Imperial College London

Joint Research
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Arginine **S**upplementation to improve **C**ardiovascular and **E**ndothelial function after **N**SAID **T**reatment (ASCENT)

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Protocol

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Protocol authorised by:

Name & Role	**Date**	**Signature**

Study Management Group

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Clinical queries
Clinical queries should be directed to the study coordinator who will direct the query to the appropriate person.

Sponsor
Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity at:

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http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice

Funder
British Heart Foundation

This protocol describes the **A**rginine **S**upplementation to improve **C**ardiovascular and **E**ndothelial function after **N**SAID **T**reatment (ASCENT) study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

COX-2	Cyclooxygenase-2
NSAIDs	nonsteroidal anti-inflammatory drugs
NO	Nitric oxide
NOS	Nitric oxide synthase
eNOS	Endothelial nitric oxide synthase
ADMA	Asymmetric dimethylarginine
BMI	Body mass index

KEYWORDS
COX-2, prostacyclin, ibuprofen, Vioxx, thrombosis, nitric oxide, ADMA, L-arginine

STUDY SUMMARY

TITLE COX-2/prostacyclin/ADMA/NO axis in the cardiovascular system

DESIGN Two arm, single centre mechanistic study. Subjects will be computer randomised to either arm.

AIMS

- To perform a systemic analysis of how COX-2 inhibition by celecoxib affects vascular function and 'omic biomarkers including those associated with the COX-2/prostacyclin/ADMA axis in healthy male volunteers
- To investigate how this is altered by L-arginine supplementation

OUTCOME MEASURES

Primary endpoint: endothelial function measured using EndoPAT

Secondary endpoints: Blood pressure, cardiovascular biomarkers, eicosanoid and methylarginine/amine levels, plasma proteome and blood transcriptome

POPULATION Healthy male volunteers

ELIGIBILITY

Inclusion Criteria	Exclusion Criteria
• Male, 18-40 years old • No abnormal findings on medical history, screening, physical examination, haematology, biochemistry, urinalysis and vital signs (sitting blood pressure, resting pulse rate, respiratory rate and body temperature within two weeks of commencement date • Normal fasting lipid profile (Soverson et al, 1994) • Non-smoking • Clear venous access in upper limbs • BMI 18-30 • No history or signs of drug abuse • No other medication 4 weeks before or during study • Informed written consent	• Any history of allergy to NSAIDs or arginine • Significant medical conditions • Pulse rate<50 beats/minute • Sitting systolic blood pressure <80 or >160 mmHg • Sitting diastolic blood pressure <60 or >100 mmHg • Baseline endothelial dysfunction (as defined by EndoPAT; LnRHI<0.51) • Participation in other clinical study 8 weeks before or during study • Donation of blood 8 weeks before or during study • COVID-19 vaccination within 4 weeks • Positive COVID-19 test

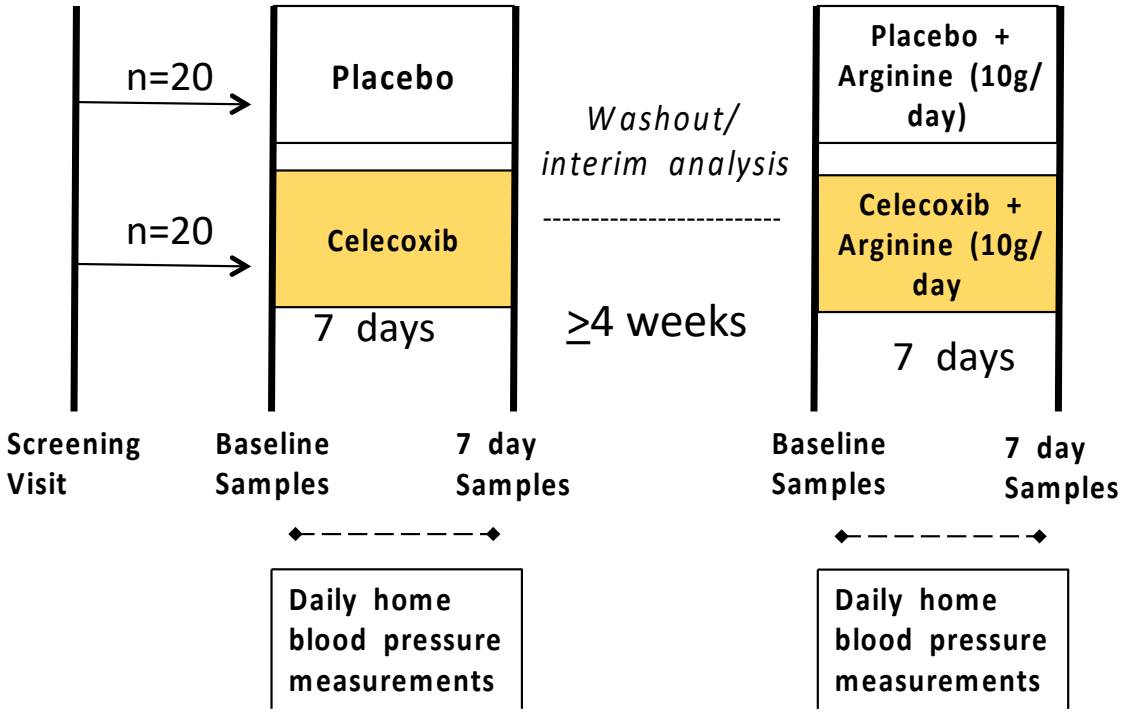
TREATMENT

Celecoxib: 200 mg b.i.d (400mg total/day)
L-arginine 5g b.i.d. (10g total/day)

Celecoxib, L-arginine or placebo will be dispensed against an individual prescription by a registered pharmacist. Drugs will be stored at the Imperial/NIHR Clinical Research Facility. Drugs or placebo will be administered orally.

DURATION 7 days of active treatment in each study arm (see flowchart below)

STUDY FLOWCHART AND TIMELINE



Exam	Visit number				
	0 (Screening)	1	2	3	4
Informed consent	x				
Medical history	x				
Physical exam/questionnaire	x				
Blood pressure	x	x	x	x	x
Heart rate	x	x	x	x	x
Breathing rate	x				
Temperature	x				
Height	x				
Weight	x				
Urine sample	x	x	x	x	x
Blood sample 50ml	x	x	x	x	x
EndoPat	x	x	x	x	x
Study medication(s) dispensed		x		x	

ANALYSIS/STORAGE OF SAMPLES

Samples will be stored in freezers or refrigerators or other appropriate place within secure, swipe access only facilities at Imperial College London. Results obtained during the study which will be stored on University Computers (including University Laptops) in a pseudonymised format; as part of our study we will measure gene expression and a large number of different proteins and other mediators in the volunteer's blood. Under GDPR (Recital 34) the gene expression data that we measure is classified as 'identifiable personal data'. However, the type of analysis that we are performing involves RNA and not genomic DNA which means that it would not be possible for a 'motivated individual with no special skill in genetics and no access to non-public datasets' to be able to identify individuals. Furthermore, we have performed a data-protection risk assessment on this study.

We will also send samples for analysis to collaborators within and outside of the EEA. All samples and data will be coded before leaving the study team. For some measurements the samples will be sent to service providers under contracts and for others to academic collaborators. All samples would be pseudonymised before leaving the study team and analysis conducted either done under contract for payment and/or as part of an academic collaboration. Where required a transfer agreement will be in place. Samples will be stored for future use subject to any requirement for future ethical approval.

1. INTRODUCTION

1.1. BACKGROUND

Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in inflammation and cancer. However, COX-2 in discreet regions including areas of the vasculature and in the kidney[1, 2] is a powerful cardiovascular protective pathway that works via formation of prostacyclin. In blood vessels, prostacyclin acts on smooth muscle and platelets to produce vasodilation and platelet inhibition, and in the kidney, regulates renal function and vasoactive hormone pathways to indirectly affect the systemic vasculature. These cardiovascular protective roles of COX-2 and prostacyclin are illustrated by the fact that nonsteroidal anti-inflammatory drugs (NSAIDs), which work by blocking COX-2 and include some of the world's most commonly taken drugs, cause the high profile cardiovascular side effects that have led to black box warnings on all drugs in this class[2, 3].

It was initially thought that cardiovascular side effects were limited to drugs that selectively target COX-2[3-5], such as Vioxx® (rofecoxib) and Celebrex® (celecoxib), introduced in the early 2000's[6, 7]. However, as a result of subsequent epidemiology analyses[8, 9] and, most recently, the publication of two large clinical cardiovascular outcome studies, SCOT[10] and PRECISION[11], it is clear that traditional NSAIDs, including ibuprofen and naproxen, carry at least as great a cardiovascular risk as the COX-2 selective drug celecoxib with ibuprofen, which is remarkably still an over the counter medication, emerging as significantly more toxic to the kidney[11].

The scale of use of NSAIDs (estimated to be regularly taken by 20-30% of the population) and the degree of associated risk (increased by 30%[12]) means that these drugs are a significant risk factor for heart attacks and strokes in their own right, potentially accounting for up to 50,000 of the 458,000 heart attacks and strokes in the UK each year (Jan 2016). Moreover, we now know that this risk emerges with just 1-2 weeks of use[13]. Concern over adverse cardiovascular events caused by NSAIDs has led to cautious prescribing of COX-2 selective NSAIDs in favour of older NSAIDs, which are more toxic to the gut, or opioids, which carry problems of tolerance, addiction and additional health burdens, and to the withdrawal in Europe of celecoxib for the prevention of colon cancer.

Mediators of protection: the power of arginine as a therapy

Endothelial nitric oxide synthase (eNOS) is expressed throughout the vasculature where it protects the entire cardiovascular system against atherosclerosis, thrombosis and hypertension. The power of eNOS and COX-2 together in protecting the cardiovascular system is biologically enhanced since NO and prostacyclin work additively to maintain vascular function and in a synergistic manner against thrombosis[14]. Thus, this pathway, which links COX-2 with prostacyclin and eNOS, provides a plausible mechanism that explains how NSAIDs precipitate cardiovascular side effects. This is supported by our[15] and others'[16] previously published findings showing that loss of COX-2 in mice is sufficient to impair systemic eNOS function in blood vessels *ex vivo*[15, 16] and that this was rescued by supplying the eNOS substrate L-arginine *in vitro*[15].

Whilst this provides essential proof of concept data we now need to complete and expand this work. In addition to basic science research, we need to translate these findings into man. We will do this by conducting a clinical study in healthy male volunteers using a study design model based on our previous work[15] that will determine the effects of COX-2 inhibition on cardiovascular endpoints and their sensitivity to reversal by L-arginine.

These data will provide essential information on (i) how COX-2 inhibition and the COX-2/prostacyclin/ADMA axis effect vascular function and (ii) the potential of L-arginine as a rescue therapy for NSAID cardiovascular toxicity.

1.2. RATIONALE FOR CURRENT STUDY

Hypothesis: COX-2 inhibition compromises endothelial function, which can be prevented by L-arginine.

2. STUDY OBJECTIVES

Investigate arginine as a means of preventing or alleviating the detrimental effects of celecoxib on measured functional endpoints and biomarkers.

3. STUDY DESIGN

Two arm, single centre mechanistic study.
Forty healthy male volunteers, twenty in each arm.

Primary endpoint: Endothelial function measured using EndoPAT

Secondary endpoints: Sitting blood pressure, cardiovascular biomarkers, eicosanoid and methylarginine/amine levels, plasma proteome and blood transcriptome.

Functional Endpoints	Biomarkers
• EndoPAT- endothelial function • Blood pressure	• Methylarginines- ADMA, SDMA and L-NMMA • Amino acids- full range including L-arginine, L-ornithine, L-citrulline. • Eicosanoids- full range including prostacyclin and PGI-M • Cardiovascular Biomarkers- thromboxane, endothelin-1, nitric oxide, renin, angiotensin, apolipoproteins, D-dimer, Sicam-1 and CRP • Renal biomarkers- creatinine, urea, cystatin C, KIM-1 and albumin

Healthy male volunteers will be randomized to 7 days' treatment with celecoxib (200mg b.i.d.; the standard maximum anti-inflammatory dose) or placebo in a double blinded format. Samples and measurements will be made at baseline and after the final dose. Following a washout of at least 4 weeks, L-arginine (Lamberts Healthcare Limited) may be administered in a dose of 5g b.i.d. (10g total/day) as per the study flowchart (page 5). A rational approach was taken in selecting the arginine dose since 10g/day increases plasma arginine by ≈40%[17]. At each visit, the primary endpoint will be endothelial function, measured using EndoPAT, which measures changes in flow-mediated endothelial function in the microvasculature. Importantly, EndoPAT is clinically validated with scores that correlate with cardiovascular risk[18]. Additionally, at each study visit, blood pressure, cardiovascular biomarkers (by ELISA) and

eicosanoid and methylarginine/amine levels (by GC/LCMS/MS) will be measured. Samples will also be collected for measurement of 'omic' biomarkers including transcriptomics, proteomics, lipidomics and metabolomics. Additionally volunteers will monitor and record their blood pressure readings daily using a home monitoring device and protocols validated and suggested (respectively) by the British and Irish Hypertension Society. In vivo aspects of this study will be performed using validated protocols[15] and using facilities and staff at the NIHR/Wellcome Trust Imperial College Clinical Research Facility.

4. PARTICIPANT ENTRY

Pre-registration evaluations and study evaluations

We will advertise for volunteers within Imperial College London by email advertisements, noticeboards and social media and using the established healthy volunteer database at the NIHR/Wellcome Trust Imperial College Clinical Research Facility. A research nurse or other suitably qualified person will explain the study to interested volunteers and go through the participant information sheet with them, giving the volunteer an opportunity to ask any questions regarding the study. If the volunteer consents to the study they will sign the study consent form and will be given a copy of the participant information sheet to keep.

The volunteer will then undergo a screening assessment to check eligibility (fulfilment of inclusion/exclusion criteria). This will include:

- Medical history questionnaire, including questions of history of drug abuse
- Sitting blood pressure, heart rate, respiratory rate and temperature
- Height and weight to calculate BMI
- Blood sample to measure routine haematology and biochemistry (including lipid profile)
- Urine sample to measure glucose and specific gravity
- EndoPAT measurement to confirm normal endothelial function
- Participants will be asked not to change their lifestyle while they are taking part in the study. However, they will be asked not to take any other medications including paracetamol and ibuprofen for the duration of the study.
- Once enrolled to the study subjects will undergo blood pressure, heart rate and EndoPAT tests. Urine and blood will be collected for study analysis.

Eligible participants who have had a COVID-19 vaccination will need to wait at least 4 weeks before entering into any phase of the study.

Participants will be provided with home COVID-19 rapid lateral flow kits. Participants will be asked to perform a test prior to each study visit. Participants with a positive test will be advised to follow government advice, perform PCR test and self-isolate. They will be excluded from the study.

Inclusion/exclusion criteria

Inclusion Criteria	Exclusion Criteria
• Male, 18-40 years old	• Any history of allergy to NSAIDs or arginine • Significant medical conditions

• No abnormal findings on medical history, screening, physical examination, haematology, biochemistry, urinalysis and vital signs (sitting blood pressure, resting pulse rate, respiratory rate and body temperature within two weeks of commencement date • Normal fasting lipid profile (Soverson et al, 1994) • Non-smoking • Clear venous access in upper limbs • BMI 18-30 • No history or signs of drug abuse • No other medication 4 weeks before or during study • Informed written consent	• Pulse rate<50 beats/minute • Sitting systolic blood pressure <80 or >160 mmHg • Sitting diastolic blood pressure <60 or >100 mmHg • Baseline endothelial dysfunction (as defined by EndoPAT; LnRHI<0.51) • Participation in other clinical study 8 weeks before or during study • Donation of blood 8 weeks before or during study • COVID-19 vaccination within 4 weeks • Positive COVID-19 test

Withdrawal criteria

Participants will be free to withdraw from taking part at any time, and they will be asked to provide a reason for withdrawal which will be recorded but their right to withdraw without providing a reason will be respected. Information collected from participants who have withdrawn may be used, but the investigators will attempt to replace the withdrawn participants with someone who completes the full protocol.

If a subject consents but then fails to fulfil the inclusion/exclusion they will be considered a 'screen failure' and their results will not be used. If screening, or during study visits, results need further follow-up then a medically qualified investigator (Prof Collins or Dr Ricky Vaja) will contact the general practitioner of the volunteer to request further follow-up.

As the study evaluates, (i) a well-known and relatively safe drug (celecoxib) used in many clinical trial settings, is available on prescription world-wide, and has a similar mechanism of action to ibuprofen and (ii) a drug/supplement (L-arginine) freely available without prescription from high street health food outlets and as a well-tolerated prescription medication for treatment of urea cycle disorders, we do not anticipate that study participants will withdraw due to the study medication.

Re-enrolment

Once participants are enrolled and randomised in the study, the study visits (visits 1 & 2 in phase 1 and visits 3 & 4 in phase 2) need to occur exactly 7 days apart. If participant attends visit 1 but is unable to attend visit 2 for a reason that is unrelated to the study medication e.g. develops an unrelated illness or cannot attend for personal reasons, the participant will have the option to re-enrol as a new participant if there is at least a 4-week washout period if they wish to do so. These participants will need to re-attend screening and re-consent and will be treated as a new participant with a new study number. To avoid double counting, any data obtained from the initial enrolment will not be used and will be superseded by the data generated by re-enrolment.

5. ADVERSE EVENTS

Definitions

Adverse Event (AE): any untoward medical occurrence in a clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement will be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, will also be considered serious.

Reporting procedures
All adverse events will be reported. Depending on the nature of the event the reporting procedures below will be followed. Any questions concerning adverse event reporting will be directed to the Chief Investigator (Professor Jane Mitchell) and the medically qualified Investigators (Professor Peter Collins and Dr Ricky Vaja) in the first instance.

Non serious AEs
All such events, whether expected or not, will be recorded.

Serious AEs
A SAE form will be completed and sent to the medically qualified Investigator, Professor Peter Collins, within 24 hours. However, relapse and death and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs will be reported to the Imperial College Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs will be submitted within 15 days of the chief investigator/medically qualified investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator will also notify the Sponsor of all SAEs.

Contact details for reporting SAEs
jrco@imperial.ac.uk
CI email: j.a.mitchell@imperial.ac.uk
Please send SAE forms to: Prof Peter Collins
Fax: 0330 128 8771

Tel: 0330 128 8112 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

There will be no follow-up after the study has completed. The end of study definition is last subject last visit.

7. STATISTICS AND DATA ANALYSIS

Sample size: This is a novel mechanistic study where the primary endpoint is the effect of celecoxib on endothelial function measured using the EndoPAT device. We anticipate that we will require n=20-40 in each study arm to detect a difference based on previous work using similar drugs and alternative measurement approaches. For example Carlsson and Wennmalm (1983)[19] showed a reduction in endothelial function with ibuprofen (effect size: 0.80 standard deviations) and diclofenac (effect size: 1.06 standard deviations). This equates to a power of 72-92% at n=20 and 95-100% at n=40 to detect a difference between groups at a nominal significance threshold of $p<0.05$. As we are using a different drug and an alternative measurement platform, power can only be estimated broadly. As such, we will perform an interim analysis of data after studying n=20 volunteers per group during the 'wash out' period. At this point, actual effect size and variance will be determined and experimental power re-calculated. If our primary endpoint is not achieved, we may revise our endpoints and/or if additional power is required, we will reserve the option to recruit up to an additional n=20 volunteers per group. Dependent upon the results the interim analysis may also serve as a stop-go checkpoint for continuation of the L-arginine arm of the study.

Data analysis: Data will be analysed according to approaches applicable to the measurement. This includes standard parametric and non-parametric tools (including Fisher's exact tests, Student's t-tests, 1-way and 2-way ANOVAs, Mann Whitney U-tests and Kruskill-Wallis tests) as well as specialised tests for large omic data sets (including modified t-test with false discovery correction where appropriate). Across all tests differences will be considered significant where $p<0.05$.

8. REGULATORY ISSUES

Ethics Approval

The Study Coordination Centre has obtained approval from the xxx Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data

analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Confidentiality
The Chief Investigator will preserve the confidentiality of participants taking part in the study.

All participant information collected during the study will be kept strictly confidential, and any information about participants which leaves the facility will have their name and address removed. Only authorised persons such as researchers, the sponsor and research and development audit will have access to the data.

Study data will be collected using REDCap which is a mature and secure web application for clinical trial data capture. Data is stored on the secure encrypted imperial college server. All data held in REDCap is anonymised. Only members of the research team will have access to data.

Written study records will be kept in a locked cabinet. Any written study records will be pseudonymised such that data, samples and records of participants will be known by a study number with the key known only to the study team and kept separately from the psuedonymised study data. Participant Identifiable data (e.g consent forms) will also be kept in a locked cabinet with restricted access. After the study data have been analysed and published, all written and electronic study records will be kept for 10 years by Imperial College London.

Psuedonymised electronic records of the data will be kept on a computer at Imperial College London which is protected by a firewall. If the electronic data are used in further research then further REC approval will be sought.

Indemnity
Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Sponsor
Imperial College London will act as the main Sponsor for this study.

Funding
The British Heart Foundation is funding this study.

Audits
The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP.

9. STUDY MANAGEMENT

The study will be lead by the Chief Investigator, Professor Jane A. Mitchell, who has overall responsibility for the work. Professor Mitchell, Dr Nicholas Kirkby and Professor Peter Collins will work together to manage and supervise the study. The day-to-day management of the study will be co-ordinated through the NIHR Imperial Clinical research facility and executed by the research team which includes Ms Hime Gashaw, Dr Plinio Ferreira and Dr Ricky Vaja. Ms Hime Gashaw will manage the day-to-day

organisation of laboratory and other supplies. Dr Ricky Vaja and Dr Plinio Ferreira will be responsible for recruitment, screening and all of the procedures and measurements made. The Principal Investigator for the Imperial College Helathcare NHS trust site where the Imperial CRF is situated is Professor Peter Collins. Dr Ricky Vaja, together with Professor Collins, will also be responsible for ensuring that, where screening results need further follow-up, the general practitioner of the volunteer is contacted and further follow-up requested. Professor Mitchell, Dr Vaja and Professor Collins will be responsible for addressing questions concerning adverse event reporting.

The volunteer study will be performed at:
NIHR Imperial Clinical research facility
Imperial Centre for Translational and Experimental Medicine
Imperial College Healthcare NHS Trust
Hammersmith Hospital
Du Cane Road
London W12 0HS
Tel: 020 3313 8070
Email: Imperial.CRF@imperial.nhs.uk

10. PUBLICATION POLICY

Identifiable personal data will not be used when reporting the study results.
Results may be presented at national and international conferences, and published in the highest possible impact peer reviewed journal, press releases/public engagement forums.

REFERENCES

1. Kirkby NS, Zaiss AK, Urquhart P, Jiao J, Austin PJ, Al-Yamani M, Lundberg MH, MacKenzie LS, Warner TD, Nicolaou A, Herschman HR and Mitchell JA. LC-MS/MS confirms that COX-1 drives vascular prostacyclin whilst gene expression pattern reveals non-vascular sites of COX-2 expression. *PLoS One*. 2013;8:e69524.
2. Kirkby NS, Chan MV, Zaiss AK, Garcia-Vaz E, Jiao J, Berglund LM, Verdu EF, Ahmetaj-Shala B, Wallace JL, Herschman HR, Gomez MF and Mitchell JA. Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-kappaB and NFAT transcriptional pathways. *Proc Natl Acad Sci U S A*. 2016;113:434-9.
3. Antman EM, Bennett JS, Daugherty A, Furberg C, Roberts H, Taubert KA and American Heart A. Use of nonsteroidal antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association. *Circulation*. 2007;115:1634-42.
4. Funk CD and FitzGerald GA. COX-2 inhibitors and cardiovascular risk. *J Cardiovasc Pharmacol*. 2007;50:470-9.
5. Mitchell JA and Evans TW. Cyclooxygenase-2 as a therapeutic target. *Inflamm Res*. 1998;47 Suppl 2:S88-92.
6. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ and Group VS. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med*. 2000;343:1520-8, 2 p following 1528.
7. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM and Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA*. 2000;284:1247-55.
8. McGettigan P and Henry D. Cardiovascular risk with non-steroidal anti-inflammatory drugs: systematic review of population-based controlled observational studies. *PLoS Med*. 2011;8:e1001098.
9. Warner TD and Mitchell JA. COX-2 selectivity alone does not define the cardiovascular risks associated with non-steroidal anti-inflammatory drugs. *Lancet*. 2008;371:270-3.

10. MacDonald TM, Hawkey CJ, Ford I, McMurray JJV, Scheiman JM, Hallas J, Findlay E, Grobbee DE, Hobbs FDR, Ralston SH, Reid DM, Walters MR, Webster J, Ruschitzka F, Ritchie LD, Perez-Gutthann S, Connolly E, Greenlaw N, Wilson A, Wei L and Mackenzie IS. Randomized trial of switching from prescribed non-selective non-steroidal anti-inflammatory drugs to prescribed celecoxib: the Standard care vs. Celecoxib Outcome Trial (SCOT). *Eur Heart J*. 2017;38:1843-1850.
11. Nissen SE, Yeomans ND, Solomon DH, Luscher TF, Libby P, Husni ME, Graham DY, Borer JS, Wisniewski LM, Wolski KE, Wang Q, Menon V, Ruschitzka F, Gaffney M, Beckerman B, Berger MF, Bao W, Lincoff AM and Investigators PT. Cardiovascular Safety of Celecoxib, Naproxen, or Ibuprofen for Arthritis. *N Engl J Med*. 2016;375:2519-29.
12. Coxib, traditional NTC, Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, Bombardier C, Cannon C, Farkouh ME, FitzGerald GA, Goss P, Halls H, Hawk E, Hawkey C, Hennekens C, Hochberg M, Holland LE, Kearney PM, Laine L, Lanas A, Lance P, Laupacis A, Oates J, Patrono C, Schnitzer TJ, Solomon S, Tugwell P, Wilson K, Wittes J and Baigent C. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet*. 2013;382:769-79.
13. Bally M, Dendukuri N, Rich B, Nadeau L, Helin-Salmivaara A, Garbe E and Brophy JM. Risk of acute myocardial infarction with NSAIDs in real world use: bayesian meta-analysis of individual patient data. *BMJ*. 2017;357:j1909.
14. Kirkby NS, Lundberg MH, Chan MV, Vojnovic I, Solomon AB, Emerson M, Mitchell JA and Warner TD. Blockade of the purinergic P2Y12 receptor greatly increases the platelet inhibitory actions of nitric oxide. *Proc Natl Acad Sci U S A*. 2013;110:15782-7.
15. Ahmetaj-Shala B, Kirkby NS, Knowles R, Al'Yamani M, Mazi S, Wang Z, Tucker AT, Mackenzie L, Armstrong PC, Nusing RM, Tomlinson JA, Warner TD, Leiper J and Mitchell JA. Evidence that links loss of cyclooxygenase-2 with increased asymmetric dimethylarginine: novel explanation of cardiovascular side effects associated with anti-inflammatory drugs. *Circulation*. 2015;131:633-42.
16. Yu Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z, Landesberg G, Crichton I, Wu W, Pure E, Funk CD and FitzGerald GA. Vascular COX-2 modulates blood pressure and thrombosis in mice. *Sci Transl Med*. 2012;4:132ra54.
17. Schneider JY, Rothmann S, Schroder F, Langen J, Lucke T, Mariotti F, Huneau JF, Frolich JC and Tsikas D. Effects of chronic oral L-arginine administration on the L-arginine/NO pathway in patients with peripheral arterial occlusive disease or coronary artery disease: L-Arginine prevents renal loss of nitrite, the major NO reservoir. *Amino Acids*. 2015;47:1961-74.
18. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Luscher TF, Shechter M, Taddei S, Vita JA and Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation*. 2012;126:753-67.
19. Carlsson I and Wennmalm A. Effect of different prostaglandin synthesis inhibitors on post-occlusive blood flow in human forearm. *Prostaglandins*. 1983;26:241-52.