Suggested Reference


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1) Project Aims
The aims of the study are to determine the long-term health outcomes of adults who suffered fetal anaemia due to rhesus disease sufficiently severe to warrant treatment by intrauterine transfusion. Specifically, we aim to recruit participants treated with intrauterine transfusion at National Women’s Hospital between 1963 and 1992, to a study of cardiac structure and function (measured using cardiac MRI), cardiovascular risk factors (body size, blood pressure, serum lipids, glucose tolerance), heart rate variability, renal function and liver function.

2) Background
Fetal anaemia is known to induce a number of adaptations in the fetus in order to preserve myocardial and systemic oxygen supply and hence end organ oxygen consumption. In fetal sheep, chronic anaemia has been shown to increase heart to body weight ratio by 30%, increase stroke volume and cardiac output by 50% and double coronary conductance, while still preserving coronary reserve. Fetal anaemia also affects coronary architecture, with an approximately 50% increase in ventricular capillary diameter. In fetuses studied after only seven days of experimentally induced anaemia, resting coronary blood flow is elevated to that obtained after maximal coronary vasodilation in normal fetuses, and maximal coronary conductance is doubled. Importantly, when the anaemia was corrected by intrauterine transfusion, approximately half of this increased conductance persisted. Indeed, this doubling of coronary conductance has since been shown to persist into adulthood in sheep. Thus it appears that the in utero anaemia programmes the fetus for a different trajectory of cardiac development that permanently alters later cardiac conductance.

The long-term significance of these cardiovascular changes for the life of the animal are currently unknown. However, if sheep who had suffered brief fetal anaemia are subsequently exposed to five minutes of hypoxic stress as adults, they demonstrate higher indices of left ventricular systolic function, a functional advantage in terms of coronary vasodilatory reserve and cardiac function. Conversely, adult rats exposed to hypoxia in utero have been demonstrated to suffer an increased infarct area using an ischaemia-reperfusion model of myocardial infarction. It is not known if the increased coronary flow to which the anaemic fetus is exposed causes long-term negative effects on endothelial function in the coronary vasculature of the more mature adult. Nor is it known if any of these findings are relevant to human survivors of fetal anaemia.

In man, prior to the introduction of anti-rhesus immunoglobulin in the 1970s, the most common cause of severe fetal anaemia was rhesus haemolytic disease. Rhesus disease forms a clinical spectrum from mild haemolytic anaemia to severe hydrops fetalis and fetal death. Without transfusions severely affected fetuses die in utero or early in postnatal life. The first ever intrauterine transfusion (IUT) for rhesus disease was performed by Sir William Liley at National Women’s Hospital in 1963. Since then, apart from a very small number of procedures performed in Christchurch and Wellington in the late 1960s, all IUTs in New Zealand have been undertaken at National Women’s Hospital. Therefore, we have access to both the oldest, and also one of the largest reported cohorts of IUT survivors in the world. In addition, given that the technique of intraperitoneal IUT was pioneered in New Zealand, and remained the accepted standard of care throughout the world until the mid 1980s, it is likely that our cohort is representative of all adult survivors of IUT.

We therefore plan to determine the effects in adulthood of fetal anaemia by studying adult survivors of IUT born at National Women’s Hospital.

3) Research Design
Hypotheses
The null hypothesis is that fetal anaemia treated with IUT has no long-term effects on health in adulthood. Specifically, we hypothesise that adults who suffered fetal anaemia due to rhesus disease sufficiently severe to be treated with IUT will not differ from their unaffected siblings in:
• cardiac structure and function (measured using cardiac MRI)
• cardiovascular risk factors (body size, blood pressure, serum lipids, glucose tolerance)
• heart rate variability
• renal function
• liver function

Study Design
Cohort study comparing:
• Exposed group: Adults who suffered fetal anaemia due to rhesus disease and were treated with IUT at National Women’s Hospital between 1963 and 1990.
• Unexposed group: Unaffected siblings of the IUT recipients.

Pilot Study
Between February 2007 and February 2008 Professor Jane Harding, Drs Stuart Dalziel and Mariam Buskh, and medical student Iris Grooten conducted a pilot study to assess the feasibility of the proposed project. The specific objectives of the pilot study were to:
1. Assess whether adults who had received IUT for rhesus disease 20 to 40 years ago at National Women’s Hospital and their unaffected siblings could be located and would be willing to be involved in a follow up study.
2. Assess the practicality and cost of locating these adults.
3. Estimate the time and resources required for the main study.
4. Develop and test an MRI protocol that will provide appropriate data for the main study.

The pilot study established that from 1977, the details of all fetal interventions occurring at National Women’s Hospital were recorded in a central logbook, known as the “Special Procedures Book”. Prior to this date it was still possible to determine which babies received IUT from the admission records of the neonatal intensive care unit (NICU). Logbooks and admission records were located. From this information it was possible to determine the name and date of birth of mothers of possible participants, as well as the date of birth, sex and sometimes the name of the possible participant. Other databases, including the National Health Index and Births, Deaths and Marriages, could then be used to obtain current contact details. From this pilot study it was concluded that a follow up study of IUT recipients was feasible, that approximately 80 sibling pairs were likely to take part, and that possible participants could be successfully traced, had hospital records available, were willing to participate and found the study protocol acceptable.

Tracing and recruitment protocol
Attempts will be made to identify, locate and recruit to the Fetal Anaemia Study all adult survivors of IUT born between 1963 and 1992 using the following approach:
The mothers of fetuses who received IUT from 1977 to 1992 will be identified from the Special Procedures Book. For babies born prior to 1977, NICU admission books will be searched to identify all those with an admission diagnosis pertaining to any aspect of rhesus disease or its management, such as rhesus sensitisation, jaundice, exchange transfusion, anaemia, and hydrops. Archived hospital records for these babies will then be reviewed to identify any who received an IUT. For babies identified as IUT recipients, hospital records will be reviewed to confirm the baby’s date of birth, sex, and if possible, full name. Hospital records of the mothers of these babies will also be reviewed to identify mother’s date of birth and full name, and dates of birth, sex and if possible names of other children.

The National Health Index database (New Zealand Health Information Service, Wellington) will then be searched. From its inception in 1980, this database has recorded the name, date of birth, address, date of last admission and, if relevant, date of death of all individuals who have utilised a public health service in New Zealand. The database will be searched using the names and dates of birth of mothers and babies to confirm their full name, including details of any name changes or aliases and their most recent documented address. Further information regarding residential and postal addresses, and phone numbers of mothers and babies will be sought through an electronic search of the New Zealand electoral roll, telephone directory, and if necessary, through social networking internet sites.

Initial contact with the possible participants will be made via an introductory letter, unless no address is available, in which case initial contact will be via telephone or email. A patient information sheet will also be included. A simple yes/no box will be provided on a self-addressed, freepost form, so that the possible participant can respond to the inquiry. If no response is received within two weeks, a second letter will be sent to the same address. If no response is received after a further two weeks, efforts will be made to contact the subject by telephone. If the above attempts at locating a possible participant are unsuccessful, their mothers and/or siblings will be sought to see if they can assist in locating the possible participant. Finally, the National Deaths Register will be searched for all IUT recipients who cannot be located and who may have died prior to the inception of the National Health Index database in 1980.

Potential unexposed sibling participants will be contacted via exposed participants.

Inclusion criteria

- Exposed participants: IUT recipient born in New Zealand between 1963 and 1992, with whom contact is successfully made.
• Unexposed siblings: Siblings of exposed participants who were not affected in any way by rhesus disease.

**Exclusion criteria**
• residence outside New Zealand with no intention to return within the study timeframe
• no unexposed sibling available
• pregnancy
• medical condition preventing participation
• contraindication to MRI or gadolinium contrast

Participants with asthma requiring regular preventive medication will not undergo perfusion imaging with adenosine, but will complete the remainder of the study protocol.

**Study protocol**
All suitable participants, whether exposed or unexposed, will undertake the same study protocol. Prior to arrival, participants will be asked to complete a standard questionnaire about past medical history, cardiovascular risk factors and family medical history. Transport and accommodation will be funded for all participants residing outside of Auckland, and in most cases, siblings will attend for assessment on the same day.

As MRI scans can be performed on only one participant at a time, the assessment will proceed by one of two sequences, with Participant A undergoing MRI scan first (Figure 2).

<table>
<thead>
<tr>
<th>Sibling A</th>
<th>Sibling B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome, discussion, consent, study questionnaire reviewed</td>
<td>Welcome, discussion, consent, study questionnaire reviewed</td>
</tr>
<tr>
<td>Anthropometric measurements</td>
<td>Anthropometric measurements</td>
</tr>
<tr>
<td>BP, pulse, O₂ saturations recorded</td>
<td>BP, pulse, O₂ saturations recorded</td>
</tr>
<tr>
<td>Cannula inserted, blood samples taken</td>
<td>Cannula inserted, blood samples taken</td>
</tr>
<tr>
<td>Electrocardiogram performed and reviewed</td>
<td>Electrocardiogram performed and reviewed</td>
</tr>
<tr>
<td>Heart rate variability trace recorded</td>
<td>Heart rate variability trace recorded</td>
</tr>
<tr>
<td>Oral glucose tolerance test</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>Cannula removed, concluding discussion, participant thanked and farewelled</td>
<td>Cannula removed, concluding discussion, participant thanked and farewelled</td>
</tr>
</tbody>
</table>

**Figure 2:** Summary of assessment process, siblings A and B

**Consent and Anthropometry**
Written informed consent will be obtained from all participants, with particular emphasis on potential side effects from adenosine and gadolinium. A study nurse will measure and record participants’ height. Body mass index will be calculated as:

\[
\text{weight (kg)} \div \text{height}^2 (m)
\]

Body surface area will be calculated using the Mosteller formula:\(^{10}\)

\[
\sqrt{\frac{\text{weight (kg)} \times \text{height (cm)}}{3600}}
\]
Maximum occipito-frontal diameter will be measured three times using a paper tape measure. An average of these measurements will be recorded as head circumference. Following anthropometric assessment, three measurements of blood pressure, resting heart rate and oxygen saturations will be recorded with the participant sitting.

**Blood Samples**

Blood samples will be taken after an overnight fast from an intravenous cannula placed in the antecubital fossa, and analysed for plasma lipids, glucose, insulin, electrolytes, urea, creatinine, liver function tests, full blood count and blood group including rhesus status. Participants will then undergo a standard 75 g oral glucose tolerance test, with blood samples taken at 30 minutes and 120 minutes.

All blood samples will be delivered to the laboratory within 4 hours of collection. Analyses will be undertaken by LabPLUS (Auckland District Health Board) and the New Zealand Blood Service reference laboratory (Great South Road, Auckland).

**Electrocardiogram and Heart Rate Variability**

A standard 12-lead electrocardiogram will be performed at the Clinical Physiology Department, Auckland City Hospital, a short distance from the Centre for Advanced MRI. If the oral glucose tolerance test had been commenced, the participant will be transported to the Clinical Physiology Department by wheelchair to minimise physical activity which may affect glucose utilisation. The electrocardiogram will be assessed by the study doctor prior to commencement of the MRI scan, noting in particular the PR and QT intervals to ensure there is no contraindication to administration of adenosine.

Heart rate and rhythm will be recorded using a PowerLab 4/25T data acquisition system (ADInstruments, Dunedin, NZ). With the participant supine, adhesive electrodes will be attached to both wrists and ankles and recording commenced from lead II at amplitude 2mV. Participants will be asked to maintain a state of quiet wakefulness for ten to fifteen minutes during the recording.

Five minutes of heart rate variability traces will be analysed using Labchart 7 Pro analysis software (ADInstruments, Dunedin, NZ). Sections of the trace distorted by movement artefact will be excluded. Spectrum band widths will be set as follows: very low frequency <0.04 Hz; low frequency 0.04 to 0.15 Hz; high frequency 0.15 to 0.4 Hz. The variation threshold, which describes the difference in duration between successive normal R-R intervals, will be set at 50 ms. R-R interval limits defining artefacts, ectopics and normal beats will be according to appearance of beats within each recording. The following variables will be recorded for each trace: SDNN, SDNN, RMSSD, pNN50, total power, LF power, HF power, LFnu, HF power, LF/HF ratio.

**MRI Scans**

All MRI scans will be performed on a Siemens 1.5 Tesla MAGNETOM Avanto scanner with Sygno MR VB11-17 software (Siemens, Erlangen, Germany) at the Centre for Advanced MRI, University of Auckland. The MRI protocol was developed prior to the pilot study in conjunction with Prof Kent Thornburg and Drs Michael Jerosch-Herold and Craig Broberg, at the Oregon Health and Sciences University, Portland, Oregon. Two healthy volunteers were scanned in November 2007 to assist with finalisation of the protocol. Further refinements were made following the pilot study, including changes to the formulation of the adenosine infusion and addition of late enhancement (viability) imaging following perfusion imaging.

Following positioning of the participant with ECG gating on the MRI table and acquisition of scout images, standard long and short axis cardiac cines will be obtained using a steady state free-precession sequence. The participant will be asked to hold their breath for approximately 10 heart beats for each of the following views: two, three and four chamber long axis views and a stack of true short-axis views from base to apex. The short axis stack comprised 12 slices, with a slice thickness of 7 mm, and inter-slice gap of 1 to 3 mm to ensure coverage of the entire heart from base to apex. Phase contrast velocity flow mapping with appropriate velocity encoding will be performed in the proximal aorta and pulmonary
artery.

Images will be acquired for assessment of myocardial blood flow at rest, during cold pressor challenge and at maximal vasodilation with adenosine infusion. For each assessment intravenous gadolinium contrast (Omniscan, GE Healthcare, Wisconsin, USA) will be given at 0.04 mmol.kg$^{-1}$. Image acquisition will commence at the time of contrast injection and will be ECG-gated. Ten minutes will be allowed between perfusion scans for wash out of the gadolinium. Myocardial blood flow will be measured in all three states with a T1 weighted single-shot gradient echo sequence with a saturation recovery magnetisation preparation. Three slices at basal, mid ventricular and apical levels will be obtained in each heart beat for rest and cold pressor perfusion imaging. For adenosine perfusion imaging, three slices per heart beat will be obtained if the time between heart beats is long enough to allow this; otherwise only the basal and mid-level slices will be acquired.

Cold pressor challenge, used to measure flow-dependent coronary vasodilation, will be achieved by placing the participant’s hand in a bag of ice for three minutes prior to the start of the perfusion scan.

Measurement of coronary flow at maximal vasodilation will be achieved by administration of intravenous adenosine (Adenocor, Sanofi-Aventis Ltd, Paris, France) at 0.14 mg.kg$^{-1}$.min$^{-1}$. As caffeine is a pharmacological antagonist of adenosine, participants will be asked to abstain from consuming beverages containing caffeine for 24 hours prior to the assessment. To ensure administration of an equivalent volume of adenosine to each participant, a predetermined dose chart will be used to formulate a 50 ml infusion of appropriate concentration for the participant’s body weight. The infusion will be administered via a double lumen extension. Gadolinium contrast will be administered through the second lumen, thus negating the need for insertion of a second intravenous cannula. Perfusion imaging will commence three minutes after the start of the adenosine infusion, which will continue for a further twenty seconds to ensure maximal vasodilation is maintained for the first pass of contrast agent. Imaging will continue for approximately 45 seconds in total.

Blood pressure, heart rate and oxygen saturations will be recorded at the start of the MRI, prior to the administration of adenosine, one minute into the adenosine infusion and one minute after completion of the adenosine infusion. In addition, verbal communication will be maintained with the participant to monitor their tolerance of potential side effects. Common side effects to adenosine include nausea, dizziness, headache, flushing, abdominal discomfort, chest and neck tightness and shortness of breath. If a patient complains of shortness of breath, they will be monitored closely for signs of bronchospasm. The ECG trace will also be watched carefully for signs of atrioventricular block. Algorithms have been devised to assist with management in the event of significant bradycardia or bronchospasm. If a patient develops intolerable side effects, significant bronchospasm or sustained bradycardia with heart rate ≤40 beats per minute, the adenosine infusion will be discontinued.

Short axis late enhancement images will be acquired during a single breath hold ten minutes after completion of the perfusion imaging. If any abnormalities are noted, long axis slices will be acquired through the region of interest.

All MRI data will be analysed by one of two analysts, at the Auckland MRI Research Group, Department of Anatomy with Radiology, University of Auckland. Cardiac volumes will be determined by segmenting the endo- and epicardial borders of all cine images using guide-point modelling to create a 4 dimensional finite-element model of the LV, followed by calculation of the cavity and myocardial volumes by numerical integration.$^{12}$ End systole and end diastole are identified as the phases with minimum and maximum volume.

The perfusion images will be motion corrected using non-rigid registration, and the endo- and epicardial contour defined. Signal saturation and coil sensitivity effects will be corrected, and the arterial input function measured in the blood pool at the base of the LV. A B-spine analysis with appropriate regularisation will be used to calculate the MBF in a range of myocardial regions of interest.$^{13}$

All laboratory and MRI imaging and analysis staff will be blinded to the participants’ fetal anaemia status.
Perinatal and maternal obstetric data will be collected from archived hospital records. Birth weight z-score will be calculated using the LMS method. Data regarding ethnicity and lifestyle factors (smoking status, alcohol intake, physical activity) will be collected by self-reported questionnaire.

**Data Management**

All tracing and contacting information, perinatal and maternal obstetric data and clinical assessment data will be recorded in a database developed specifically for this study. Participants will be assigned an individual study code number and all data will be identified only using this code. The study database will be password protected and available only to staff directly involved with the study.

All participant information will be stored for at least ten years in locked filing cabinets at the Liggins Institute, University of Auckland, under the responsibility of the study investigators.

**Intra- and Interobserver Variability**

Clinical assessments will be undertaken by the same study nurse and one of two study doctors, with the same routine adhered to for each assessment. MRI scans will be undertaken by two of four MRI radiographers, all of whom have received training on MR imaging of myocardial perfusion and are very familiar with the study protocol. All MRI scans will be analysed by the same radiologists and all blood samples will be analysed by the same laboratory.

**Dissemination of Results to Participants**

All participants will be asked at the clinical assessment if they would like a copy of their body size, blood pressure and blood test results to be sent to them and/or their general practitioner. Results will be posted or emailed to participants, according to the individual’s preference, together with a letter thanking them for their participation. Results will be posted to general practitioners, together with a letter briefly explaining the study and asking the general practitioner to follow up any abnormal findings as required. If clinically significant incidental findings are present, the study investigator will contact participants by telephone to inform them of the abnormal finding(s) and advise them to see their general practitioner for further assessment and follow up.

MRI scans results will not routinely be provided to participants. However, the study investigator will notify participants by telephone of clinically significant incidental findings as soon as these are identified and advise them to see their general practitioner for further assessment and follow up. Offer will also be made to notify the participant’s general practitioner of the abnormal finding(s). Following contact by the study investigator, a letter will be sent to the participant by the Centre for Advanced MRI together with an electronic copy of the MRI scan.

All participants will be asked at the clinical assessment if they would like to receive a copy of the final results of the study.

**Sample size**

Based on a 2008 pilot study in which we attempted to contact IUT recipients, we estimate that 80 sibling pairs may be available and consent to participate. This sample size would result in power to detect a difference of 0.45 standard deviations for cardiac end points between study groups (alpha=0.05, beta=0.2, JMP Statistical Discovery software, version 10.0.0, SAS Institute, Cary, USA). This compares well with the sheep studies, where one week of fetal anaemia resulted in an increase in maximal coronary conductance in adulthood of 2.8 standard deviations.

**Statistical analyses**

Continuous variables will be compared with unpaired t tests or Mann-Whitney tests for parametric and non-parametric data respectively. Categorical data will be compared with Chi-squared tests as appropriate. Variables with skewed distributions will be log-transformed. If the distribution remains skewed, data will be compared as medians. Differences for categorical data and normal data will be reported as relative risk (95% CI) and difference between means (95% CI) respectively. Differences between log-transformed data will be reported as a ratio of geometric means (95% CI). Differences between skewed continuous
data will be reported as difference between medians (95% CI). Primary analysis will be unadjusted. Secondary analysis will adjust for potential cofounders that are not well matched between groups (such as age, gestation at birth, birth weight z-score, amount of exercise, smoking status) using multiple linear regression.

4) Significance of Research
If this study shows that there are no long-term health effects associated with fetal anaemia treated with IUT, then the long-term safety of this treatment will be established. Each year thousands of infants are exposed to this treatment around the world. Participants treated in Auckland remain the first and thus the oldest such individuals treated for fetal anaemia with IUT in the world. As records are available in Auckland to trace these individuals, a unique opportunity exists in Auckland to investigate the long-term consequences of this treatment.

If this study confirms that fetal anaemia results in changes to cardiac conductance, flow or coronary artery architecture, this will be new knowledge regarding implications for the programming of adult disease risk. The application of these results will extend far beyond just those participants who have received IUT for rhesus disease and will be of considerable interest to obstetricians and paediatricians treating preterm infants and twins.

Preterm birth, defined as birth before 37 completed weeks gestation, currently accounts for up to 12% of all live births. The majority of preterm babies now survive, so that ex-preterm survivors are rapidly becoming a substantial proportion of the adult population, particularly in developed countries. While the short-term complications of preterm birth have been known for many decades, until recently little research focused on long-term follow up, apart from neurodevelopmental and respiratory outcomes into adolescence. There is now an increasing body of evidence suggesting long-term effects of preterm birth on cardiovascular risk factors. Dr Dalziel has shown that young adults born preterm have double the risk of later hypertension and increased insulin resistance, despite the majority of the group studied being born only moderately early at >32 weeks gestation.

Furthermore, anaemia of prematurity occurs in up to 50% of those born at <32 weeks gestation. The mechanisms underlying this anaemia are multifactorial and include inadequate red blood cell production due to immaturity of erythropoietin system, shortened erythrocyte life span, haemolysis and iatrogenic blood loss (secondary to multiple blood sampling for monitoring of unwell neonates). Although the pathophysiology of anaemia of prematurity is well understood, there is controversy regarding the timing and methods of intervention, due to concerns about the risks associated with transfusion and the expense of interventions such as erythropoietin. Information from this study will be invaluable in determining potential long-term risks associated with perinatal anaemia for this group.

Finally, multiple births, mainly twin pregnancies, account for a growing proportion of all live births and are responsible for a quarter of preterm births. In addition to the risks of anaemia of prematurity described above, twin pregnancies are also at risk of in utero twin-to-twin transfusion syndrome, whereby transfusion of blood occurs from one twin to the other via placental vascular anastomoses. This results in one twin being anaemic and the other polycythaemic. Twin-to-twin transfusion syndrome occurs in a quarter of monochorionic twin pregnancies and results in interventions to the anaemic twin ranging from IUT to preterm delivery for postnatal transfusion. The long-term consequences of these problems and their treatment are also unknown.

References


