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Increased cardiovascular risk in adult survivors of fetal anemia

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Abstract

Importance—Brief exposure to intrauterine anemia doubles coronary conductance but increases susceptibility to cardiac ischemic injury in adult sheep. The effects of fetal anemia in humans on cardiovascular outcomes in adulthood has not previously been investigated.

Objective—To compare cardiovascular disease risk factors in adult survivors of fetal anemia with that of non-anemic siblings.

Design—A retrospective observational cohort study (The Fetal Anemia Study), undertaken between January 1, 2010 and July 31, 2012.

Setting—Tertiary hospital, Auckland, New Zealand.

Participants—Exposed participants (n=95) were adults who received intrauterine transfusion at National Women's Hospital, Auckland between 1963–92 for treatment of severe anemia due to

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Author Contributions:

AHW and JEH had full access to all of the data in the study and take responsibility for the integrity for the data and the accuracy of the data analysis.

AHW and SRD contributed to the study design, data collection, analysis and interpretation. AHW wrote the first draft of the manuscript, and contributed to subsequent revisions. BRC and AAY contributed to the design of the MRI protocol, analysis of MRI data. KLT contributed to the study design, data interpretation. JEH contributed to the study design, data analysis and interpretation, and to writing all versions of the manuscript, and had overall responsibility for the study. All authors contributed to the final manuscript.

Conflict of Interest Disclosures:

Drs Cowan and Young report that they have received personal fees from Siemens AG Healthcare, outside the submitted work. No other conflicts of interest declared.

Additional Contributions:

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rhesus disease, resident in New Zealand and with a suitable non-anemic sibling. Unexposed participants (n=92) were siblings of exposed participants. Of potentially eligible exposed participants, 86% agreed to participate.

Exposure—Severe fetal anemia secondary to rhesus disease vs no intrauterine anemia.

Main Outcomes—Height, weight, body mass index, blood pressure, fasting lipid concentrations, heart rate variability, and cardiac MRI-determined left ventricular function and myocardial blood flow at rest, with cold pressor stress, and adenosine-induced vasodilation. Participant characteristics included gestation and weight at birth, age, and comorbid disease.

Results—Exposed participants were younger than unexposed (mean±SD: 33.7±9.3 vs 40.1±10.9 years, $p<0.001$), born earlier (34.3±1.7 vs 39.5±2.1 weeks, $p<0.001$), had decreased high-density lipoprotein concentration (−0.12 mmol/l, −0.24 to 0.00, $p=0.04$), and increased low to high frequency heart rate variability ratio (ratio of geometric means 1.53, 1.04 to 2.25, $p=0.03$). Exposed participants also had smaller left ventricular volumes (end diastolic volume/body surface area, difference between adjusted means −6.09 ml, 95% CI −9.75 to −2.42, $p=0.001$), increased relative left ventricular wall thickness (0.007 mm.m²/ml, 0.001 to 0.012, $p=0.01$), and reduced myocardial blood flow at rest (ratio of geometric means 0.89, 0.84 to 0.95, $p<0.001$), but not with cold pressor stress or adenosine-induced vasodilation.

Conclusions and Relevance—Adult survivors of fetal anemia have an unfavorable lipid profile, augmented sympathetic tone, smaller, thicker walled hearts, and evidence of coronary endothelial dysfunction. Fetal anemia may be associated with increased cardiovascular disease risk in adulthood.

Cardiovascular disease is the leading cause of death worldwide,¹ with estimated health care costs in the USA of \$315 billion in 2010.²

A plethora of evidence supports the association between adverse conditions before birth and cardiovascular disease in later life, the so-called Developmental Origins of Health and Disease.³ For example, low birth weight and preterm birth are associated with hypertension, impaired glucose tolerance, adverse lipid metabolism, and obesity in adulthood.^{4–9} Understanding the mechanisms underlying these associations may help in the development of strategies to reduce the global burden of cardiovascular disease.

One such mechanism may be anemia during the intrauterine period of cardiac plasticity. Near term fetal sheep whose hematocrit was reduced by half for only seven days had a 50% increase in cardiac output, 30% increase in heart to body mass ratio, six-fold increase in coronary blood flow, and a doubling of coronary conductance, suggesting growth of coronary resistance vessels.^{10,11} Importantly, these changes persist into early adulthood.¹¹ Furthermore, although exposure to fetal anemia resulted in adult sheep with improved indices of left ventricular systolic function during hypoxemia, suggesting a possible functional advantage,¹² increased infarct size following ischemia-reperfusion injury has also been reported, suggesting decreased tolerance to myocardial ischemia.¹³ As the effect of fetal anemia on human cardiac development and cardiovascular risk is unknown, our objective was to measure cardiac structure and function, and cardiovascular risk factors in adults exposed to severe fetal anemia.

The technique of intrauterine transfusion for treatment of severe fetal anemia was pioneered at National Women's Hospital, Auckland, New Zealand in 1963.¹⁵ We traced adult survivors of intrauterine transfusion treated at this hospital, and thus the oldest such cohort globally, and compared cardiovascular risk factors, left ventricular volumes and mass, and myocardial blood flow with that of their unexposed siblings.

Methods

Study Design

We undertook this cohort study at the University of Auckland, Auckland, New Zealand, between January 1, 2010 and June 30, 2012. The study was approved by the New Zealand Multi-Region Ethics Committee and participants provided written informed consent.

Participants

The study participants were adults who had received intrauterine transfusion for rhesus disease ("exposed") at National Women's Hospital, Auckland, New Zealand, and their unexposed siblings. Intrauterine transfusion recipients born between 1963 and 1992 were traced from hospital archives, and siblings via the exposed participants. Exclusion criteria were residence outside New Zealand with no intention to return within the study timeframe, no unexposed sibling available, pregnant, medical condition preventing participation, and contraindication to magnetic resonance imaging (MRI) or gadolinium contrast (Figure 1).

Study Interventions and Measurements

The study protocol is available online. (URL:<http://hdl.handle.net/2292/22774>) All assessments were performed on the same day, with siblings attending together wherever possible. Height, weight, and head circumference were measured using standard techniques, and body surface area was calculated.¹⁶ 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 data acquisition system (ADInstruments, Dunedin, NZ). Five-minute traces without movement artefact were analyzed using Labchart 7 Pro analysis software (ADInstruments, Dunedin, NZ) and standard spectrum bandwidth settings.¹⁷ Variables recorded were: standard deviation of normal R-R intervals, percentage of R-R intervals that differ by >50 ms from the preceding interval, total power, low frequency power in standard and normalized units, high frequency power in standard and normalized units, and the low to high frequency power ratio.

Cardiac MRI was performed on a 1.5 Tesla Avanto scanner (VB11 to VB17 software, Siemens, Erlangen, Germany) to determine left ventricular end-diastolic, end-systolic, and stroke volumes, left ventricular mass, and myocardial blood flow. Steady state free-precession images were obtained during approximately 10 heartbeat breath-holds for two,

three and four chamber long axis slices and a stack of 12 short-axis views from base to apex (slice thickness 7 mm; inter-slice gap of 1 to 3 mm).

Perfusion images were acquired using gadolinium contrast (0.04 mmol/kg) for quantification of myocardial blood flow at rest, during cold pressor challenge and at maximal vasodilation after a 3-minute adenosine infusion (0.14 mg/kg/min). Ten minutes was allowed between perfusion scans for gadolinium wash-out. Three or four short axis images were acquired using a T1 weighted single-shot gradient echo sequence with a saturation recovery magnetization preparation.¹⁸

Cardiac volumes were determined by segmenting the endo- and epicardial borders of all cine images using guide-point modeling to create a four-dimensional finite-element model of the left ventricle, followed by calculation of the cavity and myocardial volumes by numerical integration.¹⁹ End-systole and -diastole were identified as the phases with minimum and maximum volume.

The perfusion images were motion corrected using non-rigid registration, and the endo- and epicardial contour defined. Signal saturation and coil sensitivity effects were corrected, and the arterial input function measured in the blood pool at the base of the left ventricle. A B-spline analysis with appropriate regularization was used to calculate the myocardial blood flow in a range of myocardial regions of interest.²⁰

All laboratory and MRI imaging and analysis staff were blinded to the participants' fetal anemia status.

Perinatal and maternal obstetric data were collected from archived hospital records. Birth weight z-score was calculated using the LMS method.²¹ Data regarding ethnicity and lifestyle factors were self-reported by questionnaire.

Statistical Analysis

Based on a 2008 pilot study, we estimated that 80 sibling pairs may 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).

Continuous data were tested for normality using the Shapiro-Wilk test and skewed data were log-transformed to approximate a normal distribution where possible. Continuous variables were compared with one-way analysis of variance or Wilcoxon tests and categorical data were compared with Chi squared tests. 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 mean (SD) for unadjusted data, mean (95% CI) for adjusted data, 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.²² Correction for multiple comparisons was not applied as this was an exploratory

investigation designed to identify potentially important associations within the data set that could provide the basis for future investigations.²³

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, 89 were excluded, and 95 (86%) of the remaining 110 agreed to participate and were studied, together with 92 unexposed siblings (88 sibling groups).

Maternal and perinatal characteristics of exposed participants were similar to the 133 adult survivors of intrauterine transfusion who did not participate. 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. Sex distribution and ethnicity were similar (Table 1).

Cardiovascular Disease Risk Factors

Exposed and unexposed participants reported similar tobacco and alcohol use, physical activity and indices of socio-economic 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 ratio, but fasting high-density lipoprotein concentration was lower in exposed participants (difference between adjusted means -0.12 mmol/l, 95% CI -0.24 to 0.00 ; $p=0.04$) (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) versus 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 the 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. Normalized 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 left ventricular end-diastolic, end-systolic and stroke volumes than their unexposed siblings after adjustment for age, sex, birth weight z-score and sibship. Lower left ventricular mass index in exposed participants did not reach statistical significance ($p=0.06$, Table 3). In addition, end diastolic wall thickness to volume ratio was higher in exposed than in unexposed participants when assessed globally, in the septum and in the free wall.

Exposed participants had lower myocardial blood flow at rest, but not with cold pressor or adenosine-induced stress (Table 3). Mean arterial pressure and heart rate prior to myocardial blood flow 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, lipid status, heart rate variability parameters, left ventricular volumes, mass or myocardial blood flow. In exposed participants there was also no association between these variables and potential indicators of the severity of fetal anemia (number of intrauterine transfusions, presence of hydrops at birth, cord blood bilirubin or hemoglobin concentrations, postnatal highest bilirubin and lowest hemoglobin concentration, data not shown).

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 anemia is associated with reduced left ventricular volumes, increased wall thickness to volume ratio, lower high-density lipoprotein concentration, altered sympathovagal tone, and reduced myocardial blood flow at rest in adulthood; findings which suggest that exposure to fetal anemia may confer increased risk of cardiovascular disease.^{24–27} As the technique of intrauterine transfusion was pioneered in New Zealand in 1963,¹⁵ our cohort was recruited from the oldest reported group of intrauterine transfusion recipients in the world, and these findings will be relevant to thousands of fetal anemia survivors throughout the world.

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 unfavorable early life events may not only programme individuals for specific diseases in later life, but also influence the age of onset of these illnesses, accelerate ageing and shorten survival.^{28–30} Thus, our findings suggest that adult survivors of fetal anemia may be at risk of earlier onset or greater severity of cardiovascular disease with ageing.

This is the first report of cardiovascular outcomes in adulthood following fetal anemia. One previous study reported lower left ventricular mass, and a trend to lower left ventricular end-diastolic dimension ($p=0.053$) in 25 intrauterine transfusion recipients aged 3 to 15 years assessed by echocardiography and compared to age and sex-matched controls.³¹ These results are in keeping with ours, although our findings provide statistically stronger evidence of smaller left ventricular 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 left ventricular size.

Although several studies in sheep have suggested that exposure to fetal anemia may result in alterations to coronary vessel architecture and increased susceptibility to myocardial damage following ischemic insult,^{11,13} ours is the first study to report myocardial blood flow in human survivors of fetal anemia. Contrary to reports from sheep, we found no evidence of increased coronary flow with adenosine-induced vasodilation, suggesting that human coronary microcirculation structure is not altered by exposure to fetal anemia. However, as resting coronary flow is controlled by arteriolar resistance, lower myocardial blood flow at rest and altered sympathovagal tone suggest that endothelial function may be impaired in exposed participants, consistent with reports of increased susceptibility to ischemic myocardial injury in previously anemic sheep.¹³

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, hypercholesterolemia, 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 minimize the impact of social, familial and genetic diversity. However, this strategy resulted in two unavoidable, and potentially important, differences between groups.

Firstly, as rhesus disease is uncommon in first-born children, most unexposed participants were older than their exposed siblings. Since left ventricular volumes and myocardial blood flow decrease with increasing age,^{32,33} we adjusted for age in our statistical analysis. However, as left ventricular volumes and myocardial blood flow 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.

Secondly, exposed participants were born at an earlier gestation, and of lower birth weight than unexposed participants. A recent MRI investigation of left ventricular 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 left ventricular mass index compared to age and sex-matched term-born controls.³⁴ 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.

Our findings have several potentially important implications. Firstly, they suggest that consideration should be given to appropriate advice regarding lifestyle and clinical oversight of survivors of fetal anemia. While our findings do not permit quantification of the absolute increase in cardiovascular risk to which survivors of fetal anemia may be exposed, further assessment of endothelial function and exercise capacity may help clarify whether the cardiovascular changes observed are of functional significance. 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.

Our findings also 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 hemoglobin, 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 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. There is considerable controversy regarding transfusion thresholds for preterm infants, and two large randomized controlled trials are currently under way to investigate neurodevelopmental outcome in preterm infants treated with “liberal” or “restrictive” transfusion policies.^{35,36} Our findings suggest that consideration should also be given to the assessment of cardiovascular outcomes in babies recruited to these trials.

Conclusions

Adult survivors of fetal anemia have smaller, relatively thicker walled left ventricles, altered sympathovagal tone, decreased myocardial blood flow and an unfavorable lipid profile in comparison to unexposed siblings. This study provides the first evidence in humans that exposure to fetal anemia may confer increased risk of cardiovascular disease in later life.

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Figure 1. Selection of participants and reasons for exclusion

IUT=intrauterine transfusion. 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

	Exposed participants	Unexposed participants		Exposed non-participants	
	(N=95)	(N=92)	p-value *	(N=133)	p-value *
Age at time of study - yr	33.7 (9.3)	40.1 (10.9)	<0.001	33.6 (8.5)	0.80
Male sex – no. (%)	51 (54%)	41 (45%)	0.21	87 (65%)	0.07
Ethnic origin – no. (%)					
NZ European	78 (82%)	78 (85%)	0.67	unknown	–
Maori	13 (14%)	9 (10%)			
Other	4 (4%)	5 (5%)			
Maternal age at delivery - yr	29.9 (4.5)	24.0 (4.4)	<0.001	29.4 (5.3)	0.77
Gestation at birth - weeks	34.5 (1.6)	39.5 (2.1)	<0.001	34.5 (1.6)	1.00
Birth <37 weeks – no. (%)	93 (98%)	10 (11%)	<0.001	130 (98%)	0.94
Normal vaginal delivery – no. (%)	40 (42%)	66 (72%)	<0.001	56 (42%)	0.84
Birth weight - kg	2.5 (0.5)	3.3 (0.6)	<0.001	2.5 (0.5)	0.16
Birth weight z-score	0.8 (1.2)	–0.1 (1.2)	<0.001	0.7 (1.2)	0.10

Data are mean (SD) or no. (%).

* for comparison with exposed participants.

Table 2

Body size, blood pressure, glucose tolerance, fasting lipids, and heart rate variability in exposed and unexposed participants

	Unadjusted			Adjusted [†]			p-value
	Exposed	N	Unexposed	N	Exposed	Unexposed	
Body size							
Body mass index - kg/m ²	26.6 (5.5)	95	26.0 (4.6)	92	26.8 (25.7 to 27.8)	25.8 (24.7 to 26.9)	1.0 (−0.6 to 2.6)
Body surface area - m ²	1.97 (0.24)	95	1.92 (0.23)	92	1.95 (1.91 to 1.99)	1.94 (1.90 to 1.98)	0.01 (−0.05 to 0.07)
Blood pressure							
Systolic - mmHg	122.7 (13.9)	95	125.0 (14.4)	92	123.3 (120.5 to 126.2)	124.5 (121.7 to 127.4)	−1.2 (−5.5 to 3.1)
Diastolic - mmHg	73.0 (9.9)	95	74.3 (10.7)	92	73.9 (71.9 to 75.9)	73.5 (71.5 to 75.5)	0.4 (−2.7 to 3.4)
Glucose tolerance							
Fasting glucose - mmol/l	4.71 (0.51)	93 [‡]	4.88 (0.59)	92	4.76 (4.65 to 4.87)	4.84 (4.73 to 4.96)	−0.08 (−0.25 to 0.09)
120 minute glucose - mmol/l	5.13 (1.87)	93 [‡]	5.64 (2.09)	92	5.21 (4.78 to 5.64)	5.56 (5.12 to 5.99)	−0.35 (−1.00 to 0.31)
Fasting lipids							
Total cholesterol - mmol/l	4.75 (0.88)	95	5.01 (0.82)	92	4.85 (4.68 to 5.03)	4.90 (4.72 to 5.08)	−0.05 (−0.32 to 0.22)
HDL cholesterol - mmol/l	1.42 (0.33)	95	1.59 (0.44)	92	1.44 (1.37 to 1.52)	1.56 (1.49 to 1.64)	−0.12 (−0.24 to 0.00)
LDL cholesterol - mmol/l	2.85 (0.80)	95	2.94 (0.81)	92	2.93 (2.76 to 3.09)	2.86 (2.69 to 3.03)	0.07 (−0.19 to 0.32)
Triglycerides - mmol/l	1.05 (0.52)	95	1.09 (0.69)	92	1.05 (0.92 to 1.18)	1.09 (0.96 to 1.23)	−0.04 (−0.24 to 0.15)
Total cholesterol/HDL ratio	3.50 (1.03)	95	3.40 (1.08)	92	3.52 (3.30 to 3.74)	3.38 (3.16 to 3.60)	0.14 (−0.19 to 0.48)
Heart rate variability							
SDNN - ms	51.0 (45.6 to 57.1)	55	41.3 (35.9 to 47.6)	56	47.6 (41.9 to 54.0)	44.3 (39.1 to 50.1)	1.08 (0.89 to 1.30) [*]
pNN50	9.4 (6.2 to 14.3)	55	5.9 (3.5 to 9.8)	56	7.6 (4.9 to 11.9)	7.5 (4.7 to 11.9)	1.02 (0.51 to 2.02) [*]
Total power - ms ²	2333 (1783 to 3053)	55	1492 (1138 to 1957)	56	2001 (1530 to 2616)	1738 (1332 to 2267)	1.15 (0.77 to 1.72) [*]
LF - ms ²	726 (564 to 936)	55	412 (301 to 562)	56	630 (475 to 835)	475 (359 to 628)	1.33 (0.87 to 2.03) [*]
LF _{nu}	53 (47 to 60)	55	46 (40 to 52)	56	54 (48 to 61)	45 (40 to 51)	1.21 (1.00 to 1.46) [*]
HF - ms ²	496 (355 to 693)	55	383 (263 to 559)	56	406 (284 to 579)	467 (328 to 665)	0.87 (0.51 to 1.49) [*]
HF _{nu}	36 (32 to 41)	55	42 (37 to 49)	56	35 (30 to 40)	44 (38 to 51)	0.79 (0.64 to 0.97) [*]
LF/HF ratio	1.46 (1.15 to 1.87)	55	1.07 (0.83 to 1.38)	56	1.55 (1.20 to 2.01)	1.02 (0.79 to 1.31)	1.53 (1.04 to 2.25) [*]

Unadjusted data are mean (SD) or geometric mean (95% CI). Adjusted data are mean or geometric mean (95% CI), and difference between means (95% CI) or ratio of geometric means (95% CI). A 95% CI for a ratio of geometric means is non-significant if it includes 1.

[†] Adjusted for age, sex, birth weight z-score and sibship.

[‡] 2 participants with previous diagnosis of diabetes excluded. HDL=high-density lipoprotein, LDL=low-density lipoprotein, SDNN=standard deviation of normal R-R (NN) intervals, pNN50=percentage of NN intervals that differ by >50 ms from the preceding interval, LF=low frequency power, LFNu=low frequency power expressed in normalized units, HF=high frequency power, HFnu=high frequency power expressed in normalized units.

Table 3

Left ventricular volumes and mass, myocardial blood flow at rest, with cold pressor stress, and with adenosine-induced stress, and myocardial perfusion reserve index in exposed and unexposed participants

	Unadjusted				Adjusted [†]				p-value
	Exposed	N	Unexposed	N	Exposed	Unexposed	Difference		
Indexed left ventricular volumes and mass									
EDV/BSA - ml/m ²	82.3 (12.7)	95	82.1 (15.0)	91	79.3 (76.9 to 81.7)	85.4 (83.0 to 87.9)	-6.1 (-9.7 to -2.4)	0.001	
ESV/BSA - ml/m ²	31.3 (7.3)	95	31.1 (8.7)	91	29.8 (28.3 to 31.2)	32.9 (31.4 to 34.3)	-3.1 (-5.3 to -1.0)	0.005	
SV/BSA - ml/m ²	50.9 (7.5)	95	50.9 (7.9)	91	49.5 (48.0 to 50.9)	52.5 (51.0 to 54.0)	-3.0 (-5.3 to -0.8)	0.007	
LV mass/BSA - g/m ²	66.2 (11.2)	95	65.2 (12.8)	91	64.7 (62.9 to 66.4)	67.2 (65.4 to 69.0)	-2.5 (-5.2 to 0.2)	0.06	
End diastolic wall thickness to volume ratio [‡]									
Global [§] - mm.m ² /ml	0.091±0.018	95	0.091±0.019	91	0.094 (0.091 to 0.098)	0.088 (0.084 to 0.091)	0.007 (0.001 to 0.012)	0.01	
Septal [¶] - mm.m ² /ml	0.096±0.018	95	0.096±0.020	91	0.099 (0.095 to 0.103)	0.092 (0.089 to 0.096)	0.007 (0.001 to 0.012)	0.02	
Free wall $\parallel$ - mm.m ² /ml	0.096±0.019	95	0.095±0.020	91	0.099 (0.095 to 0.103)	0.092 (0.088 to 0.095)	0.008 (0.002 to 0.013)	0.01	
Myocardial Blood Flow At rest									
Global [§] - ml/min/g	0.74 (0.70 to 0.79)	94	0.85 (0.81 to 0.89)	90	0.74 (0.70 to 0.78)	0.85 (0.81 to 0.90)	0.86 (0.80 to 0.94)*	<0.001	
Septal [¶] - ml/min/g	1.02 (0.95 to 1.09)	94	1.14 (1.06 to 1.21)	90	1.00 (0.94 to 1.07)	1.15 (1.07 to 1.23)	0.87 (0.79 to 0.97)*	0.01	
Free wall $\parallel$ - ml/min/g	0.75 (0.71 to 0.80)	94	0.85 (0.81 to 0.89)	90	0.74 (0.71 to 0.79)	0.85 (0.81 to 0.90)	0.87 (0.80 to 0.95)*	0.001	
With cold pressor stress									
Global [§] - ml/min/g	1.04 (0.97 to 1.12)	89	1.11 (1.05 to 1.18)	89	1.06 (0.99 to 1.13)	1.09 (1.02 to 1.16)	0.97 (0.88 to 1.07)*	0.58	
Septal [¶] - ml/min/g	1.29 (1.22 to 1.38)	89	1.39 (1.31 to 1.49)	89	1.31 (1.23 to 1.40)	1.37 (1.28 to 1.46)	0.96 (0.87 to 1.06)*	0.39	
Free wall $\parallel$ - ml/min/g	1.08 (1.02 to 1.15)	89	1.14 (1.07 to 1.21)	89	1.10 (1.04 to 1.16)	1.12 (1.05 to 1.19)	0.98 (0.90 to 1.07)*	0.68	
With adenosine stress									
Global [§] - ml/min/g	2.73 (2.58 to 2.88)	83	2.86 (2.68 to 3.06)	72	2.67 (2.55 to 2.89)	2.88 (2.69 to 3.08)	0.94 (0.86 to 1.04)*	0.24	
Septal [¶] - ml/min/g	2.97 (2.81 to 3.14)	83	3.16 (2.95 to 3.38)	72	2.98 (2.80 to 3.17)	3.15 (2.94 to 3.37)	0.95 (0.86 to 1.04)*	0.27	
Free wall $\parallel$ - ml/min/g	2.82 (2.67 to 2.98)	83	2.86 (2.67 to 3.06)	72	2.80 (2.63 to 2.97)	2.89 (2.71 to 3.09)	0.97 (0.88 to 1.06)*	0.49	

Data are mean or geometric mean (95% CI) and differences between means (95% CI) or

* ratio of geometric means (95% CI). A 95% CI for a ratio of geometric means is non-significant if it includes 1. EDV=end diastolic volume, BSA=body surface area, ESV=end systolic volume, SV=stroke volume,

[‡] Adjusted for age, sex, birth weight z-score and sibship.

[‡] End diastolic wall thickness to volume ratio=end diastolic wall thickness/indexed EDV.

[§] Average from all slices.

[¶] Average from inferoseptal and anteroseptal regions from all slices.

^{||} Average from anterior, antero-lateral, infero-lateral and inferior regions from all slices.