Long-term cardiovascular outcome following fetal anaemia and intrauterine transfusion: a cohort study

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ABSTRACT
Objective To compare long-term cardiovascular outcomes in survivors of fetal anaemia and intrauterine transfusion with those of non-anaemic siblings.

Design Retrospective cohort study.

Setting Auckland, New Zealand.

Participants Adults who received intrauterine transfusion for anaemia due to rhesus disease (exposed) and their unexposed sibling(s).

Exposure Fetal anaemia requiring intrauterine transfusion.

Main outcome measures Anthropometry, blood pressure, lipids, heart rate variability and cardiac MRI, including myocardial perfusion.

Results Exposed participants (n=95) were younger than unexposed (n=92, mean±SD 33.7±9.3 vs 40.1 ±10.9 years) and born at earlier gestation (34.3±1.7 vs 39.5±2.1 weeks). Exposed participants had smaller left ventricular volumes (end-diastolic volume/body surface area, difference between adjusted means −6.1, 95% CI −9.7 to −2.4 mL/m2), increased relative left ventricular wall thickness (difference between adjusted means 0.007, 95% CI 0.001 to 0.012 mm.mm−2/mL) and decreased myocardial perfusion at rest (ratio of geometric means 0.86, 95% CI 0.80 to 0.94). Exposed participants also had increased low frequency-to-high frequency ratio on assessment of heart rate variability (ratio of geometric means 1.53, 95% CI 1.04 to 2.25) and reduced high-density lipoprotein concentration (difference between adjusted means −0.12, 95% CI −0.24 to 0.00 mmol/L).

Conclusions This study provides the first evidence in humans that cardiovascular development is altered following exposure to fetal anaemia and intrauterine transfusion, with persistence of these changes into adulthood potentially indicating increased risk of cardiovascular disease. These findings are relevant to the long-term health of intrauterine transfusion recipients, and may potentially also have implications for adults born preterm who were exposed to anaemia at a similar postconceptual age.

INTRODUCTION
A plethora of evidence supports the association between adverse conditions before birth and cardiovascular disease in later life. For example, low birth weight and preterm birth are associated with hypertension, impaired glucose tolerance, adverse lipid metabolism and obesity in adulthood.1–4 Understanding the mechanisms underlying these associations may help to develop strategies to reduce the global burden of cardiovascular disease. Anaemia and its treatment with intrauterine transfusion during the period of cardiac plasticity may be one such mechanism. Near-term fetal sheep whose haematocrit was reduced by half for 7 days had a 50% increase in cardiac output, 30% increase in heart to body mass ratio, sixfold increase in coronary blood flow and a doubling of coronary conductance, suggesting growth of coronary resistance vessels, with these changes persisting into early adulthood.5–6 Furthermore, exposure to fetal anaemia resulted in increased infarct size following ischaemia-reperfusion injury in adult sheep, suggesting decreased tolerance to myocardial ischaemia.7 In humans, decreased left ventricular (LV) mass and left atrial area has been reported in children exposed to fetal anaemia and intrauterine transfusion,8 and preterm birth has been associated with altered LV geometry and increased mass.9 However, the effect of fetal anaemia and intrauterine transfusion on cardiovascular outcome in adult humans is unknown. Our objective was to measure cardiac structure and function, and cardiovascular risk factors in adults exposed to fetal anaemia severe enough to require treatment with intrauterine transfusion.

The technique of intrauterine transfusion for treatment of severe fetal anaemia was pioneered at National Women’s Hospital, Auckland, New Zealand...
Zealand in 1963. We traced adult survivors of intrauterine transfusion treated at this hospital, and compared cardiovascular risk factors, LV volumes and mass and myocardial perfusion with that of unexposed siblings.

**METHODS**

**Participants**

Study participants were adults born between 1963 and 1992, who received intrauterine transfusion for rhesus disease (‘exposed’) at National Women’s Hospital, and their unexposed siblings. Intrauterine transfusion recipients were traced from hospital archives, and siblings via exposed participants. Exclusion criteria were residence outside New Zealand with no intention to return within the study timeframe, no unexposed sibling available, pregnancy, medical condition preventing participation and contraindication to MRI or gadolinium contrast. The study was approved by the New Zealand Multi-Region Ethics Committee and participants provided written informed consent.

**Study protocol**

All assessments were performed on the same day, with siblings attending together wherever possible, between January 2010 and July 2012. Height, weight and head circumference were measured using standard techniques, and body surface area calculated. Blood pressure was the mean of three measures with the participant sitting, using a digital device (GE Dash 4000, GE Healthcare, Wisconsin, USA). Blood samples were taken for fasting lipids and glucose. Participants then underwent a standard 2-hour 75 g oral glucose tolerance test.

Heart rate variability data were collected from lead II at amplitude 2 mV with participants supine during a period of quiet wakefulness using a PowerLab 4/25T system (ADInstruments, Dunedin, NZ). Five-minute traces without movement artefact were analysed using LabChart 7 Pro-analysis software (ADInstruments) and standard spectrum bandwidth settings.

Cardiac MRI was performed on a 1.5 Tesla Avanto scanner (VB11 to VB17 software, Siemens, Erlangen, Germany) to determine LV end-diastolic, end-systolic and stroke volumes, LV mass and myocardial perfusion. The study protocol, including MRI details, is available online (URL: http://hdl.handle.net/2292/22774) and in the online supplementary methods document.

All laboratory, MRI and analysis staff were blinded to participants’ fetal anaemia status.

Perinatal and maternal obstetric data were collected from archived hospital records, and birth weight z-score calculated.

Data regarding ethnicity and lifestyle factors were self-reported by questionnaire. Questions relevant to the data reported in this manuscript are included in the online supplementary methods document.

**Statistical analysis**

Based on a pilot study, we estimated that 80 sibling pairs would be available to participate. This sample size would result in power to detect a difference of 0.45 SDs for cardiac end points between study groups ($\alpha=0.05$, $\beta=0.2$, JMP V10.0.0, SAS Institute, Cary, North Carolina, USA).

Continuous data were log-transformed to approximate a normal distribution where required. Groups were compared using analysis of variance or Wilcoxon tests for continuous data and $\chi^2$ tests for categorical data. Multiple regression analyses were performed with participants nested within sibling groups, and adjusted for age, sex and birth weight z-score.

Data are presented as adjusted mean (95% CI), antilog-transformed geometric mean (95% CI), median (range), number (percentage), difference between means (95% CI) and ratio of geometric means (95% CI). If the 95% CI for a ratio of geometric means includes 1, there is no significant difference between groups. As this was an exploratory analysis designed to identify potentially important associations for future investigation, no correction was made for multiple comparisons.

**RESULTS**

Four hundred and seventy-three fetuses received intrauterine transfusion at National Women’s Hospital between 1963 and 1992, of whom 228 were presumed to have survived to adulthood (figure 1). Of these, 199 were successfully contacted, 92 were excluded and 95 (89%) of the remaining 107 were studied, together with 92 unexposed siblings. As there was more than one exposed or unexposed participant in some families, this equated to 88 sibling groups.

Sex distribution and ethnicity of exposed and unexposed participants were similar. However, exposed participants were younger than unexposed siblings, born at an earlier gestation, and of lower birth weight, but had higher birth weight z-scores (table 1).

Maternal and perinatal characteristics of exposed participants were similar to the 133 adult survivors of intrauterine transfusion who did not participate (table 1). Of exposed participants, 24 (25%) received antenatal corticosteroids, 25 (26%) required ventilation in the first 24 hours, 81 (85%) received at least one exchange transfusion, 54 (57%) received phototherapy and 59 (62%) received at least one postnatal top-up transfusion.

**Cardiovascular disease risk factors**

Exposed and unexposed participants reported similar tobacco and alcohol use, physical activity and indices of socioeconomic status (data not shown), and were similar in body size, blood pressure and glucose tolerance. They also had similar fasting cholesterol, low-density lipoprotein and triglyceride concentrations and total cholesterol/high-density lipoprotein (HDL) ratio, but fasting HDL concentration was lower in exposed participants (table 2).

Heart rate variability was assessed in 55 (58%) exposed and 56 (61%) unexposed participants, comprising 54 (61%) sibling groups due to limited availability of equipment. Participants who underwent heart rate variability assessment were older than the remainder of the cohort (mean±SD 40.8±8.8 vs 31.0±10.3 years, p<0.001), but there were no other differences between those who underwent this assessment and those who did not. Normalised low frequency power and low-to-high frequency ratio were higher in exposed participants (ratio of geometric means 1.21, 95% CI 1.00 to 1.46; p=0.05 and 1.53, 95% CI 1.04 to 2.25; p=0.03, respectively), indicating increased sympathetic tone. Normalised high frequency power was lower in exposed participants (ratio of geometric means 0.79, 95% CI 0.64 to 0.97; p=0.03), indicating decreased parasympathetic tone.

**Cardiac MRI findings**

Exposed participants had smaller indexed LV end-diastolic, end-systolic and stroke volumes than their unexposed siblings after adjustment for age, sex, birth weight z-score and sibship. Lower LV mass index in exposed participants did not reach statistical significance (p=0.06, table 3). Relative LV wall thickness was higher in exposed than in unexposed participants when assessed globally, and in the septum and free wall.

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Exposed participants had lower myocardial perfusion at rest, but not with cold pressor or adenosine-induced stress (figure 2). Mean arterial pressure and heart rate prior to myocardial perfusion assessment at rest and during adenosine-induced stress were similar in both groups.

**Potential confounders**

There was no relationship in exposed or unexposed participants between gestational age at birth and blood pressure, glucose tolerance, fasting lipid concentrations, heart rate variability parameters, LV volumes, mass or myocardial perfusion. In exposed participants, there was also no association between these variables and potential indicators of the severity of fetal anaemia (number of intrauterine transfusions, presence of hydrops at birth, cord blood bilirubin or haemoglobin concentrations, postnatal highest bilirubin and lowest haemoglobin concentration, see online supplementary tables S1 and S2).

![Figure 1](Selection of participants and reasons for exclusion. Neonatal death=death from birth to 28 days post-term equivalent age. Infant death=death from 28 days to 12 months post-term equivalent age.)

**Table 1** Baseline characteristics of study cohort

<table>
<thead>
<tr>
<th></th>
<th>Exposed participants (n=95)</th>
<th>Unexposed participants (n=92)</th>
<th>p Value*</th>
<th>Exposed non-participants (n=133)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of study (years)</td>
<td>33.7 (9.3)</td>
<td>40.1 (10.9)</td>
<td>&lt;0.001</td>
<td>33.6 (8.5)</td>
<td>0.80</td>
</tr>
<tr>
<td>Male sex n (%)</td>
<td>51 (54%)</td>
<td>41 (45%)</td>
<td>0.21</td>
<td>87 (65%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Ethnic origin n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>78 (82%)</td>
<td>78 (85%)</td>
<td>0.67</td>
<td>Unknown</td>
<td>–</td>
</tr>
<tr>
<td>Maori</td>
<td>13 (14%)</td>
<td>9 (10%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (4%)</td>
<td>5 (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age at delivery (years)</td>
<td>29.9 (4.5)</td>
<td>24.0 (4.4)</td>
<td>&lt;0.001</td>
<td>29.4 (5.3)</td>
<td>0.77</td>
</tr>
<tr>
<td>Gestation at birth (weeks)</td>
<td>34.5 (1.6)</td>
<td>39.5 (2.1)</td>
<td>&lt;0.001</td>
<td>34.5 (1.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Birth &lt;37 weeks n (%)</td>
<td>93 (98%)</td>
<td>10 (11%)</td>
<td>&lt;0.001</td>
<td>130 (98%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Normal vaginal delivery, n (%)</td>
<td>40 (42%)</td>
<td>66 (72%)</td>
<td>&lt;0.001</td>
<td>56 (42%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.5 (0.5)</td>
<td>3.3 (0.6)</td>
<td>&lt;0.001</td>
<td>2.5 (0.5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>0.8 (1.2)</td>
<td>−0.1 (1.2)</td>
<td>&lt;0.001</td>
<td>0.7 (1.2)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n (%). *For comparison with exposed participants.

As age, sex and birth weight z-score were independent predictors of outcome, these variables were corrected for in the statistical analysis. Exploratory analysis revealed that age at the time of scan was the most important contributor to differences between adjusted and unadjusted outcomes.

**DISCUSSION**

We have shown that fetal anaemia treated with intrauterine transfusion is associated with reduced LV volumes, increased relative LV wall thickness, altered sympathovagal tone, reduced myocardial perfusion at rest and lower HDL concentration in adulthood, findings which suggest that exposure to fetal anaemia and intrauterine transfusion may confer increased risk of cardiovascular disease.\(^\text{16–19}\) As the technique of intrauterine transfusion was pioneered in New Zealand in 1963,\(^\text{10}\) our cohort was recruited from the oldest group of intrauterine transfusion recipients in the world, and these findings will be relevant to many thousands of fetal anaemia survivors.

Since all exposed participants were younger than 50 years, we did not expect to find high rates of cardiovascular disease. However, a growing body of evidence indicates that unfavourable early life events may programme individuals for specific diseases in later life, and also influence the age of onset of these illnesses, accelerate ageing and shorten survival.\(^\text{20–21}\) Thus, our findings suggest that adult survivors of fetal anaemia and intrauterine transfusion may be at risk of earlier onset cardiovascular disease with ageing.

This is the first report of cardiovascular outcomes in adulthood following fetal anaemia and intrauterine transfusion. One previous study reported lower LV mass, and a trend to lower LV end-diastolic dimension (p=0.053) in 25 intrauterine transfusion recipients aged 3–15 years assessed by echocardiography and compared with age-matched and sex-matched controls.\(^\text{8}\) These results are in keeping with ours, although our findings provide statistically stronger evidence of smaller LV volumes in exposed participants, due to advantages in study design including larger sample size and use of sibling controls. In addition, our study was conducted in adults, in whom maturation of the cardiovascular system is complete, and cardiac MRI provided more accurate assessment of LV size. Data from fetal sheep suggest that exposure to fetal anaemia alters cardiomyocyte maturation, resulting in fewer and larger cardiomyocytes, with final cardiomyocyte...
endowment at birth determined by the balance between the timing of exposure to anaemia and its correction by intrauterine transfusion,\(^4\)\(^,\)\(^7\)\(^,\)\(^22\)\(^,\)\(^23\) observations which may help explain our findings of decreased LV size in exposed participants.

Although several studies in sheep have suggested that fetal anaemia results in alterations to coronary vessel architecture and increased susceptibility to myocardial damage following ischaemia, our findings are the first report of myocardial perfusion in human survivors of fetal anaemia and intrauterine transfusion. Contrary to reports from sheep, we found no evidence of increased myocardial perfusion with adenosine-induced vasodilation, suggesting that adult human coronary microcirculation structure is not altered by exposure to fetal anaemia and intrauterine transfusion. However, as resting coronary flow is controlled by arteriolar resistance, lower myocardial perfusion at rest and altered sympathovagal tone suggest that endothelial function may be impaired in exposed participants. As endothelial dysfunction is an independent risk factor for future cardiovascular events,\(^24\) our findings suggest that exposed participants may be at increased risk of myocardial ischaemia, consistent with reports of increased susceptibility to ischaemic myocardial injury in previously anaemic sheep.\(^7\)
Identification and tracing of exposed participants for this study was made more difficult by the length of time since intrauterine transfusion, reliance on archived paper-based medical records and, in many cases, a complete lack of records for affected infants. Despite these difficulties, nearly 90% of the adult survivors in our cohort of intrauterine transfusion recipients were successfully contacted, although over half of those contacted had no suitable sibling or were unable to participate. However, as we found no differences in the maternal and perinatal characteristics of exposed participants and non-participants, this is unlikely to have affected our findings.

It is possible that other differences between exposed and unexposed participants confounded our findings. Both groups were similar for several important cardiovascular risk factors, including smoking, hypertension, diabetes, hypercholesterolaemia, exercise participation and body habitus. These factors were therefore unlikely to have influenced outcomes. Furthermore, by using unexposed siblings as our comparison group, we hoped to minimise the impact of social, familial and genetic diversity. However, this strategy resulted in two unavoidable, and potentially important, differences between groups.

First, as rhesus disease is uncommon in first-born children, most unexposed participants were older than their exposed siblings. Since LV volumes and myocardial perfusion decrease with increasing age,25 we adjusted for age in our statistical analysis. However, as adjusted LV volumes and myocardial perfusion were lower in exposed participants than in their older unexposed siblings, the opposite outcome to that expected based on age alone, it is unlikely that differences in age account for our findings.

Second, exposed participants were born at an earlier gestation, and of lower birth weight than unexposed participants. A recent MRI investigation of LV function in young adults born preterm at a mean gestation of 30.3 weeks reported a 10% reduction in indexed end-diastolic, end-systolic and stroke volumes, and a 30% increase in LV mass index compared with age-matched and sex-matched term-born controls.9 The authors concluded that these alterations in cardiac structure were determined solely by gestational age. In contrast, in our cohort we found that birth weight z-score was an independent predictor of the outcomes of interest, but gestational age was not. Furthermore, on multiple logistic regression, only gestational age was significantly associated with exposed/unexposed status (p<0.001). Thus, it was neither possible nor necessary to adjust for gestational age in our analysis.

In addition, it is possible that differences in perinatal factors, such as receipt of antenatal corticosteroids, postnatal exchange and top-up transfusions, phototherapy and requirement for ventilation after birth contributed to the observed differences in cardiovascular outcomes between exposed and unexposed participants. As most unexposed siblings were born at term gestation after an uncomplicated pregnancy, it is unlikely that these perinatal factors were present in this group. However, within exposed participants there was no association between perinatal markers of severity of fetal anaemia and the outcomes of interest.

There are well-documented limitations of self-reported questionnaires.26 However, ours was completed by all study participants and was based on large national and internationally validated questionnaires.27–30

Our findings have several potentially important implications. First, they suggest that consideration should be given to appropriate advice regarding lifestyle and clinical oversight of survivors of fetal anaemia and intrauterine transfusion. While this work is exploratory and our findings do not permit quantification of the absolute increase in cardiovascular risk to which these individuals may be exposed, pending further investigation it would seem prudent to advise they follow routine advice regarding monitoring of cardiovascular risk factors and maintenance of a healthy lifestyle. Further assessment of endothelial function and exercise capacity may help clarify the significance of the cardiovascular changes observed in this study. However, follow-up into their sixth decade of life and beyond will be required to ascertain if the increased cardiovascular risk identified in our cohort translates into actual cardiovascular events in later life.

As the treatment of fetal anaemia has changed over the last 50 years, particularly with the advent of ultrasound and intra-vascular transfusion, we are uncertain whether these findings would be similar in a younger, more recently recruited cohort. However, our findings do suggest that further research is...
warranted regarding the optimal approach to intrauterine transfusion of anemic fetuses. As modern diagnostic techniques can provide detailed information regarding the severity of fetal anemia, cardiovascular compromise, growth and the response of the fetus to treatment, it should be possible to correlate factors such as lowest intrauterine haemoglobin, presence of hydrops and volume of blood transfused with cardiovascular outcomes in future cohorts. Such information may help clarify appropriate transfusion thresholds and optimal timing of intervention for future treatment of anemic fetuses.

Furthermore, our findings may have implications for the management of postnatal anemia in preterm infants, as it is possible that cardiovascular changes similar to those seen in adults who were anemic in utero may also result when anemia occurs ex utero at the same developmental stage. Although increased LV mass and altered LV geometry has been documented in adult survivors of preterm birth, the influence of postnatal anemia on cardiovascular outcome was not investigated. There is considerable controversy regarding transfusion thresholds for preterm infants, and two large randomised controlled trials are currently under way to investigate neurodevelopmental outcome in preterm infants treated with ‘liberal’ or ‘restrictive’ transfusion policies. Our findings suggest that consideration should also be given to the assessment of cardiovascular outcomes in babies recruited to these trials.

In conclusion, compared with unexposed siblings, adult survivors of fetal anemia and intrauterine transfusion have smaller and relatively thicker walled left ventricles, decreased myocardial perfusion at rest suggestive of coronary endothelial dysfunction and augmented sympathetic tone. This study provides the first evidence in humans that exposure to fetal anemia and intrauterine transfusion is associated with altered cardiovascular development that may confer increased risk of cardiovascular disease in later life.

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Contributors All authors contributed to the study design and reviewed the draft manuscript. In addition, AHW and SRD contributed to the literature search, study design, data collection, analysis and interpretation; BRC and AAY contributed to the design of the MRI protocol and analysis of MRI data; KLT contributed to data interpretation and JEH contributed to data analysis and interpretation, approved the final manuscript as submitted and had overall responsibility for the study.

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Competing interests None declared.

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Ethics approval New Zealand Multi-Region Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Unpublished data relating to the perinatal characteristics of the study cohort, and the socioeconomic, demographic and self-reported health status of study participants are available to authors JH, SD and AW. No other unpublished data are available.

REFERENCES