581	.625	-2.830	2.167
	4	.571	.185	.091	-.223	1.365
	1	-2.293*	.411	.031	-4.061	-.525
4	2	-.902	.643	.295	-3.667	1.862
	3	-.571	.185	.091	-1.365	.223

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

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