

**Peripheral chemoreflex regulation of sympathetic outflow and venous function in human hypertension**

Study Protocol

5th May 2020

Version 2

**Table of Contents**

[1. ADMINISTRATIVE INFORMATION 4](#_Toc40271510)

[1.1 Full/long study title 4](#_Toc40271511)

[1.2 Short study title 4](#_Toc40271512)

[1.3 Research reference numbers 4](#_Toc40271513)

[1.4 Key study contacts 5](#_Toc40271514)

[1.5 Study summary 7](#_Toc40271515)

[1.6 Funding and support in kind 8](#_Toc40271516)

[2 INTRODUCTION 9](#_Toc40271517)

[2.1 Background 9](#_Toc40271518)

[2.2 Aims and objectives 11](#_Toc40271519)

[2.2.1 Central Hypothesis 11](#_Toc40271520)

[2.2.2 Primary Objective 11](#_Toc40271521)

[2.2.3 Specific hypothesis 1 11](#_Toc40271522)

[2.2.4 Specific hypothesis 2 11](#_Toc40271523)

[2.3 Outcome measures 11](#_Toc40271524)

[2.3.1 Primary Outcome 12](#_Toc40271525)

[2.3.2 Secondary Outcome 12](#_Toc40271526)

[2.4 Expected value of results 12](#_Toc40271527)

[3 STUDY DESIGN 12](#_Toc40271528)

[3.1 Study setting 12](#_Toc40271529)

[3.2 Participants 12](#_Toc40271530)

[3.2.1 Inclusion criteria 12](#_Toc40271531)

[3.2.2 Exclusion criteria 12](#_Toc40271532)

[3.2.3 Recruitment 13](#_Toc40271533)

[3.2.4 Consent 13](#_Toc40271534)

[3.3 Study visits 14](#_Toc40271535)

[3.3.1 Familiarisation / screening visit 14](#_Toc40271536)

[3.3.2 Study 1: Experimental protocol 14](#_Toc40271537)

[3.3.3 Study 2: Experimental protocol 15](#_Toc40271538)

[3.3.4 Measured variables and assessment procedures 16](#_Toc40271539)

[3.4 Data analysis 17](#_Toc40271540)

[3.4.1 Data analysis 17](#_Toc40271541)

[3.4.2 Statistical analyses 18](#_Toc40271542)

[3.4.3 Size of sample 18](#_Toc40271543)

[4 RESPONSIVENESS TO MĀORI 18](#_Toc40271544)

[4.1 Potential benefit to Māori 18](#_Toc40271545)

[4.2 Management of cultural issues 19](#_Toc40271546)

[4.3 Study consultation process 19](#_Toc40271547)

[5 ETHICAL AND REGULATORY CONSIDERATIONS 20](#_Toc40271548)

[5.1 Assessment and management of risk 20](#_Toc40271549)

[5.2 Data protection and patient confidentiality 21](#_Toc40271550)

[5.3 Research ethics committee review and reports 22](#_Toc40271551)

[5.4 Peer review 22](#_Toc40271552)

[5.5 Protocol compliance 22](#_Toc40271553)

[5.6 Amendments 22](#_Toc40271554)

[6. FINANCE 22](#_Toc40271555)

[6.1 Funding 22](#_Toc40271556)

[6.2 Reimbursement to participants 22](#_Toc40271557)

[7 DISSEMINIATION POLICY 23](#_Toc40271558)

[7.1 Dissemination policy 23](#_Toc40271559)

[7.2 Authorship eligibility guidelines 23](#_Toc40271560)

[7.2.1. Definition of authorship 23](#_Toc40271561)

[7.2.2. Procedure 23](#_Toc40271562)

[8 REFERENCES 25](#_Toc40271563)

[9 APPENDIX 31](#_Toc40271564)

[9.1 APPENDIX 1: Amendment History 31](#_Toc40271565)

# 1. ADMINISTRATIVE INFORMATION

## 1.1 Full/long study title

Peripheral chemoreflex regulation of sympathetic outflow and venous function in human hypertension

## 1.2 Short study title

Peripheral chemoreflex and the veins in hypertension

## 1.3 Research reference numbers

|  |  |
| --- | --- |
| HDEC Number: | 20/NTA/29 |
| ADHB-RCC Number: | TBC |
| UTN Number: | U1111-1248-3119 |

## 1.4 Key study contacts

|  |  |
| --- | --- |
| Chief Investigator | Dr James P Fisher (BSc, PhD)Associate Professor of PhysiologyFaculty of Medical and Health Sciences, Department of Physiology,University of Auckland | 85 Park Road, Grafton, Auckland 1023 Tel: 09-373 7599 | Ext 86320Email: jp.fisher@auckland.ac.nz  |
| Primary contact | Dr James P. Fisher(details as above) |
| Sponsor | The University of AucklandOffice of Research Strategy and IntegrityEmail: humanethics@auckland.ac.nz |
| Funder(s) | University of Auckland |
| Key Protocol Contributors / Co-investigators | Prof. Julian F.R. Paton (BSc, PhD)Professor of Translational PhysiologyFaculty of Medical and Health Sciences, Department of Physiology,University of Auckland | 85 Park Road, Grafton, Auckland 1023 Email: j.paton@auckland.ac.nzDr. Fiona McBrydeSenior Lecturer in PhysiologyFaculty of Medical and Health Sciences, Department of Physiology,University of Auckland | 85 Park Road, Grafton, Auckland 1023 Email: f.mcbryde@auckland.ac.nzDr. Mathew Dawes (BSc, MBBS, MRCP, PhD)Senior Lecturer/SMODepartment of MedicineUniversity of AucklandFaculty of Medical and Health Sciences, Level 12, Auckland Hospital Support Building,Park Avenue, Grafton,Auckland 1142Email: m.dawes@auckland.ac.nz Dr. Ana Luiza Carrari SayeghPostdoctoral Research FellowFaculty of Medical and Health Sciences, Department of Physiology, University of Auckland, 85 Park Road, Grafton, Auckland 1023. |

## 1.5 Study summary

|  |  |
| --- | --- |
| Study Design | Single centre, case control (study 1), placebo controlled (study 2), interventional study. |
| Study Participants | Patients with essential hypertension and healthy individuals |
| Planned Size of Sample  | Up to 80 total  |
| Planned Study Period | 2 years |
| Research Question/Aim(s) | High blood pressure (hypertension) affects one in three people, and remarkably ~50% of treated patients remain hypertensive. We need to better understand the mechanisms regulating blood pressure in hypertensive patients if new therapies are to be devised. Current treatments target the heart and arterial resistance, but the ‘forgotten’ venous circulation has been largely neglected. We hypothesize that high levels of sympathetic activity in hypertension result in profound constriction of the veins, and that ameliorating this may be an effective way to help control arterial pressure. Our extensive work in hypertensive animal models indicates that the carotid bodies develop tonicity and heightened reflex sensitivity due to excessive ATP bioavailability acting via purinergic (P2) receptors, which drive sympathetic outflows. We wish to determine whether a non-selective P2 receptor blocker reduces peripheral chemoreflex sensitivity, sympathetic activity, venous tone and blood pressure in humans with hypertension. This project will provide novel mechanistic insights into carotid chemoreflex regulation and the neural control of the venous circulation. Translating insights from our animal models of hypertension into the human setting will lay the groundwork for future studies, potentially leading to a paradigm shift in the future treatment of hypertension. |
| Key MeSH Terms | Chemoreceptor cells, hypertension, sympathetic nervous system |

## 1.6 Funding and support in kind

|  |
| --- |
| **FUNDER(S)**(Names and contact details of ALL organisations providing funding and/or support in kind for this study) |
| **University of Auckland** – Faculty Research Development Fund. |

# 2 INTRODUCTION

## 2.1 Background

**Hypertension – the ‘silent killer’**

It is estimated that one-third of the adult population in New Zealand suffers from hypertension1, while worldwide ~1 billion people are currently affected2. Hypertension is a powerful independent predisposing factor for coronary heart disease, stroke and heart failure3, and thus has a considerable socio-economic cost4. However, despite treatment only ~50% of patients have adequate BP control, both in New Zealand5 and elsewhere6. There have been no new safe anti-hypertensive medications for >20 years7 and *a better understanding of the mechanisms regulating BP in hypertensive patients is required if new therapies are to be devised*.

There is accumulating evidence that sympathetic activation plays a causative role in both triggering and sustaining the hypertensive state8-13, and also elicits end-organ damage14, 15. As such, the sympathetic nervous system constitutes an important therapeutic target in hypertension, but has proven largely resistant to current frontline anti-hypertensive medications13. Thus, to develop effective countermeasures it is essential to identify the mechanisms driving the increase in sympathetic nerve activity (SNA), and the associated end-organ responses, in hypertension16. This may have wider implications for other diseases in which sympathetic overdrive exists (e.g. heart failure, sleep apnea, rheumatoid arthritis)14, 17.

***The chemoreflex as a novel therapeutic target in hypertension***

Peripheral chemoreceptors are located in the carotid and aortic bodies and are responsive to decreases in the partial pressure of oxygen (PO2), and increases in both partial pressure of carbon dioxide (PCO2) and H+ ions18. Activation of the carotid chemoreceptors cause marked reflex increases in ventilatory drive and SNA directed towards the heart, kidneys and *peripheral vasculature*18. The carotid bodies have recently emerged as a potential therapeutic target for treating hypertension19, 20 and heart failure21-23. Recent studies have demonstrated the development of aberrant discharge and raised peripheral chemoreflex sensitivity in hypertension20, 24, 25, that drives a long-term increase in SNA resulting in a chronically sustained neurally-mediated hypertension20, 25-27. Unilateral carotid body resection was shown to lower BP in ~60% patients with drug resistant hypertension19. However, removing the carotid bodies is an irreversible procedure with a risk of unwanted side-effects, therefore it is more desirable (and safer) to pharmacologically assuage carotid body aberrant signaling.

**The ‘forgotten’ circulation**

Cardiovascular research and therapeutic interventions have focused overwhelmingly on the heart and arterial circulation, while *investigation and understanding of the venous circulation has been neglected28-30*. The sympathetic nervous system has an underappreciated influence on venous tone31 and while the arterial system has been well studied32, there is a near-complete dearth of data on the autonomic control of venoconstrictor tone in hypertension. The veins store two-thirds of the blood volume, but rather than being a passive reservoir, they contain vascular smooth muscle, adrenergic receptors (especially on mesenteric veins) and are richly innervated by the sympathetic nerves which constantly adjust venous compliance33. Our pilot data in rats shows that sympathetic outflow to venous beds is distinct from that to the arterial circulation, suggesting differential control (Fig 1). Thus small changes in venous tone can very rapidly mobilize venous blood into the arterial circulation34, 35, with the potential to greatly impact BP and the pathogenesis of hypertension33.

 **Fig 1**. Venous sympathetic activity (V; ∫=integrated). We show that whilst venous sympathetic activity is under baroreceptor (‘Baro stim’) control, but displays an activity pattern distinct from lumbar and thoracic activity.

**Hypertension, the peripheral chemoreflex and venous function**

**Fig 2**. Venous tone (mean circulatory filling pressure) can be reduced in spontaneously hypertensive rat by carotid body denervation.

Reduced venous compliance and capacitance are reported in several hypertensive animal models*36-40*. Increased venoconstrictor tone in the spontaneously hypertensive rat is abolished by ganglionic blockade36, suggesting that it is of a sympathetic origin. Furthermore, an augmented adrenergic responsiveness has been observed in an experimental rabbit model of hypertension41. Data from our research team has shown that venous tone can be reduced in hypertensive rats by denervating the carotid chemoreceptors (see Fig 2).

In studies of human hypertension decreased venous compliance has been reported42, 43, although not universally44, 45. These discrepant findings may relate to the method used, i.e., isolated veins vs. whole-limbs, with the latter approach being inferior as vasoconstriction may mimic venoconstriction by reducing pressure in small post-capillary venules46. High-resolution ultrasound methods now provide a reproducible method for non-invasively assessing human vein function47, 48. However, no studies have used this approach in human hypertension to assess either venous compliance or responsiveness to sympathetic activation. Moreover, *the tonic influence of the carotid chemoreflex on the venous system has not been assessed in human health or disease*. Given the known increase in carotid chemoreflex tonicity/sensitivity with hypertension20, 24, 25 that drives SNA and raises BP20, 25-27, it is important to understand whether venous tone is elevated in human hypertension due to carotid chemoreflex-mediated sympathoexcitation and whether ameliorating carotid body activity might cause venodilatation to lower arterial blood pressure.

**Purinergic signaling mechanisms**

Although there are a multitude of transmitters and receptors within the carotid body49, adenosine tri-phosphate (ATP) is a major player50. ATP is released from glomus type I cells in response to hypoxia. ATP stimulates P2 receptors that are either ligand-gated ion channels (P2X) or G protein-coupled P2Y receptors51. Importantly, the local blockade of P2X3 receptors within the carotid body has been demonstrated to lower SNA, whereas their systemic blockade reduced blood pressure in hypertensive rats, suggesting that this tonicity was driving aberrant sympathetic vasoconstriction (of arterioles)20. Pyridoxine hydrochloride (i.e. vitamin B6) can be safely given to humans and is converted into pyridoxal-5-phosphate52, 53, a non-selective P2 receptor blocker54. Interestingly, vitamin B6 deficiency has been associated with hypertension in epidemiological studies55, 56, but the mechanism is unclear. Vitamin B6 deficient rats exhibit sympathoexcitation and hypertension, while dietary vitamin B6 supplementation reduces SNA and BP in rats57, 58.

To date, only a single study has considered the effect of pyridoxine supplementation (5 mg·kg-1·day-1, 4 weeks) on office BP in hypertensive individuals and noted a hypotensive effect59. However, no placebo control group was included, thus non-pyridoxine specific effects cannot be ruled out. The mechanism for this reduction remains enigmatic and is a focus of this application as we wish to assess whether it is acting on the carotid body.

## 2.2 Aims and objectives

### 2.2.1 Central Hypothesis

In human hypertension, peripheral chemoreflex sensitivity is heightened due to dysfunctional purinergic signaling, causing sympathetic overdrive and augmented venoconstrictor tone.

### 2.2.2 Primary Objective

Determine the influence of the peripheral chemoreflex on venous function, and reveal whether pharmacological attenuation of purinergic signaling reduces peripheral chemoreflex sensitivity, sympathetic nerve activity and venoconstrictor tone, in human hypertension.

### *2.2.3 Specific hypothesis 1*

In human hypertension, venous compilance is decreased and that the responses to chemoreflex activation (hypoxia) and inactivation (dopamine) are augmented, revealing hyper-sensitivity and generation of aberrant tonicity.

### *2.2.4 Specific hypothesis 2*

Pyridoxine reduces peripheral chemoreflex sensitivity, SNA, venoconstriction and BP in humans with hypertension.

## 2.3 Outcome measures

### 2.3.1 Primary Outcome

* Venous compliance

### 2.3.2 Secondary Outcome

* Muscle SNA and ventilatory responses to hypoxia (i.e., peripheral chemoreflex sensitivity)

## 2.4 Expected value of results

An understanding of new mechanisms by which BP is controlled in hypertension is critically important as the problem is worsening and it triggers subsequent cardiovascular diseases. Controlling BP would save and prolong lives and reduce hospitalizations. In this project, our world-class team will investigate a largely neglected mechanism of BP control and an entirely novel therapeutic target. Those presently available are poorly tolerated. We believe that pharmacological suppression of purinergic signaling will lower peripheral chemoreflex sensitivity, SNA, venoconstrictor tone and thus BP in hypertension. This is highly relevant as there is a P2X2/3 receptor antagonist in clinical trials for chronic cough60, which may become available for testing in humans with hypertension. A positive outcome from these studies would help entice a future study using this selective antagonist in Auckland, which could provide new BP treatment and the first treatment to curb excessive sympathetic activity in hypertensive patients.

# 3 STUDY DESIGN

## 3.1 Study setting

Data will be collected in the Human Cardiorespiratory Physiology Laboratory, Department of Respiratory Physiology, Auckland City Hospital, Auckland DHB.

## 3.2 Participants

### 3.2.1 Inclusion criteria

* Patients with essential hypertension (At least Stage 2 hypertension; untreated office SBP ≥140 mmHg or DBP ≥90 mmHg);
* Normotensive controls (office SBP < 120 mmHg and DBP < 80 mmHg);
* Men and women;
* Aged over 18 years;
* Body mass index <35 kg/m2.

### 3.2.2 Exclusion criteria

* Significant arrhythmias (e.g., atrial fibrillation, previous VT / significant ventricular ectopy)
* Hemodynamically significant valvular heart disease (e.g., stenosis, mechanical valve replacement)
* Severe left ventricular systolic dysfunction
* Recent acute coronary syndrome (<12 months) (e.g., MI, angioplasty, unstable angina)
* Previous coronary artery bypass surgery
* Secondary causes of hypertension (e.g., phaeochromocytoma)
* Recent stroke/TIA (<12 months)
* Current smoker
* Body mass index <18 kg/m2.
* Current pregnancy
* Current user of recreational drugs
* Current abuser of alcohol
* Inability to fully or appropriately provide consent (e.g., language issue, reading capability)
* Underlying medical conditions, which in the opinion of the Investigator place the participant at unacceptably high risk for participating in the study.

Chronic and systemic illness including:

* Severe respiratory disease (e.g., chronic obstructive pulmonary disease);
* Severe, uncontrolled type II diabetes;
* Current treatment for cancer or complete remission <5 years
* Connective tissue or inflammatory disease
* Neurological / psychiatric disease (e.g., peripheral neuropathy, dementia, Parkisnon’s, epilepsy)
* Infection or pyrexial illness
* Uncontrolled thyroid disorders
* Renal impairment (e.g., eGFR <60)
* Liver disease

### 3.2.3 Recruitment

* Patients with essential hypertension will be recruited from specialist tertiary hypertensive clinics and GP surgeries across the region. We have formed a clinical hypertension research network spanning private practice and 5 district health boards around the Auckland region;
* Normotensive controls will be recruited from the local communities using posters, email announcements and social media sites;
* These recruitment materials will direct interested potential participants to seek telephone or email contact with the research team;
* A member of the research team will then answer any questions and forward a Participant Information Sheet. A follow up call or email exchange will be initiated to ascertain if the potential participant meets the inclusion criteria (outlined in the Participant Information Sheet) and agrees to participate, an appointment for the experimental study visit will be booked.

### 3.2.4 Consent

A member of the research team will obtain written informed consent using the Consent Form. A Participant Information Sheet will be given to each potential participant prior to recruitment and the risks and benefits of participating in the study will be clearly explained (as described above). The participant will be given ample time to read the information sheet and the opportunity to enquire about details on the study. All questions or concerns should be answered to the satisfaction of the participant. It will be explained that they are free to decline to take part and will be informed about their right to withdraw from the study at any time. If the individual agrees to take part in the study they will be asked to sign and date the Consent Form that will also be signed and dated by the Investigator. Throughout the study the individual will have the opportunity to ask questions about the study and any new information that may be relevant to the participant’s willingness to continue participation in the study will be shared in a timely manner allowing them to opt out.

## 3.3 Study visits

To address the specific hypothesis advanced, two inter-related studies are proposed.

Study 1: *Is venous compliance reduced, and responses to chemoreflex activation [hypoxia] and inactivation [dopamine] augmented, in human hypertension?* Patients with hypertension (n=20) and normotensive controls (n=20) will attend the laboratory for one familiarisation / screening visit and one experimental visit. As described in detail below, at the experimental visit venous compliance will be assessed47, 48 under control, hypoxic (chemoreflex activation; PETO2 ~45 mmHg) and low-dose dopamine (chemoreflex inactivation; 2 µg·min-1·kg-1)61 conditions. Low-dose dopamine will be prepared by Auckland Pharmacy Clinical Trials Department, Auckland District Hospital.

Study 2: *Does pyridoxine administration reduce peripheral chemoreflex sensitivity, SNA, and BP, and enhance venous compliance, in human hypertension?* Hypertensive participants (n=40) will attend the laboratory for one familiarisation / screening visit and two experimental sessions spaced by 4 weeks. As described in detail below, at the experimental visits chemoreflex activation and venous compliance will be assessed47, 48. Between experimental sessions participants will be 1:1 randomized to take either an oral pyridoxine supplement (5 mg·kg-1·day-1)59 or placebo pill for 4 weeks, according to double-blind design. Supplement preparation, blinding and dispensing will be undertaken by Auckland Pharmacy Clinical Trials Department, Auckland District Hospital.

### 3.3.1 Familiarisation / screening visit

An initial familiarisation visit will be conducted (~45 min) where an investigator will explain the nature of the procedures, answer any questions and obtained written informed consent (as described above). Subsequently, anthropometric (height, weight, hip-to-waist ratio), demographic, clinical information will be collected (Health Screening Questionnaire attached).

### 3.3.2 Study 1: Experimental protocol

Patients with hypertension and normotensive controls will attend the laboratory for one experimental visit (~2 hours). Experimental sessions will be conducted at the Human Cardiorespiratory Physiology Laboratory, Level 7, Respiratory Physiology Department, Auckland City Hospital, Auckland DHB. This visit will be scheduled ~2-7 days after the initial familiarisation / screening visit, as appropriate. However, premenopausal women will be studied during the first five days of their menstrual cycle (early follicular phase) or during the placebo/no-hormone phase of oral contraceptive use, as appropriate. Patients will withhold their morning medications until the end of the experimental session in order to limit confounding effects. The procedures will be reviewed again with the participant and remaining questions answered. Prior to the study visit participants will have been provided with a participant information leaflet, advising them of the following pre-study stipulations:

* No food intake for 2 hours prior to the study.
* No caffeine (e.g., coffee, coke, red bull) for 12 hours before the study.
* No alcohol on the day before the study and the day of the study.
* No exercise after 8:00pm the evening before the study and no exercise on the day of the study.
* No ‘over the counter’ (e.g. paracetamol) or cardioactive medications (beta-blocker, ACE inhibitor, angiotensin receptor blockers, calcium antagonists, diuretics (e.g., spironolactone), alpha blockers) on the morning of the study. Patients are advised to bring these medications [if needed to,] to the study appointment so they can take the usual medication immediately after the research tests (by late morning).

Participants will be asked to lie in a semi−recumbent position on a bed and an intravenous catheter positioned into a superficial arm/hand vein. Following this participants will be instrumented for monitoring of heart rate, BP, respiration and monitoring of the diameter of a vein in the lower leg (i.e., popliteal or long saphenous vein). These measurement procedures are described in detail below (Section 3.3.4). Vein compliance will be assessed using Doppler ultrasound and a standard proximal cuff deflation protocol47, 48. This vein compliance procedure will be repeated under three conditions: control (saline), chemoreflex activation (hypoxia; PETO2 45 mmHg) and during chemoreflex inactivation (low-dose dopamine; 2 µg·min-1·kg-1)61. Conditions will be isocapnic, separated by ~20 min and randomized. The vein compliance assessment protocol is described in detail below, along with the chemoreflex inactivation protocols. Low-dose dopamine will be prepared by Auckland Pharmacy Clinical Trials Department, Auckland District Hospital.

### 3.3.3 Study 2: Experimental protocol

Hypertensive participants will attend the laboratory for two identical experimental sessions spaced by 4 weeks (~2 hours each). Experimental sessions will be conducted at the Human Cardiorespiratory Physiology Laboratory, Level 7, Respiratory Physiology Department, Auckland City Hospital, Auckland DHB. The first experimental visit will be scheduled ~2-7 days after the initial familiarisation / screening visit, as appropriate. Patients will withhold their morning medications until the end of the experimental session in order to limit confounding effects. The procedures will be reviewed again with the participant and remaining questions answered. Prior to the experimental visit participants will be advised to follow the same pre-study stipulations as stated for Study 1.

As for study 1, participants will be asked to lie in a semi−recumbent position on a bed, an intravenous catheter positioned into a superficial arm/hand vein, and a venous blood sample obtained (~20 ml) for plasma biochemistry, catecholamines, and pyridoxine (plus metabolites; e.g., pyridoxal-5-phosphate) measures. Following this participants will be instrumented for monitoring of heart rate, BP and respiration. Lower limb vein compliance will be assessed using Doppler ultrasound and a standard proximal cuff deflation protocol47, 48, as described below. A muscle sympathetic nerve activity recording will then be obtained. After a baseline of 20 min, a hypoxic stimulus will be delivered while isocapnia is maintained for 5 min (i.e., PETO2 45 mmHg and PETCO2 ~40 mmHg) chemoreflex sensitivity will be calculated as the peak increase in ventilation and muscle SNA62.

At the end of the experimental session, patients will be provided with a 24-hr BP monitor. They will be asked to wear this over the following 24-hr period and return the device using the postage materials provided.

Between experimental sessions participants will be 1:1 randomized to take either an oral pyridoxine supplement (5 mg·kg-1·day-1)59 or placebo pill for 4 weeks, according to a double-blind design. Supplement preparation, blinding and dispensing will be undertaken by Auckland Pharmacy Clinical Trials Department, Auckland District Hospital.

### 3.3.4 Measured variables and assessment procedures

The investigators have extensive experience of conducting human physiological investigations and using the experimental techniques and procedures outlined below (e.g. 17, 19, 63-66).

Breathing monitoring: Participants will wear a mouthpiece/noseclip or oronasal mask (Hans Rudolph) attached to a heated pneumotachograph connected to a differential pressure transducer to measure bidirectional airflow (Hans Rudolph). Ports will allow the measurement of the percentage of CO2 and O2 (ADInstruments) in the expirate. Respiratory chest movements will be monitored with a belt placed around the thorax.

Cardiovascular monitoring: Brachial BP will be measured with a clinically validated automated sphygmomanometer (Omron), using a cuff wrapped around the upper arm. Beat-to-beat BP will be measured using finger photoplethysmography, using a small lightweight cuff wrapped around the finger. This will also be used to estimate stroke volume (SV) using a pulse contour method (Finometer PRO, Finapres Medical Systems)67. Heart rate will be measured using standard electrocardiogram involving the placement of several sticky patch electrodes on the collarbones and chest (standard 3 lead ECG). Cardiac output will be calculated as SV x HR. Arterial oxygen saturation will be monitored continuously and non-invasively with finger pulse oximetry (ADInstruments).

Sympathetic nerve monitoring: A small, sterile wire (unipolar tungsten microelectrodes, tip measuring 1-5 ųm) will be inserted near the fibular head on the outside of the leg, to obtain a multiunit recording of postganglionic muscle SNA from the peroneal nerve, using published techniques68, 69. When the nerve is located participants may notice a transient tingling sensation. The participants will be asked to keep their leg as still as possible during the study. This ‘microneurography’ technique has been used over the last ~50 years to measure nerve activity. It is a safe procedure with minimal side effects. Established approaches will be used to process and verify an acceptable recording of muscle SNA68, 69. Multiunit sympathetic bursts will first be identified by visual inspection and further evaluated using an interactive computer analysis program70. The raw neurogram (filtered and amplified) will be analysed offline for the detection of single unit sympathetic action potentials using action potential shape recognition software71. We have successfully performed the microneurography technique without adverse events in studies of healthy subjects64, 72, 73 type II diabetics74, chronic heart failure75, rheumatoid arthritis17 and hypertension76, 77.

Venous compliance assessment: Lower limb vein diameter will determined using high-resolution Doppler ultrasound (uSmart 3300, Terason) coupled with automated edge detection and wall tracking of arterial images72, 78. This ultrasound examination is similar to the scan done for pregnant women, but a large vein is examined. This is a simple and safe procedure and involves a probe being put on the participants’ skin over the region of interest with the help of a ‘water jelly’. Commercially available software will be used for vessel edge detection and tracking of high-resolution arterial ultrasound images.

A resting longitudinal ultrasound image of a lower limb vein will be obtained and pressure-diameter relationships will be generated using an established method47, 48. Specifically, a venous collecting cuff (~24 cm width) is placed around the thigh proximal to the knee and connected to a rapid cuff inflator / external air source (D.E. Hokanson). The venous collecting cuff is then inflated to 60 mmHg for 8 min. Ultrasound images will be recorded for 10 s at the beginning of each minute during the 8-min occlusion period. After this 8-min period, collecting cuff pressure will be manually reduced at a rate of 1 mmHg/s from 60 to 0 mmHg. Longitudinal ultrasound images will be measured continuously during the deflation period. Venous compliance calculations are described below (3.4.1. Data Analysis).

Low-dose dopamine (Chemoreflex inactivation): Systemic administration of low-dose dopamine (i.e. 2 μg kg−1 min−1) is an established method of acutely reducing the responsiveness of the carotid chemoreceptors in humans79-81. The authors have experience of using this approach to investigate the influence of human hypertension66 and high altitude hypoxia64 on carotid chemoreflex sensitivity. Dopamine infusion will be commenced a minimum of 10 min before any data collection.

Blood samples: Blood samples will be processed (e.g., centrifuged) and plasma/ serum stored at the Auckland Regional Tissue Bank (<https://www.aucklandregionaltissuebank.ac.nz/>). Samples will be assessed for standard clinical biochemistry (e.g., glucose, cholesterol), catecholamines and pyridoxine plus metabolites (e.g., pyridoxal-5-phosphate).

## 3.4 Data analysis

### 3.4.1 Data analysis

Body mass index (BMI) will be expressed as the ratio between participant’s weight and the square of their height. Analogue signals for ECG, BP, sympathetic nerve activity and respiration will be sampled simultaneously, and beat-to-beat or breath-by-breath time series derived, before averages are calculated for each experimental period (ADInstruments). Venous compliance will be calculated from the longitudinal ultrasound images measured continuously during the cuff deflation protocol. Vein cross-sectional area (CSA) will be calculated from the diameter measurements (with the equation ϖ \* radius2) and plotted against cuff pressure. A quadratic regression will be applied to model the data (SigmaPlot, Chicago, IL). The regression parameters 1 and 2 will be used as an estimate of venous compliance 48. The following equations were used: 1) popliteal vein CSA = 0 + 1 (cuff pressure) + 2 (cuff pressure)2, and 2) popliteal vein compliance = 1 + 2 2 (cuff pressure). The regression parameter 0 will be used as an estimate of the capacitance response for each trial.

### 3.4.2 Statistical analyses

Anthropometric (e.g., BMI) and demographic (e.g., age) information gathered at primary screening will be quantified using basic statistics (mean, SD, Median, IQR) and graphical presentations (boxplots, histograms, scatter plots). Likewise levels of primary and secondary outcomes will be similarly reported. Normal distribution will be evaluated using Shapiro-Wilk tests. Comparisons of normally distributed physiological variables between groups will be made using a t-test, and non-normally distributed data evaluated using a Mann–Whitney U test. In the event of potential confounding differences in baseline characteristics, analysis of covariance (ANCOVA) will be employed. Statistical analysis will performed using Sigmaplot 13.0 (Systat Software Inc, London, UK). Significance will be set at *p* < 0.05. Normally distributed data will be presented as mean (SD) while non-normally distributed data will be presented as median [interquartile range].

### 3.4.3 Size of sample

For *Study 1*, based on Young et al48 who reported a popliteal vein compliance of -0.045±0.0185 mm2·100mm-2·mmHg-1 (mean±SD) in normotensives, a sample size of n=20 per group (i.e., n=20 hypertension, n=20 normotension) would yield a minimal detectable difference of 0.0168 mm2·100mm-2·mmHg-1 at 80% power and 5% alpha.

For *Study 2*, based on Aybak et al59, n=20 per group (n=40 total) would yield a detectable difference of 9 mmHg in mean BP following treatment (unpaired design) assuming a SD of 10 mmHg, at 80% power and 5% alpha.

# 4 RESPONSIVENESS TO MĀORI

## 4.1 Potential benefit to Māori

Hypertension (high BP) is of pandemic proportion. Despite being a modifiable risk factor for the prevention of stroke, cardiac arrest, heart failure and renal failure, at least one in three people have hypertension in New Zealand and the prevalence is greater in Māori. Given that there has been a complete dearth of new anti-hypertensive medications over the last 15 years, a better understanding of the mechanisms regulating BP in hypertensive patients is required if new therapeutic approaches are to be devised. The present study will provide important new information regarding the involvement of central and peripheral chemoreflexes dysfunction in the genesis of high BP. Such information may lead to future therapeutic studies targeting central and peripheral chemoreflexes via pharmacological, lifestyle and device-based approaches.

## 4.2 Management of cultural issues

The researchers understand the importance of appropriate communication with Māori participants. We acknowledge that kanohi ki te kanohi is the most effective recruitment tool in Māori. When recruiting in studies such as this, research staff will always arrange an initial face to face meeting with the participants. Participants are also invited to bring whānau with them to this meeting. This meeting gives participants the opportunity to discuss the study and ask any questions they may have before deciding whether they would like to be involved. Participants will be given as much time as they need to consider participation in the study, including time to discuss with whānau. The protection of taonga of health and information are of primary importance in this study. The researchers recognise that knowledge belongs to the tangata whenua, and must not be removed or handed on without their express approval. Taonga is protected in this study through informed consent, confidentiality of patient information and approval by the Ethics Committee.

We understand that many Māori consider their blood to be tapu and that participation in this type of study requires careful consideration. When donating a biological sample, such as blood, it may be appropriate to discuss participation with whānau / family member. We have included an optional section on the consent form for a whānau / family member to sign to indicate their support. In the Participant Information Sheet we also provide the contact details for the administrator for He Kamaka Waiora (Māori Health Team) should additional guidance be required. Finally, in the Consent Form an option is provided whereby a karakia can be requested to accompany the destruction of any unused samples at the end of this study.

## 4.3 Study consultation process

The planning and conduct of the investigators research program has been critically informed by several sources in terms of Māori responsiveness. In the first instance this has been provided by the ‘Responsiveness to Māori (R2M) team' sitting within the Office of the Tumuaki, Faculty of Medical and Health Sciences, University of Auckland. The researchers have also received valuable guidance from Dr Helen Wihongi, Director - Māori Health Research, He Kamaka Waiora I Waitematā and Auckland DHB. In addition, during the process of receiving research approval for Auckland DHB, this project will have been supported by the Māori Research Review Committee.

The investigators are affiliated with the new Centre of Heart Research (Manaaki Mānawa) which is a strategic investment priority of the University of Auckland. At the core of the Centre’s governance structure is a Māori Advisory Committee which has broad representation across clinical and academic disciplines. The investigators will provide regular updates on the progress of their research to the Advisory Committee and draw on their expertise and networks to identify effective ways to share our findings with Māori communities including iwi and particularly those whānau affected by hypertension.

# 5 ETHICAL AND REGULATORY CONSIDERATIONS

## 5.1 Assessment and management of risk

The protocols and physiological measures used in this project are well established and used in research laboratories around the world. The research team are experienced with all the procedures employed (e.g. 17, 19, 63-66). Therefore the risks associated with the study are low. To further minimise the risk associated with this investigation, studies will be undertaken in a dedicated clinical research laboratory at Auckland City Hospital, with medically trained personnel in close proximity, along with crash cart facilities, in the unlikely event that they are required.

Exposure to hypoxia (for peripheral chemoreflex activation) can cause mild hyperventilation and feelings of shortness of breath, light-headedness and discomfort. To minimise the risk of this, participants will be carefully familiarized (e.g., trained to rapidly remove mouthpiece if necessary), key physiological parameters (BP, arterial oxygen saturation) will be continuously monitored, and a trial will be terminated if arterial oxygen saturation <80%. In the unlikely event that any unusual sensations do not dissipate upon removal of the mouthpiece/mask and return to normal breathing, oxygen can be administered.

The patients will receive an intravenous low-dose dopamine infusion at a rate of 2 μg/kg/min. This will require siting of an intravenous cannula by a research nurse or doctor. The dose of dopamine used in the study is lower than that often used in a clinical setting (routinely used in the coronary care units at a starting dose of 2.5 μg/kg/min), and we would expect dopamine at this dose to be well tolerated. Low-dose dopamine has been used to inhibit chemoreceptor function in other physiological studies without adverse effect 79, 81-91. Unlike high doses which stimulate alpha and beta adrenergic receptors in the cardiovascular system, lower doses are typically thought to target dopaminergic (D2) receptors which mediate carotid body activity 81. The documented side effects of dopamine according to the New Zealand Formulary are: nausea, vomiting, chest pain, palpitation, tachycardia, vasoconstriction, hypotension, dyspnoea, headache; *less commonly* bradycardia, hypertension, gangrene, mydriasis; *rarely* fatal ventricular arrhythmias (at high doses only). A medical doctor will be present at all times during the dopamine infusion and recovery period. The half-life of dopamine is <2 minutes in the body, so side effects can be rapidly limited by cessation of the infusion.

Vitamin B6 is a water-soluble vitamin that is present in many foods. It is converted into pyridoxal-5-phosphate52, 53, an active coenzyme forms of vitamin B6, which we are interested in because it has been identified as a non-selective P2 receptor blocker54. In this study patients will receive oral pyridoxine supplement (5 mg·kg-1·day-1 for 4 weeks), which we consider to be low risk. Severe and progressive sensory neuropathy has been identified with chronic administration of higher oral pyridoxine doses in the range of 1–6 g per day for 12–40 months 92. In contrast, studies of patients treated with vitamin B6 (average dose of 200 mg/day) for up to 5 years found no evidence of this effect 93. The New Zealand Formulary also states that nausea, headache and somnolence have been reported. The Patient Information Sheet will advise participants to inform the study team if they experience these issues and found them concerning, or in the unlikely event that they experience numbness or tingling of the fingers or toes.

The potential risks of microneurography include a brief tiredness in the leg after the procedure. There is also a potential risk of temporary pins­and­needles sensation or increased sensitivity to touch in the leg following microneurography, which subsides after 24­48 hours. However, these side effects are infrequent, affecting approximately 1 in 10 participants. Since 1979, nerve recordings have been performed on thousands of subjects throughout the world (Europe, United States, South America, and Australasia) without complications. The Chief Investigator has previously performed nerve recordings in approximately 200 subjects without complications.

## 5.2 Data protection and patient confidentiality

The participants name (i.e., a direct identifier) will appear on the Consent Form. Participants will be coded with a Participant Information Number and this will be used to store the other study data in a deidentified format. Consent Forms will be maintained at the University of Auckland and/or Auckland DHB in a locked filing cabinet in a department with security-limited access, along with all paper records (e.g., health history forms). De-identified data in an electronic format will be kept on secure and password protected University of Auckland servers. This electronic data includes the physiological signals (e.g., ECG, BP, sympathetic nerve activity, respiration and blood vessel function). Once deidentified the risk of reidentification is extremely low. Access to paper and electronic records will be restricted to the researchers, along with the Sponsor, regulatory authorities and the Health and Disability Ethics Committee. Analysis will take place by the study team led by Dr James P Fisher (using deidentified data). Blood samples will be stored by Auckland Regional Biobank (<https://www.aucklandregionaltissuebank.ac.nz/>). Auckland Regional Biobank will store the tissue specifically for this project. They will coordinate the appropriate analyses of these samples as specified for this project, but will not store this material for future unspecified use (i.e., Pathway 3 will be taken). Samples will be stored in a deidentified manner and risk of reidentification is negligible. The results of this study will be reported in professional publications and meetings, but will not be published in a form that identifies individual participants. No health data will be transferred to individuals in another country.

In accordance with New Zealand law, study data (paper and electronic) will be securely stored for a minimum period of ten years after which they will be destroyed. Paper records will be maintained at the University of Auckland and/or Auckland DHB in a locked filing cabinet in a department with security-limited access. De-identified data in an electronic format will be kept on secure and password protected University of Auckland servers. Auckland Regional Biobank has robust procedures in place for the storage and destruction of data and samples (<https://www.aucklandregionaltissuebank.ac.nz/governance/>) .

## 5.3 Research ethics committee review and reports

Before the start of the study, approval will be sought from the Health and Disability Ethics Committee (HDEC) for the study protocol, informed consent forms and other relevant documents (e.g., advertisements). Substantial amendments that require review by HDEC will not be implemented until the HDEC grants a favourable opinion for the study. All correspondence with the HDEC will be retained. The Chief Investigator’s will produce the annual reports as required and notify the HDEC of the end of the study.

Authorisation for this study also be obtained by the Sponsor (University of Auckland), and research approval will also be obtained by the Auckland DHB Research Review Committee.

## 5.4 Peer review

This study has been extensively discussed and is supported by a multidisciplinary team who has a specific interest in this population. The quality of this research study has also been assessed and approved by several independent scientific reviewers during the process of receiving competitively awarded funding from the Auckland Medical Research Foundation. As such, the study has undergone high quality peer review that is independent, expert and proportionate in accordance with HDEC guidelines.

## 5.5 Protocol compliance

Accidental protocol deviations will be documented and reported to the Chief Investigator immediately. Frequently recurring protocol deviations will not be accepted and appropriate action taken.

## 5.6 Amendments

In the event that an amendment to the protocol is required an application to the HDEC will be submitted in accordance with the latest guidelines. The amendment history will be tracked in the Study Protocol appendix.

# 6. FINANCE

## 6.1 Funding

Funding for this study is provided by the University of Auckland. This will provide postdoctoral student funding to undertake the project and running costs. All the required scientific equipment is available.

## 6.2 Reimbursement to participants

Travel expenses and subsistence (to cover meal/beverages) will be provided to the participants in the form of a voucher ($50) for each experimental session undertaken.

# 7 DISSEMINIATION POLICY

## 7.1 Dissemination policy

The sponsor owns the data arising from the study and has responsibility for its dissemination. The study findings will be made freely available to the broader scientific community as soon as possible. An electronic copy of each paper that is accepted for publication in a peer-reviewed journal will be deposited within PubMed Central, within 6 months of publication. Participants that express an interest will be notified of the results of the study (e.g., by provision of the publication or bespoke presentation).

## 7.2 Authorship eligibility guidelines

### 7.2.1. Definition of authorship

An author is considered to be someone who has made substantive intellectual contribution to a study. Many journals consider it best practice that everyone who is listed as an author should have made a substantial, direct, intellectual contribution to the work. Honorary or guest authorship is not acceptable.

### 7.2.2. Procedure

 The baseline criteria for this research for both authorship and acknowledgments for peer reviewed publications and conference contributions is that:

**i.** Authors must meet all of the following criteria:

* substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data
* drafting the article or revising it critically for important intellectual content
* final approval of the version to be published

**ii.** No-one should be omitted from the authorship list if he/she meets the three criteria in ‘i’ above.

**iii.** Some journals allow authorship of multi-centre projects to be attributed to a group. However, all members of the group who are named as authors must still fully meet the above criteria for authorship in ‘i’ above.

**iv.** Other collaborators or members of the research group who may have contributed to some but not all of the criteria in ‘i’ above will be listed in the Acknowledgments (see vi below).

**v.** The individual authors will jointly make decisions about authorship before submitting the manuscript for publication. The lead author, corresponding author or the guarantor must be prepared to explain the presence and order of these individuals to the editor of a journal. Authorship and order of authorship (see 7 below) will be agreed in advance, in the early stages of the research.

**vi.** All contributors who do not meet the criteria for authorship will be listed in an Acknowledgments section. Examples of those who might be acknowledged include:

* persons who have contributed materially to the paper but whose contributions do not justify authorship. These may be listed under such headings as “participating investigators” and their function or contribution should be described - for example, “served as scientific advisors,” “critically reviewed the study proposal,” or “collected data/material”. Because readers may infer their endorsement of the data and conclusions, these persons must give written permission to be acknowledged
* a person who provided purely technical help, provided general comments on the manuscript or writing assistance, or a departmental chair who provided general support
* editors can ask corresponding authors to declare whether they had assistance with study design, data collection, data analysis, or manuscript preparation. Authors should therefore disclose in the Acknowledgements section the identity of any individuals who provided this assistance and any entities that supported the work in the published article
* financial support should also be acknowledged and, if appropriate, the grant identified
* material or logistical support, in particular giving recognition to support provided in developing countries, should always be acknowledged

**vii.** Order of authorship

* the authors shall decide the order of authorship together. Contributors should discuss authorship issues frankly at the start of the work for each anticipated publication and not wait to raise concerns at submission time
* authors shall specify in their manuscript a description of the contributions of each author and how they have assigned the order in which they are listed so that readers can interpret their roles correctly
* the corresponding author or guarantor shall prepare a concise, written description of how the order of authorship was decided
* examples of authorship order include:
* descending order of contribution
* placing the person who took the lead in writing the manuscript or doing the research first and the most experienced contributor in the field last
* alphabetical
* random order

**viii.** If an individual leaves the project the question of contribution to publications and authorship should be discussed in advance of their departure to minimise misunderstandings and to agree how this will be managed.

# 8 REFERENCES

1. McLean RM, Williams S, Mann JI, Miller JC and Parnell WR. Blood pressure and hypertension in New Zealand: results from the 2008/09 Adult Nutrition Survey. *N Z Med J*. 2013;126:66-79.

2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK and He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217-23.

3. Kannel W and Wilson PWE. Cardiovascular risk factors and hypertension. In: J. L. Izzo and H. R. Black, eds. *Hypertension primer: the essentials of high blood pressure.* 3rd ed.: Council for High Blood Pressure Research (American Heart Association); 2003: 235-239.

4. McKeigue PM, Ferrie JE, Pierpoint T and Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation*. 1993;87:152-61.

5. McKeigue PM, Shah B and Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet*. 1991;337:382-6.

6. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB, American Heart Association Statistics C and Stroke Statistics S. Executive summary: heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*. 2014;129:399-410.

7. Wexler RR, Greenlee WJ, Irvin JD, Goldberg MR, Prendergast K, Smith RD and Timmermans PB. Nonpeptide angiotensin II receptor antagonists: the next generation in antihypertensive therapy. *J Med Chem*. 1996;39:625-56.

8. Grassi G. Role of the sympathetic nervous system in human hypertension. *Journal of hypertension*. 1998;16:1979-87.

9. Esler M, Julius S, Zweifler A, Randall O, Harburg E, Gardiner H and DeQuattro V. Mild high-renin essential hypertension. Neurogenic human hypertension? *The New England journal of medicine*. 1977;296:405-11.

10. Goldstein DS. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension*. 1983;5:86-99.

11. Esler M, Lambert G and Jennings G. Increased regional sympathetic nervous activity in human hypertension: causes and consequences. *J Hypertens Suppl*. 1990;8:S53-7.

12. Abboud FM. The sympathetic system in hypertension. State-of-the-art review. *Hypertension*. 1982;4:208-25.

13. Grassi G and Mancia G. Neurogenic hypertension: is the enigma of its origin near the solution? *Hypertension*. 2004;43:154-5.

14. Fisher JP, Young CN and Fadel PJ. Central sympathetic overactivity: maladies and mechanisms. *Auton Neurosci*. 2009;148:5-15.

15. Burns J, Sivananthan MU, Ball SG, Mackintosh AF, Mary DA and Greenwood JP. Relationship between central sympathetic drive and magnetic resonance imaging-determined left ventricular mass in essential hypertension. *Circulation*. 2007;115:1999-2005.

16. Grassi G. Counteracting the sympathetic nervous system in essential hypertension. *Current opinion in nephrology and hypertension*. 2004;13:513-9.

17. Adlan AM, Paton JF, Lip GY, Kitas GD and Fisher JP. Increased sympathetic nerve activity and reduced cardiac baroreflex sensitivity in rheumatoid arthritis. *J Physiol*. 2017;595:967-981.

18. Guyenet PG. Regulation of breathing and autonomic outflows by chemoreceptors. *Compr Physiol*. 2014;4:1511-62.

19. Narkiewicz K, Ratcliffe LE, Hart EC, Briant LJ, Chrostowska M, Wolf J, Szyndler A, Hering D, Abdala AP, Manghat N, Burchell AE, Durant C, Lobo MD, Sobotka PA, Patel NK, Leiter JC, Engelman ZJ, Nightingale AK and Paton JF. Unilateral Carotid Body Resection in Resistant Hypertension: A Safety and Feasibility Trial. *JACC Basic Transl Sci*. 2016;1:313-324.

20. Pijacka W, Moraes DJ, Ratcliffe LE, Nightingale AK, Hart EC, da Silva MP, Machado BH, McBryde FD, Abdala AP, Ford AP and Paton JF. Purinergic receptors in the carotid body as a new drug target for controlling hypertension. *Nat Med*. 2016;22:1151-1159.

21. Marcus NJ, Del Rio R, Schultz EP, Xia XH and Schultz HD. Carotid body denervation improves autonomic and cardiac function and attenuates disordered breathing in congestive heart failure. *J Physiol*. 2014;592:391-408.

22. Niewinski P, Janczak D, Rucinski A, Jazwiec P, Sobotka PA, Engelman ZJ, Fudim M, Tubek S, Jankowska EA, Banasiak W, Hart EC, Paton JF and Ponikowski P. Carotid body removal for treatment of chronic systolic heart failure. *Int J Cardiol*. 2013;168:2506-9.

23. Niewinski P, Janczak D, Rucinski A, Tubek S, Engelman ZJ, Piesiak P, Jazwiec P, Banasiak W, Fudim M, Sobotka PA, Javaheri S, Hart EC, Paton JF and Ponikowski P. Carotid body resection for sympathetic modulation in systolic heart failure: results from first-in-man study. *Eur J Heart Fail*. 2017;19:391-400.

24. Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, Sobotka PA, Gourine AV and Paton JF. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. *J Physiol*. 2012;590:4269-77.

25. McBryde FD, Abdala AP, Hendy EB, Pijacka W, Marvar P, Moraes DJ, Sobotka PA and Paton JF. The carotid body as a putative therapeutic target for the treatment of neurogenic hypertension. *Nat Commun*. 2013;4:2395.

26. Moraes DJ, Machado BH and Paton JF. Carotid body overactivity induces respiratory neurone channelopathy contributing to neurogenic hypertension. *J Physiol*. 2015;593:3055-63.

27. Sinski M, Lewandowski J, Przybylski J, Bidiuk J, Abramczyk P, Ciarka A and Gaciong Z. Tonic activity of carotid body chemoreceptors contributes to the increased sympathetic drive in essential hypertension. *Hypertens Res*. 2012;35:487-91.

28. Aya HD, Carsetti A, Bazurro S, Bastoni D, Malbrain ML and Cecconi M. From cardiac output to blood flow auto-regulation in shock. *Anaesthesiol Intensive Ther*. 2015;47 Spec No:s56-62.

29. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci*. 2006;7:335-46.

30. Szenasi A, Dornyei G, Racz A, Debreczeni B and Koller A. [Regulation of vasomotor tone of small skeletal muscle veins by intrinsic mechanisms]. *Orv Hetil*. 2016;157:805-12.

31. Tabrizchi R and Pang CC. Effects of drugs on body venous tone, as reflected by mean circulatory filling pressure. *Cardiovasc Res*. 1992;26:443-8.

32. McEniery CM, Wilkinson IB and Avolio AP. Age, hypertension and arterial function. *Clin Exp Pharmacol Physiol*. 2007;34:665-71.

33. Rothe CF. Reflex control of veins and vascular capacitance. *Physiol Rev*. 1983;63:1281-342.

34. Safar ME and London GM. Venous system in essential hypertension. *Clin Sci (Lond)*. 1985;69:497-504.

35. Olsen H and Lanne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *Am J Physiol*. 1998;275:H878-86.

36. Fink GD, Johnson RJ and Galligan JJ. Mechanisms of increased venous smooth muscle tone in desoxycorticosterone acetate-salt hypertension. *Hypertension*. 2000;35:464-9.

37. Overbeck HW. Hemodynamics of early experimental renal hypertension in dogs. Normal limb blood flow, elevated limb vascular resistance, and decreased venous compliance. *Circ Res*. 1972;31:653-63.

38. Simon G. Altered venous function in hypertensive rats. *Circ Res*. 1976;38:412-8.

39. Simon G, Pamnani MB, Dunkel JF and Overbeck HW. Mesenteric hemodynamics in early experimental renal hypertension in dogs. *Circ Res*. 1975;36:791-8.

40. Yamamoto J, Trippodo NC, MacPhee AA and Frohlich ED. Decreased total venous capacity in Goldblatt hypertensive rats. *Am J Physiol*. 1981;240:H487-92.

41. Bevan JA, Bevan RD, Pegram BL, Purdy RE and Su C. Increased responsiveness of veins to adrenergic stimulation in experimental hypertension. *Blood Vessels*. 1974;11:241-4.

42. London GM, Safar ME, Simon AC, Alexandre JM, Levenson JA and Weiss YA. Total effective compliance, cardiac output and fluid volumes in essential hypertension. *Circulation*. 1978;57:995-1000.

43. Takeshita A and Mark AL. Decreased venous distensibility in borderline hypertension. *Hypertension*. 1979;1:202-6.

44. Houston DS, Fernandez PG and Snedden W. Forearm vascular responses in normotensives and hypertensives after sublingual nifedipine. *Clin Invest Med*. 1985;8:56-61.

45. Wood JE. Peripheral venous and arteriolar responses to infusions of angiotensin in normal and hypertensive subjects. *Circ Res*. 1961;9:768-74.

46. Rothe CF. Physiology of venous return. An unappreciated boost to the heart. *Arch Intern Med*. 1986;146:977-82.

47. de Groot PC, Bleeker MW and Hopman MT. Ultrasound: a reproducible method to measure conduit vein compliance. *J Appl Physiol (1985)*. 2005;98:1878-83.

48. Young CN, Prasad RY, Fullenkamp AM, Stillbower ME, Farquhar WB and Edwards DG. Ultrasound assessment of popliteal vein compliance during a short deflation protocol. *J Appl Physiol (1985)*. 2008;104:1374-80.

49. Nurse CA. Synaptic and paracrine mechanisms at carotid body arterial chemoreceptors. *J Physiol*. 2014;592:3419-26.

50. Prasad M, Fearon IM, Zhang M, Laing M, Vollmer C and Nurse CA. Expression of P2X2 and P2X3 receptor subunits in rat carotid body afferent neurones: role in chemosensory signalling. *J Physiol*. 2001;537:667-77.

51. Ralevic V and Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998;50:413-92.

52. Solomon LR and Hillman RS. Regulation of vitamin B6 metabolism in human red cells. *Am J Clin Nutr*. 1979;32:1824-31.

53. Jansonius JN. Structure, evolution and action of vitamin B6-dependent enzymes. *Curr Opin Struct Biol*. 1998;8:759-69.

54. Theriault O, Poulin H, Thomas GR, Friesen AD, Al-Shaqha WA and Chahine M. Pyridoxal-5'-phosphate (MC-1), a vitamin B6 derivative, inhibits expressed P2X receptors. *Can J Physiol Pharmacol*. 2014;92:189-96.

55. Liu R, Mi B, Zhao Y, Li Q, Yan H and Dang S. Effect of B Vitamins from Diet on Hypertension. *Arch Med Res*. 2017;48:187-194.

56. de Moraes AC, Gracia-Marco L, Iglesia I, Gonzalez-Gross M, Breidenassel C, Ferrari M, Molnar D, Gomez-Martinez S, Androutsos O, Kafatos A, Cuenca-Garcia M, Sjostrom M, Gottrand F, Widhalm K, Carvalho HB and Moreno LA. Vitamins and iron blood biomarkers are associated with blood pressure levels in European adolescents. The HELENA study. *Nutrition*. 2014;30:1294-300.

57. Olsen NS and Martindale WE. Studies on chronic vitamin B6 deficiency in the rat. I. Changes in the intact animal. *J Nutr*. 1954;53:317-27.

58. Paulose CS, Dakshinamurti K, Packer S and Stephens NL. Sympathetic stimulation and hypertension in the pyridoxine-deficient adult rat. *Hypertension*. 1988;11:387-91.

59. Aybak M, Sermet A, Ayyildiz MO and Karakilcik AZ. Effect of oral pyridoxine hydrochloride supplementation on arterial blood pressure in patients with essential hypertension. *Arzneimittelforschung*. 1995;45:1271-3.

60. Abdulqawi R, Dockry R, Holt K, Layton G, McCarthy BG, Ford AP and Smith JA. P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet*. 2015;385:1198-205.

61. Edgell H, McMurtry MS, Haykowsky MJ, Paterson I, Ezekowitz JA, Dyck JR and Stickland MK. Peripheral chemoreceptor control of cardiovascular function at rest and during exercise in heart failure patients. *J Appl Physiol (1985)*. 2015;118:839-48.

62. Pedersen ME, Dorrington KL and Robbins PA. Effects of somatostatin on the control of breathing in humans. *J Physiol*. 1999;521 Pt 1:289-97.

63. Elliott RO, Alsalahi S and Fisher JP. Impact of acute dynamic exercise on radial artery low-flow mediated constriction in humans. *Eur J Appl Physiol*. 2018;118:1463-1472.

64. Fisher JP, Fluck D, Hilty MP and Lundby C. Carotid chemoreceptor control of muscle sympathetic nerve activity in hypobaric hypoxia. *Exp Physiol*. 2018;103:77-89.

65. Junejo RT, Braz ID, Lucas SJE, Van Lieshout JJ, Lip GYH and Fisher JP. Impaired cerebrovascular reactivity in patients with atrial fibrillation. *Journal of American College of Cardiology*. 2019;73:1229-1234.

66. Niewinski P, Tubek S, Banasiak W, Paton JF and Ponikowski P. Consequences of peripheral chemoreflex inhibition with low-dose dopamine in humans. *J Physiol*. 2014;592:1295-308.

67. Bogert LW and van Lieshout JJ. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol*. 2005;90:437-46.

68. Vallbo AB, Hagbarth KE, Torebjork HE and Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev*. 1979;59:919-57.

69. Sundlof G and Wallin BG. The variability of muscle nerve sympathetic activity in resting recumbent man. *J Physiol*. 1977;272:383-97.

70. Cui J, Wilson TE and Crandall CG. Muscle sympathetic nerve activity during lower body negative pressure is accentuated in heat-stressed humans. *J Appl Physiol*. 2004;96:2103-8.

71. Murai H, Takamura M, Maruyama M, Nakano M, Ikeda T, Kobayashi D, Otowa K, Ootsuji H, Okajima M, Furusho H, Takata S and Kaneko S. Altered firing pattern of single-unit muscle sympathetic nerve activity during handgrip exercise in chronic heart failure. *J Physiol*. 2009;587:2613-22.

72. Ogoh S, Fisher JP, Young CN, Raven PB and Fadel PJ. Transfer function characteristics of the neural and peripheral arterial baroreflex arcs at rest and during postexercise muscle ischemia in humans. *Am J Physiol Heart Circ Physiol*. 2009;296:H1416-24.

73. Fisher JP, Fernandes IA, Barbosa TC, Prodel E, Coote JH, Nobrega AC and Vianna LC. Diving and exercise: the interaction of trigeminal receptors and muscle metaboreceptors on muscle sympathetic nerve activity in humans. *Am J Physiol Heart Circ Physiol*. 2015;308:H367-75.

74. Young CN, Deo S, Fisher JP, LeMaster JW and PJ F. Arterial baroreflex control of heart rate and sympathetic nerve activity in patients with type II diabetes. *FASEB J*. 2009;23:786.7.

75. Deo SH, Fisher JP, Vianna LC, Kim A, Chockalingam A, Zimmerman MC, Zucker IH and Fadel PJ. Statin therapy lowers muscle sympathetic nerve activity and oxidative stress in patients with heart failure. *Am J Physiol Heart Circ Physiol*. 2012;303:H377-85.

76. Fisher JP, McIntyre D, Farquhar WB, Pickering AE, Lip GYH and JFR P. Influence of respiratory phase on muscle sympathetic nerve activity in human hypertension. *Paper presented at: Experimental Biology*. 2011;Washington D.C.

77. Fisher JP, Reynolds RF, Farquhar WB, Pickering AE, Lip GYH and JFR P. Respiratory modulation of muscle sympathetic nerve activity in patients with hypertension. *Proc Physiol Soc*. 2010;20:PC28.

78. Radegran G. Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol*. 1997;83:1383-8.

79. Boetger CL and Ward DS. Effect of dopamine on transient ventilatory response to exercise. *J Appl Physiol (1985)*. 1986;61:2102-7.

80. Dahan A, Ward D, van den Elsen M, Temp J and Berkenbosch A. Influence of reduced carotid body drive during sustained hypoxia on hypoxic depression of ventilation in humans. *J Appl Physiol (1985)*. 1996;81:565-72.

81. Limberg JK, Johnson BD, Holbein WW, Ranadive SM, Mozer MT and Joyner MJ. Interindividual variability in the dose-specific effect of dopamine on carotid chemoreceptor sensitivity to hypoxia. *J Appl Physiol (1985)*. 2016;120:138-47.

82. Bainbridge CW and Heistad DD. Effect of haloperidol on ventilatory responses to dopamine in man. *J Pharmacol Exp Ther*. 1980;213:13-7.

83. Bascom DA, Clement ID, Dorrington KL and Robbins PA. Effects of dopamine and domperidone on ventilation during isocapnic hypoxia in humans. *Respir Physiol*. 1991;85:319-28.

84. Goldberg LI. The role of dopamine receptors in the treatment of congestive heart failure. *J Cardiovasc Pharmacol*. 1989;14 Suppl 5:S19-27.

85. Henson LC, Ward DS and Whipp BJ. Effect of dopamine on ventilatory response to incremental exercise in man. *Respir Physiol*. 1992;89:209-24.

86. Janssen C, Beloka S, Kayembe P, Deboeck G, Adamopoulos D, Naeije R and van de Borne P. Decreased ventilatory response to exercise by dopamine-induced inhibition of peripheral chemosensitivity. *Respir Physiol Neurobiol*. 2009;168:250-3.

87. Sabol SJ and Ward DS. Effect of dopamine on hypoxic-hypercapnic interaction in humans. *Anesth Analg*. 1987;66:619-24.

88. Ward DS and Bellville JW. Reduction of hypoxic ventilatory drive by dopamine. *Anesth Analg*. 1982;61:333-7.

89. Ward DS and Bellville JW. Effect of intravenous dopamine on hypercapnic ventilatory response in humans. *J Appl Physiol Respir Environ Exerc Physiol*. 1983;55:1418-25.

90. Ward DS, Voter WA and Karan S. The role of the carotid bodies in the counter-regulatory response to hypoglycemia. *Adv Exp Med Biol*. 2009;648:273-80.

91. Welsh MJ, Heistad DD and Abboud FM. Depression of ventilation by dopamine in man. Evidence for an effect on the chemoreceptor reflex. *J Clin Invest*. 1978;61:708-13.

92. Bendich A and Cohen M. Vitamin B6 safety issues. *Ann N Y Acad Sci*. 1990;585:321-30.

93. <https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/#h8>.

# 9 APPENDIX

## 9.1 APPENDIX 1: Amendment History

|  |  |  |  |
| --- | --- | --- | --- |
| Amendment number | Date of Amendment | Protocol version number | Summary of Amendment |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |