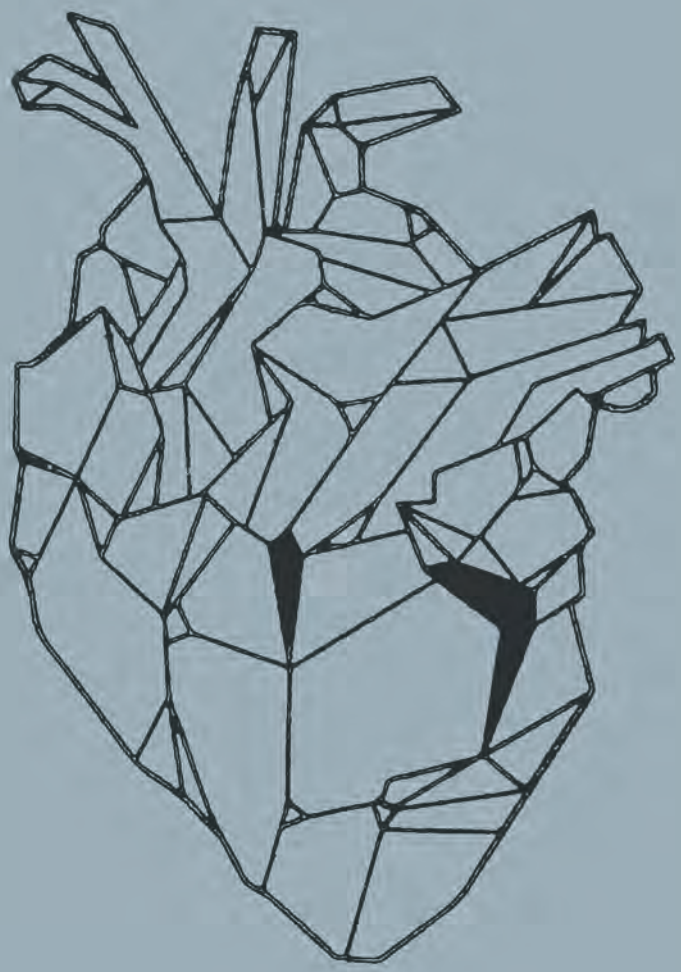
Early Life Growth, Adiposity and Cardiovascular Health in Childhood

The Generation R Study



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Early Life Growth, Adiposity and Cardiovascular Health in Childhood

The Generation R Study

Vroege groei, adipositas en cardiovasculaire gezondheid in de kindertijd

Het Generation R Onderzoek

Proefschrift

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Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. dr. R.C.M.E. Engels

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Manuscripts based on this thesis

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Toemen L, Gaillard R, van Osch-Gevers L, Helbing WA, Hofman A, Jaddoe VWV. Tracking of structural and functional cardiac measures from infancy into school-age. *Eur J Prev Cardiol. 2017 Sep;24(13):1408-1415.*

Chapter 2.2
Toemen L, de Jonge LL, Gishti O, van Osch-Gevers L, Taal HR, Steegers EA, Hofman A, Helbing WA, Jaddoe VW. Longitudinal growth during fetal life and infancy and cardiovascular outcomes at school-age. *J Hypertens. 2016 Jul;34(7):1396-406*

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Marinkovic T, **Toemen L**, Kruithof CJ, Reiss IK, van Osch-Gevers L, Hofman A, Franco OH, Jaddoe VWV. Early infant growth velocity patterns and cardiovascular and metabolic outcomes in childhood. *J Pediatr, 2017 Jul;186:57-63*

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Toemen L, Gaillard R, Roest AA, van der Geest RJ, Steegers EA, van der Lugt A, Helbing WA, Jaddoe VWV. Longitudinal fetal and childhood growth patterns are associated with cardiac measures assessed by cardiac Magnetic Resonance Imaging. The Generation R Study. *Eur J Prev Cardiol. 2019 Jul 29:2047487319866022*

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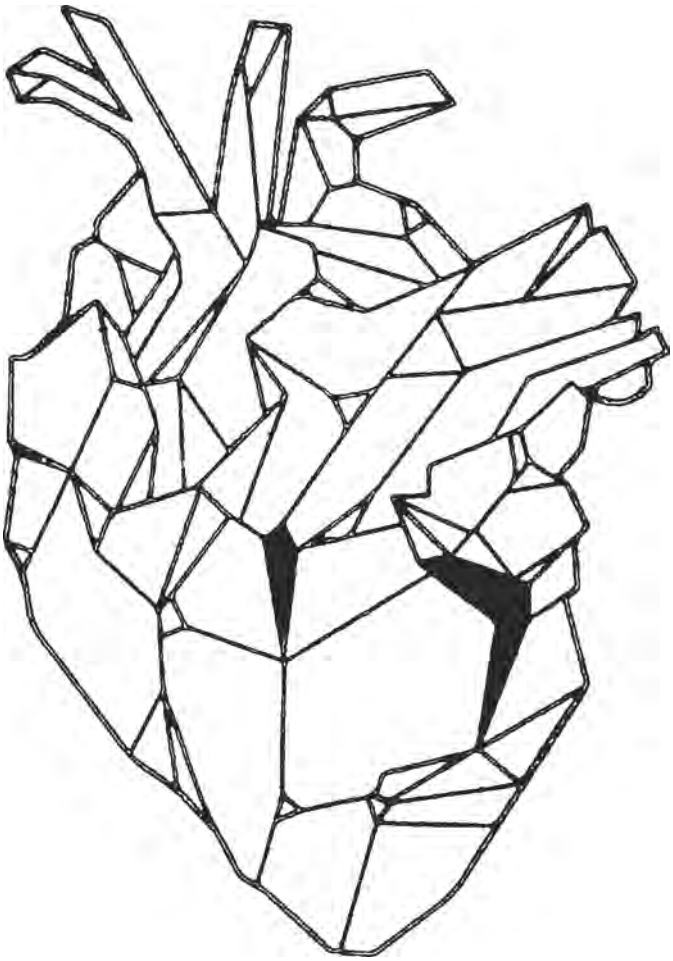
Chapter 3.3
Toemen L, Santos S, Roest AA, Vernooij MW, Helbing WA, Gaillard R, Jaddoe VWV. Pericardial adipose tissue, cardiac structures and cardiovascular risk factors in school-age children. *Submitted to European Heart J Cardiovasc Imaging.*

**Authors contributed equally*

Chapter 1 | General introduction



General introduction

Early origins of health and disease

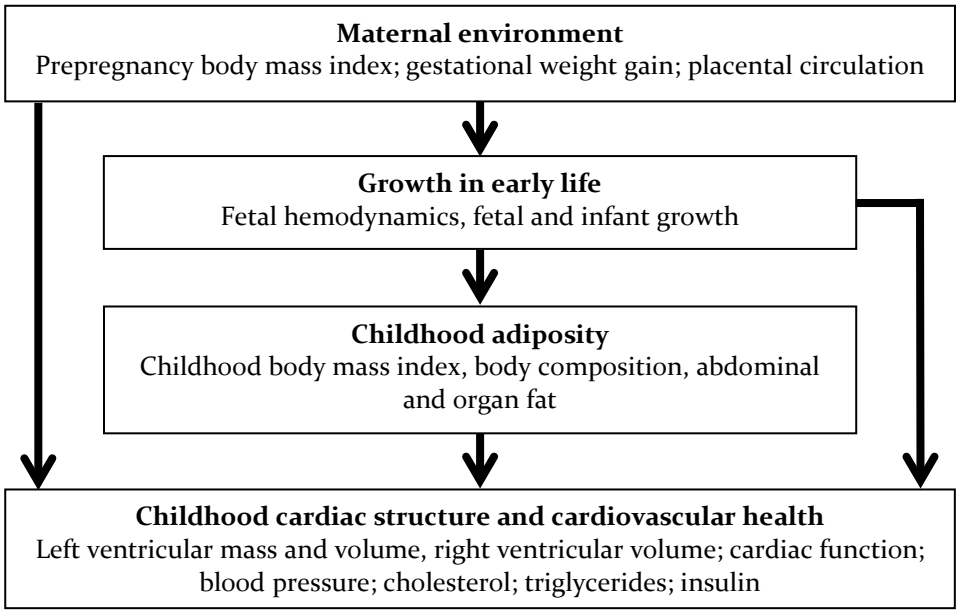
Cardiovascular disease is a major public health problem worldwide and might originate in early life.[1-3] The developmental origins hypothesis suggests that adverse exposures in fetal and postnatal life might lead to cardiovascular adaptations.[4, 5] This enables the individual to survive in the short term, but these adaptations can also predispose individuals to cardiovascular disease later in life.[5] Animal studies have shown that growth restriction in fetal life leads to adverse cardiovascular structure and function in later life.[6, 7]

Large observational studies in humans have shown that fetal growth restriction, followed by increased infant growth, were associated with cardiovascular disease.[3, 8, 9] These effects on the cardiovascular system are already present in childhood. Observational studies suggest that children with lower birth weight had higher blood pressure in childhood and altered cardiac structure.[10-12] Not only fetal life, but also early postnatal life is important for cardiovascular health. Rapid infant growth and adiposity are also associated with cardiovascular disease in adulthood, and with cardiovascular adaptations in childhood.[2, 13, 14]

Common risk factors for cardiovascular disease, such as blood pressure, lipid levels and obesity track from childhood to adulthood.[15-17] Tracking represents the maintaining of a given rank order relative to peers over time.[18] This means that children with higher blood pressure, lipid levels or obesity are more likely to also become adults with these risk factors and are therefore more likely to develop cardiovascular disease.

In summary, cardiovascular disease might originate in early life. Both an adverse fetal environment and an affluent postnatal environment seem to adversely affect cardiovascular health from childhood to adulthood. Identifying the risk factors and the sensitive periods in early life and the mechanisms through which early life affects cardiovascular health are important for future preventive strategies to ensure cardiovascular health later in the life course. Therefore, studies presented in this thesis were designed to identify fetal, infant and childhood factors associated with cardiovascular health outcomes in childhood (**Figure 1.1**). The studies are particularly focused on the role of growth and adiposity in specific early-life periods.

Figure 1.1 Overview of hypothesis for the associations of maternal, fetal, infant and childhood factors with cardiac structure and cardiovascular health in childhood



Cardiac development

In fetal life, cardiac growth is mainly determined by myocardial cell hyperplasia, while in late pregnancy or after birth this switches to myocardial cell hypertrophy.[7] Possibly, growth in utero affects cardiac size through altered hemodynamics and thus also wall stress, which can affect the maturation and sarcomere structure of cardiomyocytes.[19-21] This could affect the number, size and function of the cardiomyocytes around the time of birth, and program cardiomyocyte development after birth. A reduced number of cardiomyocytes at birth could mean the heart is more vulnerable to stress and damage later in life.[7] Postnatally, the heart grows rapidly to accommodate to the demands of the growing body. Physiological growth also occurs in response to physical activity and exercise.[7] In children, one of the main determinants of cardiac size is lean body mass.[22] Lean body mass is associated with an increase in blood volume, leading to a higher preload. Increase in volume and mass reduces wall stress that was caused by the increased demand, according to Laplace´s law.[23, 24] However, on the long term, maladaptation to increased demand can cause geometric remodeling, increasing the risk for cardiac disease. It is unclear how childhood cardiac size and geometry relate to adult cardiac morbidity and

mortality. We know that left ventricular mass tracks from childhood into young adulthood.[17, 18, 25] This could mean that early life cardiac development can place an individual at risk for later cardiac disease.

In adults, cardiac remodeling resulting in increased left ventricular mass, called hypertrophy is associated with cardiac disease and mortality.[26, 27] Based on the relationship between left ventricular size and wall thickness, one can distinguish different remodeling patterns. An increase in wall thickness, but normal left ventricular mass is called concentric remodeling. Increased left ventricular mass indicates hypertrophy, but when the wall thickness is not increased, this is called eccentric hypertrophy. When both are increased, it is called concentric hypertrophy.[24] These changes in cardiac geometry, especially the concentricity measures, add additional prognostic value to left ventricular mass in predicting cardiac disease.[27, 28] Many studies were performed using echocardiography or electrocardiography to determine hypertrophy. Cardiac Magnetic Resonance Imaging (cMRI) is a more precise method to assess cardiac measures than echocardiography and enables imaging of both left and right ventricular dimensions.[29] Right ventricular size is associated with cardiac disease independently of left ventricular mass.[30] A cMRI study in adolescents showed that preterm birth was associated with changes in cardiac geometry, which were more pronounced in the right than in the left ventricle.[31] When focusing on early factors associated with later cardiac development, it is therefore important to also study the right ventricle. By using cMRI, we were able to study childhood right ventricular volume and function, left ventricular volume, mass, function and geometry.

Cardiovascular health

Both prenatal, antenatal and postnatal life contribute to later cardiovascular health. Factors in fetal life, such as maternal under- and overnutrition, gestational diabetes, pre-eclampsia and gestational hypertension, maternal smoking and alcohol use and stress all influence fetal nutrition, growth and birth weight. The underlying etiology of these stressors leading to increased cardiovascular risk are varied and could be different in distinct pregnancy periods.[32] Children born of compromised pregnancies are not only at risk for cardiovascular disease, but also at risk for pregnancy complications, thus influencing intergenerational cardiovascular health.[32] Parental lifestyle not only influences fetal health, but also childhood lifestyle and health. For example, parental policy, role modeling and accessibility influences childhood physical activity, healthy food and junk food intake.[33] Childhood environment can also influence adult health. Higher socio-economic status and a non-smoking

environment in childhood predict better cardiovascular health in adulthood.[34]

Although cardiovascular disease usually becomes apparent later in adulthood, precursors can be observed earlier in life. Many traditional cardiovascular risk factors, such as obesity, lipid levels and blood pressure track from childhood to adulthood.[16] But increased childhood risk factors also influence adult cardiovascular health independently of adult obesity and blood pressure.[35, 36] Lifelong exposure to high levels of LDL-cholesterol increases the risk for cardiovascular events.[37] Increased blood pressure, overweight and obesity, high LDL-cholesterol and high triglycerides in adolescence are predictors of preclinical atherosclerosis.[38] Higher glucose and insulin concentrations in childhood predicted higher glucose, insulin, blood pressure, lipid concentrations and preclinical atherosclerosis in young adulthood.[39] Children with adverse glucose homeostasis were also more likely to develop diabetes, hyperglycemia, hypertriglyceridemia, and metabolic syndrome.[40] It is therefore important to study early life factors contributing to childhood cardiovascular risk status, which can possibly predict adult cardiovascular risk.

Growth and cardiovascular development

Both low and high birth weight are associated with higher body mass index, higher blood pressure and altered cardiac structure in childhood and adulthood.[11, 41-43] However, birth weight is only a proxy for fetal growth. Studies on fetal growth suggest that higher fetal growth in mid and late pregnancy are associated with lower blood pressure in childhood, while rapid infant growth is associated with higher childhood blood pressure and adverse body fat distribution.[10, 44, 45] Children with low birth weight, or with low weight at the age of 1 year have higher left ventricular mass, and altered cardiac structure in adult life.[46] Therefore, it is important to study early life growth and identify critical periods in early life.

Adiposity and cardiac development

Obesity is a growing public health problem worldwide. Obesity and excessive weight gain during pregnancy are associated with offspring obesity and an adverse cardiovascular health profile in childhood.[47, 48] Higher childhood body mass index is associated with adult left ventricular remodeling and larger left ventricular mass.[35] Left ventricular remodeling and left ventricular hypertrophy are risk factors for cardiovascular morbidity and mortality.[27, 49] Not just increased body mass index, but body composition might affect the cardiac remodeling. High body mass index reflects increases in both lean and adipose tissue. Lean body mass is most

strongly associated with the increase in left ventricular mass, that is often observed in obese adults.[50] Visceral adipose tissue is more strongly associated with cardiovascular disease than subcutaneous adipose tissue.[51] In adults, visceral adipose tissue is associated with concentric remodeling of the left ventricle, in which the left ventricular mass-to-volume ratio is increased.[52] Pericardial adipose tissue is a visceral fat depot directly attached to the heart and could possibly influence cardiac health directly. Observational studies in adults report associations of pericardial adipose tissue with higher cardiovascular morbidity and mortality and an adverse cardiovascular risk profile.[53-55] However, it remains unclear if these associations are independent of visceral and general adiposity.

In children, the associations of adiposity and body composition and cardiovascular health are not extensively studied. Obese children generally have a larger left ventricular mass and an adverse cardiovascular risk profile.[56, 57] Lean body mass is a strong predictor of childhood left ventricular mass, but the effects of specific adipose tissue depots are not clear.[22] It is important to obtain insight into the mechanisms and etiology of how body composition in childhood already influences cardiovascular health. This knowledge could help in developing more effective preventive programs and strategies to improve cardiovascular health throughout the life course.

General aim of the thesis

The general aim of this thesis was to identify early-life growth and adiposity related factors related to cardiac structures and adverse cardiovascular outcomes in children.

General design and measurements

The studies presented in this thesis were embedded in the Generation R Study, a population based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands.[58] The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood.[58] In total, 9778 mothers with a delivery date between April 2002 and January 2006 were included in the study (**Figure 1.2**). The response at baseline was 61%.[59] Assessment in early-, mid- and late pregnancy were performed in the mothers, while their partners were assessed once. Assessments included parental physical examinations, fetal ultrasound examinations and self-administered questionnaires. During the preschool period, information about anthropometrics was collected at each visit to the routine child health centers in the study area and parents received questionnaires. A

subgroup of children visited the dedicated research center for more extensive physical examinations and echocardiography.

In the school-age period, children visited the research center in the Erasmus MC – Sophia Children's hospital at the ages of 6 and 10 years for extensive physical examinations, echocardiography and body composition measurements. At the age of 10 years, children also visited our Magnetic Resonance Imaging (MRI) center for brain and total body imaging.[59] By using MRI, we were able to obtain detailed imaging of abdominal adiposity. In the 6 and 10 year follow up visits, we already collected information on total body and regional body composition by Dual-energy X-ray absorptiometry (DXA) scanner. However, studies in adults show that there are important differences between subcutaneous adipose tissue, and visceral adipose tissue in relation to cardiovascular disease.[51] Visceral adipose tissue is more strongly linked to cardiovascular morbidity and mortality.[54] With DXA-scanning, we were not able to obtain information on visceral adipose tissue, but we were able to distinguish the different adipose tissue depots with the help of MRI scanning.

Studies on the relation between cardiac structure and cardiovascular mortality and morbidity in adults often focus on left ventricular mass and concentricity of the left ventricle. Both increased left ventricular mass and increased concentricity are associated with cardiovascular disease.[28] However, with echocardiography, these measures are not obtained directly. The diameter of the left ventricle, and the thickness of the ventricular walls are measured and used to calculate the mass of the ventricle (**Figure 1.3**). With cardiac MRI scanning, instead of measuring only the diameter and thickness of the ventricle, we can obtain multiple images from the base to the apex of both the left and the right ventricle. In these images we can distinguish the ventricular cavity and the ventricular wall, and thus measure the volume (**Figure 1.4**). Therefore, cardiac MRI imaging gives more precise information on left ventricular mass, and left and right ventricular volumes.

Figure 1.2 Design and data collection in the Generation R Study

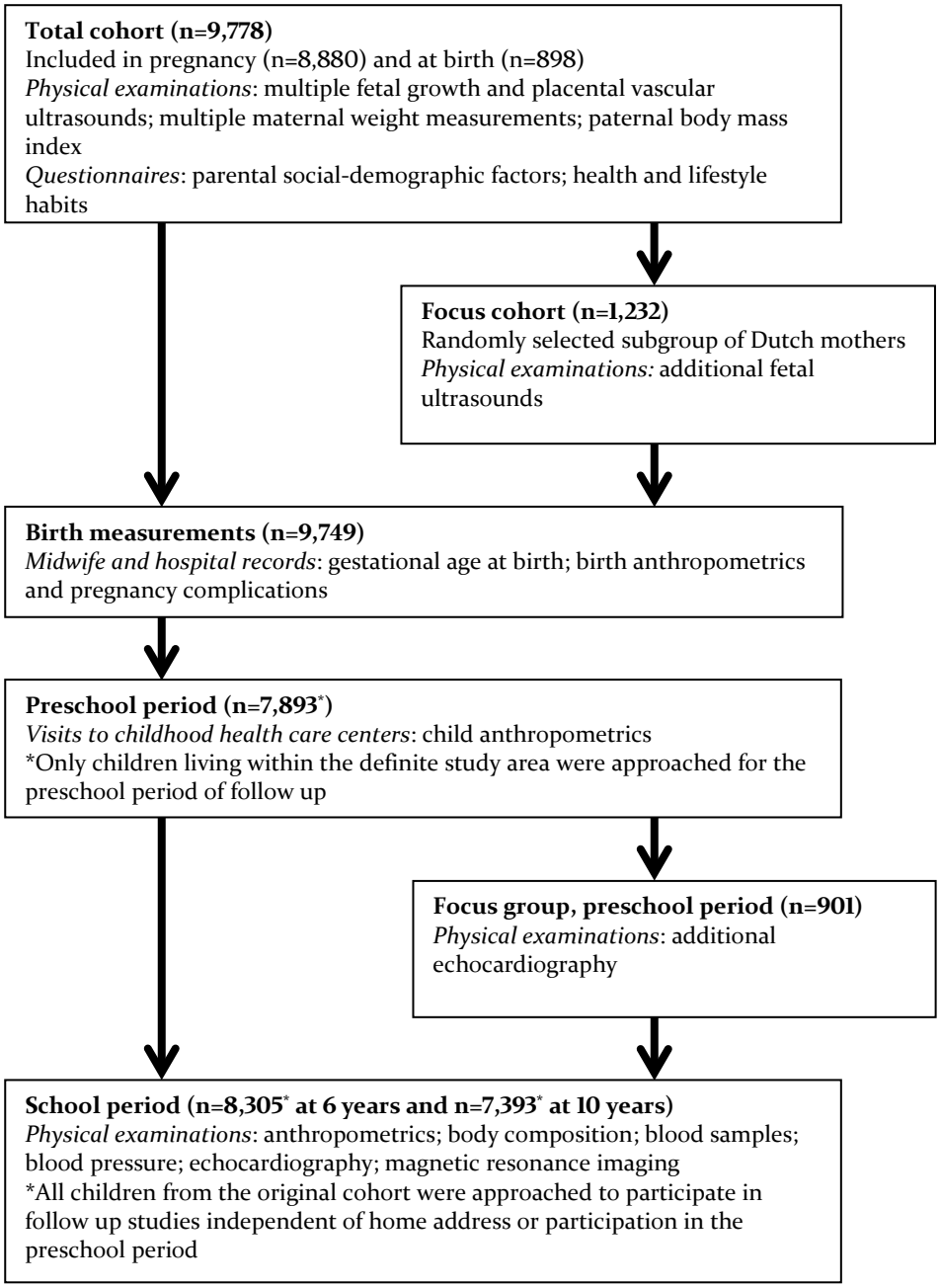
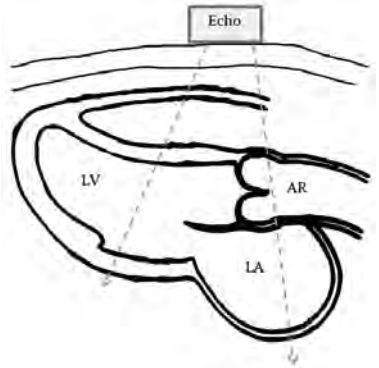


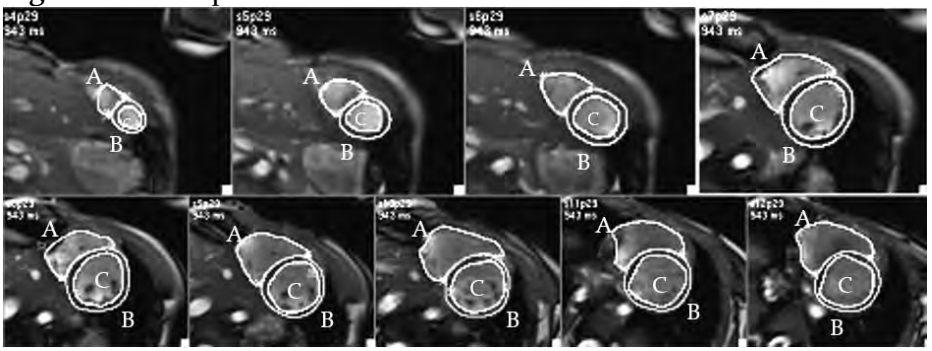
Figure 1.3 Echocardiography measurement



Abbreviations: Echo: echocardiography transducer; LV: left ventricle; AR: aortic root diameter; LA: left atrium.

The echocardiography transducer is put on the chest wall and creates a cross-section image of the heart, including the left ventricle, aortic root and the left atrium. One can measure the left ventricular wall thickness, left ventricular diameter, aortic root diameter and the left atrium diameter. A formula is used to calculate left ventricular mass and volume. However, in individuals where the shape of the ventricles differs from the norm, this formula might not be as accurate.

Figure 1.4 Example of cardiac MRI measurements



Legend: A: right ventricular outline; B: left ventricular epicardial outline; C: left ventricular endocardial outline.

Cardiac MRI uses multiple sections to image the heart from apex to base and is therefore less sensitive to deviations from normal shape. In these images, endo- and epicardial borders were semi-automatically contoured. These contours are then used to calculate right and left ventricular volumes and mass. Since we cannot distinguish the wall of the right ventricle, no right ventricular mass can be calculated, only volume.

Outline of this thesis

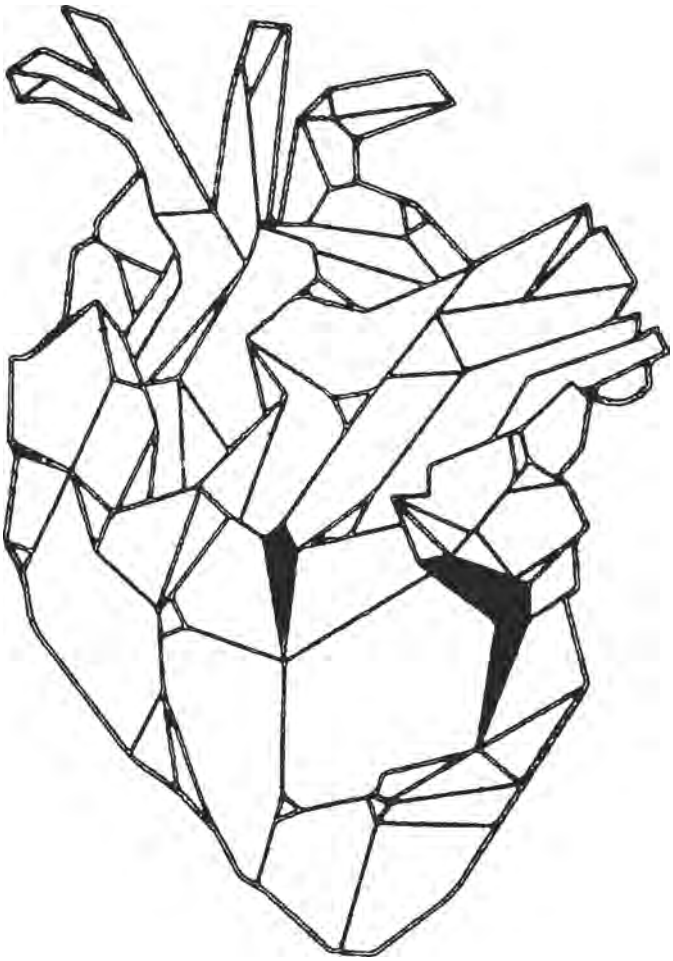
The general aim of this thesis is addressed in the several studies presented in this thesis.

In **Chapter 2** studies on fetal, infant and childhood growth on cardiovascular outcomes are described. In **Chapter 2.1** the tracking of cardiac structure from infancy to childhood is described. The influence of growth patterns in fetal life and infancy on cardiovascular health are discussed in **Chapter 2.2**, while in **Chapter 2.3** we have studied the associations of infant growth velocity patterns with cardiometabolic health in childhood. **Chapter 2.4** focusses on fetal and childhood growth and cardiac structure, while **Chapter 2.5** discusses the influence of fetal hemodynamics on childhood cardiac structure.

In **Chapter 3** we present studies on the associations between maternal and child obesity and body composition on cardiovascular health. **Chapter 3.1** discusses the associations between maternal obesity, gestational weight gain and childhood cardiovascular health. The associations of childhood adiposity and pericardial fat with cardiac structure and cardiovascular health are discussed in **Chapter 3.2** and **Chapter 3.3**, respectively.

Finally, **Chapter 4** provides a general discussion, in which the studies described in this thesis are described in a broader context. Also, implications and suggestions for future research are discussed.

Chapter 2 | Growth



Chapter 2.1

Tracking of cardiac measures from infancy to school-age

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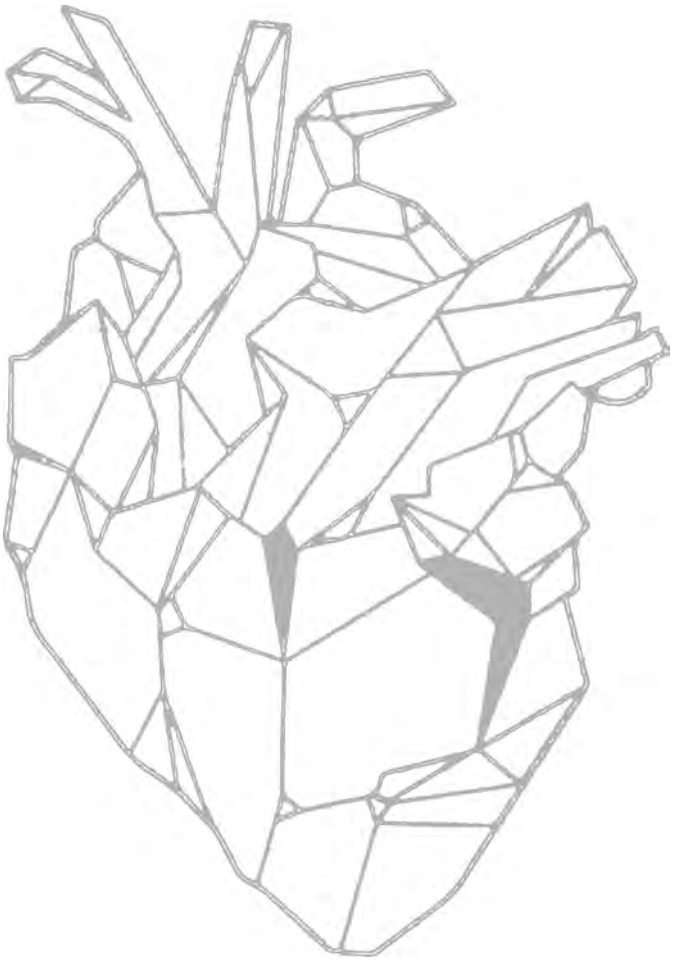


ABSTRACT

Objective: Cardiac structure and function are important predictors for cardiovascular disease in adults. Not much is known about tracking of cardiac measures, other than left ventricular mass, from early life onwards. We examined whether and to what extent cardiac measures track from infancy into school-age.
Methods: We performed a population-based prospective cohort study among 1,072 children. Aortic root diameter, left atrial diameter, left ventricular mass, relative wall thickness and fractional shortening were measured repeatedly by echocardiography. We explored tracking between infancy (1.5, 6, and 24 months) and school-age (6 and 10 years).
Results: Of all cardiac measures, aortic root diameter, left atrial diameter and left ventricular mass were significantly correlated between infancy and school-age (r=0.10-0.42, all p-values <0.01), with the strongest correlations between 24 months and 10 years. Of the different structures, aortic root diameter showed the strongest correlations. Approximately 30% of children who were in the lowest or highest quartile of a measure at the age of 1.5 months remained in that quartile at the age of 10 years. When analyzing the effects of the infant cardiac measures on the same outcomes at 10 years in conditional regression models, we observed effect estimates of the same size for the different age windows.
Conclusion: Our results suggest moderate tracking of structural cardiac measures from early infancy until school-age, which become stronger at older ages, but not of relative wall thickness or fractional shortening. Moderate tracking of cardiac structures suggests that cardiac structures are at least partly determined in early life.

INTRODUCTION

Cardiovascular disease is a major public health problem and seems to originate at least partly in early life.[18, 25] Common risk factors for cardiovascular disease, including blood pressure and lipid levels track from childhood to adulthood.[15, 16] Tracking represents the maintaining of a given rank order relative to peers over time.[18] Previous studies have shown that left ventricular mass (LVM) tracks from childhood to adulthood.[18, 25] Longitudinal studies on tracking of LVM in children from the age of 7 years until the age of 22 years show correlation coefficients in the range of 0.4 to 0.7.[25] Previously, we have reported that tracking of LVM is also present during the first two years of life.[60] Increased LVM is an independent predictor of cardiovascular disease and mortality in adults.[28, 61] Next to LVM, an increase in aortic root diameter (AOD) is associated with increased risk for heart failure, whereas an increase in left atrial diameter (LAD) is associated with cardiovascular events, such as stroke, and cardiovascular mortality in adults.[62, 63] The predictive value of increased LVM for cardiovascular events is higher when combined with information about relative wall thickness (RWT).[28] To the best of our knowledge, no previous studies have analyzed tracking of these different cardiac structural and functional measures from infancy to childhood.

We hypothesize that structural and functional cardiac measures already track from infancy onwards. Therefore, we examined the extent of tracking from infancy into school-age in a population-based prospective cohort study among 1,072 children followed from fetal life to the age of 10 years. We measured cardiac structure and function repeatedly with echocardiography at the ages of 1.5, 6, and 24 months, and 6 and 10 years. Measures included LVM, AOD, LAD, RWT and FS.

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[64] All children were born between 2002-2006. Details of this study have been described previously.[64] Detailed cardiovascular measures were performed in a subgroup of 1,106 Dutch children.[64] Of the total of 1,079 live born singleton children, we excluded 7 children from the analysis due to cardiac abnormalities (Flowchart given in **Figure S.2.1.1**). Echocardiograms were successfully performed in 85%-95% of the participating children at the different ages, with 24 months being the least

successful. Missing echocardiograms were mainly due to crying or unavailability of equipment or echo cardiographer. Written informed consent was obtained from parents of participants. The study has been approved by the local Medical Ethics Committee.

Left cardiac structures until the age of 10 years

Two-dimensional M-mode echocardiograms were performed when the children were aged 1.5, 6 and 24 months and at the age of 6 and 10 years in our dedicated research center. We used methods recommended by the American Society of Echocardiography.[65] Intraobserver and interobserver intraclass correlation coefficients (ICC) were calculated previously in 28 children with a median age of 7.5 years, (interquartile range 3.0-11.0) and varied between intraobserver ICC 0.91 to 0.99 and interobserver ICC 0.78 to 0.96.[66] We measured aortic root diameter (AOD), left atrium diameter (LAD), left ventricular end diastolic diameter (LVEDD), left ventricular posterior wall thickness (LVPWT), and interventricular septum thickness (IVS) and calculated fractional shortening (FS) and left ventricular mass (LVM).[65, 67] To assess left ventricular concentricity, we calculated relative wall thickness (RWT) as (2*LVPWT)/LVEDD.[68]

To account for differing body sizes, we additionally standardized all cardiac outcomes on body surface area (BSA) using Generalized Additive Models for Location, Size and Shape (GAMLSS) using R, version 3.2.0 (R Core Team, Vienna, Austria).[69] These models enable flexible modelling, taking into account the distribution of the response variable.[70] Worm plots and Akaike Information Criterion were used in sensitivity analyses to obtain the best model fit. Weight and length were measured at the cardiac ultrasound. BSA was computed using the Haycock formula (BSA $(m^2)=0.024265$ x weight $(kg)^{0.5378}$ x height $(cm)^{0.3964}$.[69]

Statistical analysis

First, we used One-Way ANOVA and Chi-square tests to compare childhood characteristics between boys and girls. Second, to examine whether children maintain their position in the distribution of the different cardiac structure measures, we estimated the Pearson's correlation coefficients. AOD, LAD and LVM were standardized on BSA to account for differing body sizes. Since RWT and FS are ratio's between cardiac measures and not dependent on BSA, we constructed standard deviation scores (SDS) using this formula: (observed value-mean)/standard deviation. Third, we categorized the cardiac outcomes in quartiles and calculated the percentages of children that remained in the lowest or highest quartile between the measures at 1.5 and 10 years. Finally, we

performed conditional regression analyses to identify the independent associations of the cardiac measures at the different age windows with cardiac measures at age 10 years. We used standardized residuals obtained from regression of the cardiac structure measure at a specific age window on the previous measures.[71] These standardized residuals are independent from each other and can be used in a regression model together. The R^2 of these models gives insight in the amount of variability of the cardiac measure at the age of 10 years, explained by the cardiac measures of the previous age windows combined. There was no statistical interaction for sex in relation to tracking of any of the cardiac measures. Statistical analyses were performed using SPSS version 21.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp).

RESULTS

Participant characteristics (**Table S2.1.1**) shows that boys had higher birth weight and had greater height and weight in infancy, but at school-age length and weight did not differ between boys and girls. The cardiac parameters are shown in **Table 2.1.1**.

Table 2.1.2 shows that AOD, LAD and LVM correlated across all age windows, with correlation coefficients ranging between 0.10 and 0.42 (all p-values <0.01). The measures of AOD showed the strongest correlations across all age windows (r=0.27-0.42, all p-values <0.01). The correlations across infancy to school age were highest between 24 months and 10 years. Correlations within school-age (6 to 10 years) were higher than in infancy (1.5 to 24 months). The measures of RWT and FS correlated inconsistently between periods and the correlations were weaker than the correlations of the other measures.

Figure 2.1.1 shows the distribution of children in quartiles of cardiac measures at the age of 10 for the children who were in the lowest quartile of the measure at the age of 1.5, and for the children who were in the highest quartile at 1.5 months. Of the children who were in the lowest quartile of AOD at 1.5 months 36% remained in the lowest quartile at the age of 10 years, while 8% changed to the highest quartile. Of the children who were in the highest quartile at 1.5 months, 45% remained in the highest quartile, while 10% changed to the lowest quartile. AOD showed the strongest trend. The trends of children remaining in the lowest (30%) or highest (29%) LAD quartile and LVM quartiles (29% and 37%) from 1.5 months to 10 years were less clear. The distribution of RWT and FS did not show the same clear

trend. Distribution for all infant quartiles is shown in **Table S2.1.2**. Trends found for 24 months were stronger than those observed for 1.5 months (**Figure S2.1.2**).

The results of the conditional regression analyses focused on identification of specific age windows for the cardiac outcomes at age 10 years did not show one clear age window for all outcomes (**Figure S2.1.3**). AOD at 1.5 months had the strongest, independent association with AOD at 10 years. The other periods each had an additional, but less strong effect. The explained variability (R^2) of the combined measures on AOD at 10 years was 31%. For LAD at 10 years, the strongest independent associations were observed at the age windows of 24 months and 6 years, the R^2 of the model was 21%. The effect estimates of the different age windows of LVM on the measure at 10 years were of similar size, the R^2 was 18%. RWT was in none of the age windows associated with RWT at 10 years, independently from the other age windows, the R^2 was 2%. For FS, an independent association at the age of 24 months, with FS at 10 years was seen, the R^2 of the model was 4%.

DISCUSSION

In this population-based prospective cohort study, we observed moderate tracking of AOD, LAD, and LVM between the ages of 1.5 months and 10 years. Around 30% of the children who were in the lowest or highest quartiles at the age of 1.5 months remained in the same quartile at the age of 10 years. Tracking was not consistently seen in RWT and FS.

Interpretation of main findings

Tracking can be defined as the stability of a child's rank in a distribution over time.[72] Tracking of structural and functional cardiovascular measures suggests that cardiac structure originates at least partly in early life. Adverse cardiac structure in childhood could possibly place individuals at greater risk for cardiovascular disease in later life. Tracking can also be important for identifying individuals at risk for cardiovascular disease early in life.[72] Longitudinal studies have shown tracking of common risk factors for cardiovascular disease including blood pressure, lipid levels and LVM from childhood to adulthood.[15, 16, 18, 60] Tracking of cholesterol (r=0.53) and BMI (r=0.53) showed the strongest coefficients of tracking between 8 to 21 years in a study among 354 participants, followed by tracking of triglycerides (r=0.33), diastolic blood pressure (r=0.28), HDL-cholesterol (r=0.26), and systolic blood pressure (r=0.21).[16]

Table 2.1.1 Structural and functional cardiac measures in boys and girls

	Successful measures (N)	Total group N=1,072	Boys N=553	Girls N=519	P-value
Aortic root diameter, mm					
1.5 months	737	11.7 (1.2)	12.0 (1.2)	11.5 (1.1)	<0.01
6 months	728	13.7 (1.2)	14.0 (1.2)	13.4 (1.2)	<0.01
24 months	694	16.3 (1.5)	16.7 (1.5)	16.0 (1.4)	<0.01
6 years	817	19.2 (1.8)	19.7 (1.9)	18.6 (1.6)	<0.01
10 years	781	21.7 (1.8)	22.3 (1.7)	21.2 (1.7)	<0.01
Left atrial diameter, mm					
1.5 months	740	16.8 (1.9)	17.0 (1.8)	16.6 (1.9)	0.01
6 months	731	18.0 (1.9)	18.0 (1.9)	18.0 (1.9)	0.78
24 months	690	20.6 (2.4)	20.7 (2.5)	20.5 (2.4)	0.20
6 years	812	25.0 (2.7)	25.4 (2.6)	24.6 (2.7)	<0.01
10 years	781	27.4 (2.7)	28.0 (2.6)	26.8 (2.7)	<0.01
Left ventricular mass, g					
1.5 months	659	14.5 (3.1)	15.2 (3.2)	13.8 (2.8)	<0.01
6 months	666	19.4 (4.0)	20.3 (4.0)	18.4 (3.7)	<0.01
24 months	645	31.3 (5.6)	32.6 (5.9)	30.0 (5.0)	<0.01
6 years	807	53.6 (11.1)	55.4 (11.3)	51.8 (10.7)	<0.01
10 years	779	72.5 (12.0)	75.6 (11.9)	69.5 (11.4)	<0.01
Relative wall thickness					
1.5 months	683	0.34 (0.07)	0.33 (0.07)	0.35 (0.07)	0.02
6 months	693	0.32 (0.07)	0.33 (0.07)	0.32 (0.06)	0.33
24 months	673	0.30 (0.07)	0.30 (0.07)	0.30 (0.06)	0.56
6 years	817	0.30 (0.05)	0.30 (0.05)	0.30 (0.05)	0.20
10 years	782	0.30 (0.03)	0.30 (0.03)	0.30 (0.03)	<0.01
Fractional shortening, %					
1.5 months	687	35.3 (5.0)	35.3 (4.8)	35.4 (5.3)	0.93
6 months	695	37.1 (4.7)	37.2 (4.6)	37.1 (4.8)	0.72
24 months	663	35.5 (4.6)	35.4 (4.6)	35.5 (4.7)	0.88
6 years	817	35.3 (4.5)	35.5 (4.6)	35.1 (4.6)	0.23
10 years	781	35.8 (4.5)	36.1 (4.6)	35.5 (4.3)	0.04

Values are means (SD). P-value was estimated by using One-Way ANOVA test.

Table 2.1.2 Correlation tables of different age windows of structural and functional cardiac measures

Aortic root diameter					
	1.5 months	**6 months**	**24 months**	**6 years**	**10 years**
1.5 months	1				
6 months	0.37**	1			
24 months	0.32**	0.31**	1		
6 years	0.28**	0.27**	0.40**	1	
10 years	0.33**	0.38**	0.42**	0.41**	1

Left atrial diameter					
	1.5 months	**6 months**	**24 months**	**6 years**	**10 years**
1.5 months	1				
6 months	0.23**	1			
24 months	0.14**	0.23**	1		
6 years	0.16**	0.14**	0.24**	1	
10 years	0.13**	0.10**	0.25**	0.35**	1

Left ventricular mass					
	1.5 months	**6 months**	**24 months**	**6 years**	**10 years**
1.5 months	1				
6 months	0.38**	1			
24 months	0.22**	0.24**	1		
6 years	0.20**	0.31**	0.31**	1	
10 years	0.21**	0.32**	0.33**	0.29**	1

Relative wall thickness					
	1.5 months	**6 months**	**24 months**	**6 years**	**10 years**
1.5 months	1				
6 months	0.04	1			
24 months	0.01	-0.01	1		
6 years	0.11*	0.12**	-0.01	1	
10 years	-0.03	0.03	0.17**	0.07	1

Fractional shortening					
	1.5 months	**6 months**	**24 months**	**6 years**	**10 years**
1.5 months	1				
6 months	0.15**	1			
24 months	0.10*	0.20**	1		
6 years	0.24**	0.10*	0.13**	1	
10 years	0.02	-0.04	0.16**	0.18**	1

Numbers are Pearson's correlation coefficients. * $p<0.05$; ** $p<0.01$

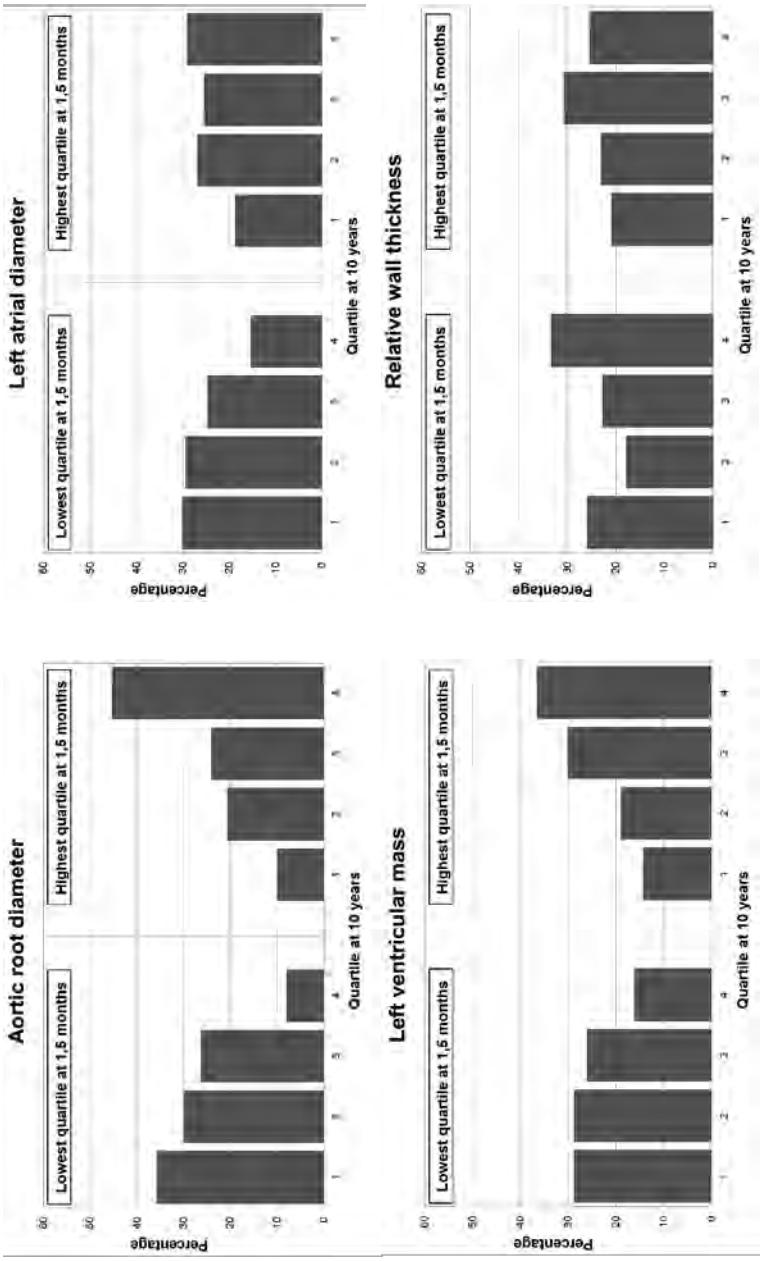
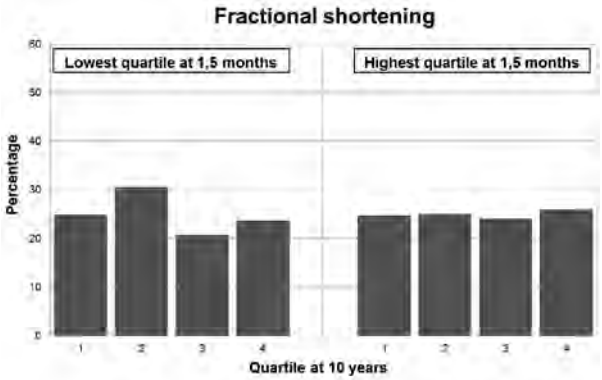


Figure 2.1.1 Quartile distribution of cardiac measures in school-age for children who were in the lowest or highest quartile at 1.5 months

Figure 2.1.1 (continued)



Bars represent the percentage of children with a cardiac measure in quartile groups, at the age of 10 years (x-axis). The first part represents the distribution of cardiac structure at the age of 10 years, for the children who were in the lowest quartile group at the age of 1.5 months; while the second part represents the distribution of cardiac structure at 10 years, for the children who were in the highest quartile group at 1.5 months of age. For example, the bar on the left shows that of the children who were in the lowest quartile group of aortic root diameter at the age of 1.5 months, over 35% was still in that quartile group at the age of 10 years. Distribution for children who were in the lowest or highest quartile at 24 months is shown in **Figure S2.1.2**.

Previously, we reported moderate tracking of cardiac structures and function between the ages of 1.5 and 24 months in the same study group as in the current study.[60] Another study describes tracking of LVM in adolescents. This study followed 231 normotensive adolescents and reported a tracking coefficient for LVM of 0.41 between the ages of 11 and 17 years.[18] In line with this study, we observed moderate tracking of LVM. In our study we observed slightly lower tracking coefficients of LVM, than in the study on adolescents. This phenomenon has also been described in tracking of blood pressure.[15, 72] Baseline age was an important predictor of tracking of blood pressure, with stronger tracking in (late) adolescence than in childhood.[15, 72]

To our knowledge, tracking of AOD, LAD, RWT and FS in children has not been studied before. In our study, we observed tracking of AOD and LAD, but we did not find consistent tracking of RWT and FS. Since AOD and LAD correlate with LVM, we expected these measures to track. Tracking of AOD was stronger than tracking of LAD and LVM. Echocardiography of LAD and LVM shows more intraobserver and interobserver variation than the measures on AOD.[66] Larger measurement error in repeated measures causes underestimation of the true tracking

coefficients, which could explain the observed differences.[72] RWT is used in clinic as an extension on LVM to determine geometry of the heart. It represents the ratio between LVPWT and LVEDD, and both are dependent on growth. We would have expected that the ratio between these two measures would be constant in a healthy child and would show tracking. However, this was not the case. The same was observed for FS. This measure is the percentage change in cavity diameter. It is possible that there is very limited variation of these measures between persons in this relatively healthy population, and that there is a high variability of the repeated measurements in a participant, due to factors such as measurement error, heart rate variability and blood pressure variability. This within person variability could be large enough to obscure any possible real tracking. Also, the explained variability of the first four measures on RWT and FS at the age of 10 years was very low. This would indicate that not the measures at earlier ages, but other factors at the time of the measurement can explain the variability. Factors associated with RWT and FS could be BMI, exercise, heart rate, and blood pressure.[35]

Various factors may affect tracking of cardiac structures. In adults, cardiac remodeling is varies between the sexes.[73] However, even though a study in 231 adolescents found that boys have a larger LVM than girls, the degree of tracking was not influenced by sex.[18] The results are comparable to our results. We also found that boys have larger cardiac structures, but no differences in the degree of tracking. In childhood and adolescence, most variation in cardiac size can be explained by lean mass and not by cardiovascular risk factors.[22, 74] In our study, boys had a higher BSA and higher lean mass index than girls, which can explain the larger cardiac structures.[75]

To determine the most important age window for cardiac tracking, we used conditional analyses. With these analyses, we could determine the effect of a measure at a given age on the measure at 10 years, independent of the effect of the measures at the other ages. We did not find one age window to be consistently stronger correlated than the other age windows. Our results suggest that that the measures at 24 months seem to be a stronger predictor in infancy for the measures at the age of 10, than the measures at 1.5 months. This finding may reflect stability of cardiac structures after the first 2 years of life, or may reflect just a shorter time interval between the ages. However, we did not observe stronger correlations between 6 and 10 years.

The observed moderate tracking is important from an etiological view point. It suggests that variation in cardiac structure partly originates in early life and might put individuals at risk for later cardiovascular

disease. However, based on this research, we cannot determine if tracking alone provides enough evidence to identify the individuals at risk in early life. More research and longer follow-up is needed to explain the variation in cardiac structure and to study whether this variation indeed leads to increased cardiovascular risk later in life, before predictive models can be created.

Study limitations

The main strength of this study is its population-based prospective study design starting from early fetal life. Also, we were able to perform echocardiography repeatedly in a large cohort of children over a time period of 10 years. Another strength is that we standardized the cardiac structural measures on BSA; this way we created SDS that were independent from body size at the time of measure. This ensured that we measured cardiac tracking, opposed to tracking of linear growth in childhood, since cardiac structures in childhood are mainly dependent on body size.[22] This study was performed in a Dutch population, making it less generalizable to other ethnicities. A limitation of our study is that for each time point 15-25% of the children did not visit the research. Of the children who did visit, we could not obtain cardiac measures in 5-15% of the children. Missing values were because of the child being uncooperative at time of measure, or because of defective equipment or absent echocardiographer. However, we do not think these missing values lead to bias, because it is very unlikely that the correlation coefficients we found would be different in the children in whom we were not able to obtain cardiac measures. As mentioned previously, measurement error in repeated measures causes underestimation of the true tracking coefficients.[72] Since measurement error is more likely in the younger children, who have smaller hearts and are less cooperative, this could have underestimated the tracking coefficients we found within infancy and from infancy to childhood. Also, measurement inaccuracies of the ventricular diameter and wall thickness could increase measurement error in the calculated measures, such as LVM, RWT and FS. Studies on tracking of cardiac structure with more precise methods, such as cardiac MRI or speckle-tracking echocardiography for cardiac function could be an interesting addition to this research field.[76]

CONCLUSION

Our study indicates that children who have a larger cardiac size measured by LVM, AOD and LAD, compared to their peers in infancy, are also more

likely to have a larger cardiac size in school-age. The strongest period for tracking across infancy to school-age seems to be between the ages of 24 months and 10 years. Our results suggest moderate tracking of structural cardiac measures from early infancy until school-age, which become stronger at older ages, but not of FS or RWT. Moderate tracking of cardiac structures suggests that cardiac structures are at least partly determined in early life. Whether early cardiac structure and functional development predicts later life cardiac disease should be further studied.

SUPPLEMENTAL MATERIAL

Table S2.1.1 Subject characteristics

Characteristics	Boys	Girls	P-value
Characteristics at birth	*N=553*	*N=519*	
Gestational age at birth, wk	40.3 (35.9-42.4)	40.3 (35.7-42.4)	0.54
Premature, n (%)	29 (5.2)	21 (4.0)	0.35
Birth weight, g	3550 (535)	3468 (538)	0.01
Low birth weight, n (%)	20 (3.6)	20 (3.9)	0.84
Small for gestational age, n (%)	36 (6.5)	34 (6.6)	0.98
Large for gestational age, n (%)	59 (10.7)	71 (13.7)	0.13
Characteristics at 1.5 months	*N=449*	*N=425*	
Age at visit, m	1.5 (1.0-3.0)	1.5 (1.0-2.8)	0.60
Weight at visit, g	5096 (746)	4777 (626)	<0.01
Length at visit, cm	57.5 (2.6)	56.4 (2.5)	<0.01
BSA at visit, m^2	0.29 (0.03)	0.28 (0.02)	<0.01
Characteristics at 6 months	*N=449*	*N=426*	
Age at visit, m	6.3 (5.5-8.1)	6.3 (5.5-8.4)	0.59
Weight at visit, g	8200 (862)	7644 (815)	<0.01
Length at visit, cm	69.5 (2.5)	67.8 (2.5)	<0.01
BSA at visit, m^2	0.40 (0.03)	0.39 (0.03)	<0.01
Characteristics at 24 months	*N=427*	*N=404*	
Age at visit, m	25.1 (23.7-28.1)	25.1 (23.5-28.4)	0.43
Weight at visit, kg	12.88 (1.39)	12.40 (1.33)	<0.01
Length at visit, cm	89.6 (3.3)	88.4 (3.2)	<0.01
BSA at visit, m^2	0.57 (0.04)	0.56 (0.04)	<0.01
Characteristics at 6 years	*N=438*	*N=437*	
Age at visit, y	5.9 (5.7-6.6)	5.9 (5.7-6.6)	0.42
Weight at visit, kg	22.63 (3.03)	22.44 (3.40)	0.37
Length at visit, cm	119.2 (5.1)	118.6 (5.3)	0.07
BSA at visit, m^2	0.86 (0.07)	0.86 (0.08)	0.24
Characteristics at 10 years	*N=424*	*N=425*	
Age at visit, y	9.8 (8.9-10.5)	9.8 (9.2-10.6)	0.04
Weight at visit, kg	34.5 (5.7)	35.0 (6.5)	0.31
Length at visit, cm	142.5 (6.2)	142.1 (6.6)	0.37
BSA at visit, m^2	1.16 (0.1)	1.17 (0.1)	0.45

N, number; Wk, weeks; M, months; Y, years. Values are means (SD) or medians (95% range). P-value was estimated by using One-Way ANOVA test, Kruskal-Wallis test and Chi-square test.

Table S2.1.2 Distribution of cardiac measures in school-age for children for the different quartiles of the cardiac measure at 1.5 months

Quartile of cardiac outcome at the age of 1.5 months	**Quartile of cardiac outcome at the age of 10 years**			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Aortic root diameter				
Quartile 1	49 (35.8)	41 (29.9)	36 (26.3)	11 (8.0)
Quartile 2	42 (32.1)	38 (29.0)	29 (22.1)	22 (16.8)
Quartile 3	24 (17.5)	40 (29.2)	33 (24.1)	40 (29.2)
Quartile 4	14 (9.9)	29 (20.6)	34 (24.1)	64 (45.4)
Left atrial diameter				
Quartile 1	39 (30.2)	38 (29.5)	32 (24.8)	20 (15.5)
Quartile 2	34 (24.8)	33 (24.1)	37 (27.0)	33 (24.1)
Quartile 3	34 (23.0)	33 (22.3)	36 (24.3)	45 (30.4)
Quartile 4	25 (18.7)	36 (26.9)	34 (25.4)	39 (29.1)
Left ventricular mass				
Quartile 1	32 (28.8)	32 (28.8)	29 (26.1)	18 (16.2)
Quartile 2	33 (25.2)	35 (26.7)	38 (29.0)	25 (19.1)
Quartile 3	31 (25.8)	32 (26.7)	25 (20.8)	32 (26.7)
Quartile 4	18 (14.3)	24 (19.0)	38 (30.2)	46 (36.5)
Relative wall thickness				
Quartile 1	32 (26.0)	22 (17.9)	28 (22.8)	41 (33.3)
Quartile 2	34 (27.6)	31 (25.2)	33 (26.8)	25 (20.3)
Quartile 3	32 (23.5)	43 (31.6)	32 (23.5)	29 (21.3)
Quartile 4	28 (20.9)	31 (23.1)	41 (30.6)	34 (25.4)
Fractional shortening				
Quartile 1	52 (38.0)	48 (35.0)	28 (20.4)	9 (6.6)
Quartile 2	42 (28.6)	44 (29.9)	36 (24.5)	25 (17.0)
Quartile 3	32 (22.9)	32 (22.9)	36 (25.7)	40 (28.6)
Quartile 4	11 (8.9)	17 (13.8)	30 (24.4)	65 (52.8)

Values are numbers (%) and represent the distribution of children in quartiles of cardiac measures at the age of 10 for the children in the 4 quartiles of the measure at the age of 1.5 months.

Figure S2.1.1 Flow chart of participants included in the analysis

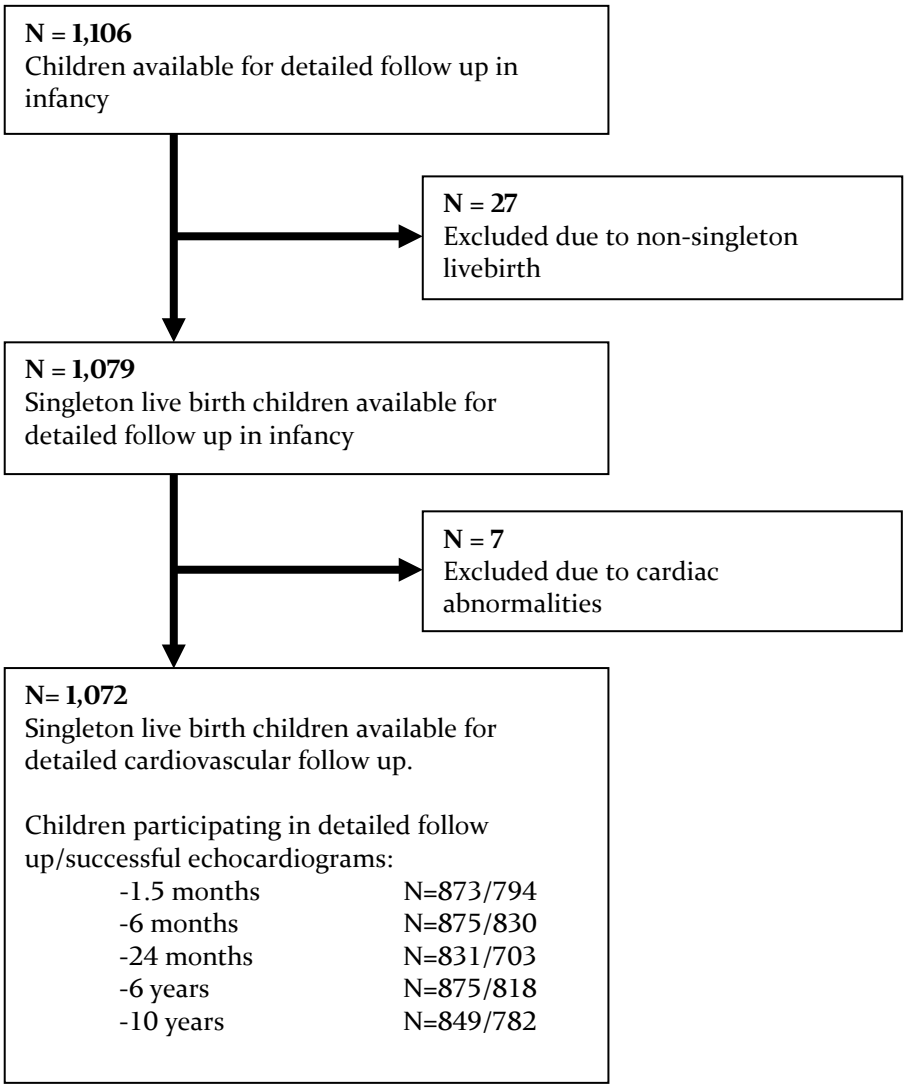


Figure S2.1.2 Quartile distribution of cardiac measures in school-age for children who were in the lowest or highest quartile at 24 months

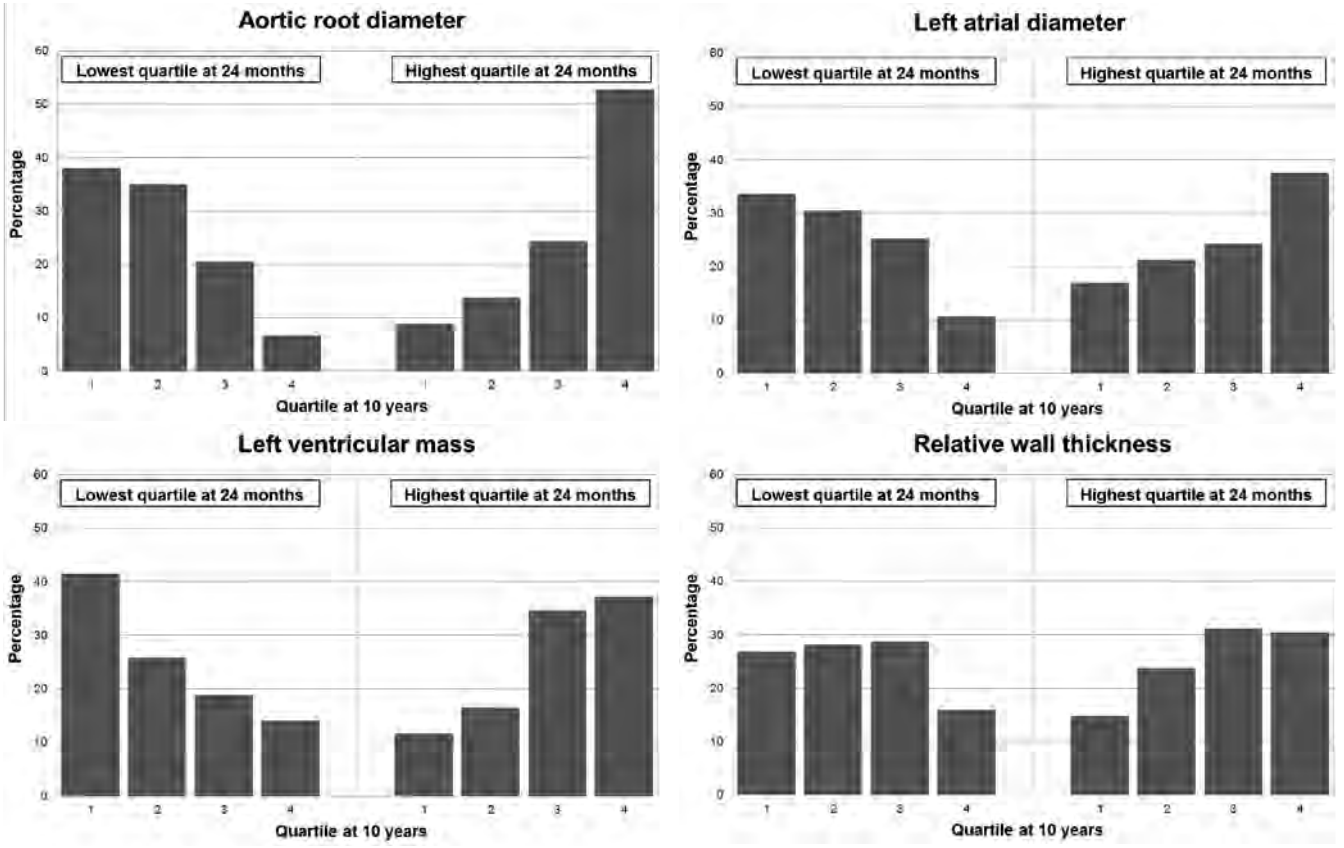
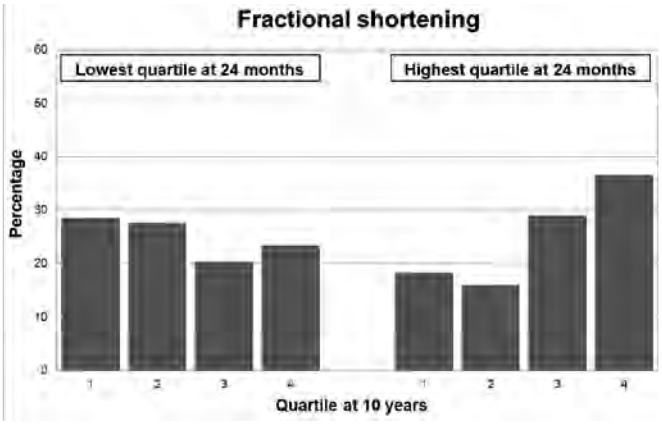
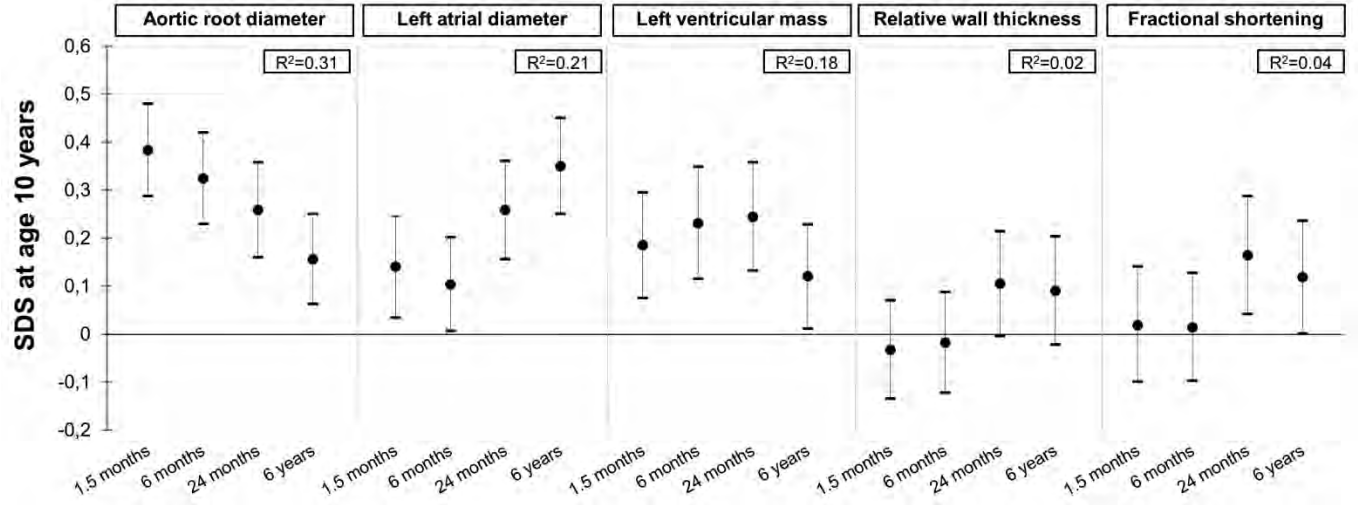


Figure S2.1.2 (continued)



Bars represent the percentage of children with a cardiac measure in quartile groups, at the age of 10 years (x-axis). The first part represents the distribution of cardiac structure at the age of 10 years, for the children who were in the lowest quartile group at the age of 24 months; while the second part represents the distribution of cardiac structure at 10 years, for the children who were in the highest quartile group at 24 months of age. For example, the bar on the left shows that of the children who were in the lowest quartile group of aortic root diameter at the age of 24 months, over 38% was still in that quartile group at the age of 10 years.

Figure S2.1.3 Conditional analyses of different age windows on structural and functional cardiac measure at 10 years



Conditional models for cardiac structure and function. Values are linear regression coefficients (95% CI) that reflect the difference in cardiac structure or function at 10 years per standardized residual for each of the time points, independent from the other time points. For example, the estimate at the 6 months point for aortic root diameter represents the association of 1 SDS larger standardized residual of aortic root diameter at 6 months on the size of aortic root diameter at 10 years; this association is independent from aortic root diameter at the other time points. R^2 reflects the explained variation of the cardiac measure at the age of 10 years, by all the previous measures combined.
Aortic root diameter, left atrial diameter and left ventricular mass are standardized on BSA.

Chapter 2.2

Longitudinal growth during fetal life and infancy and cardiovascular health at school-age

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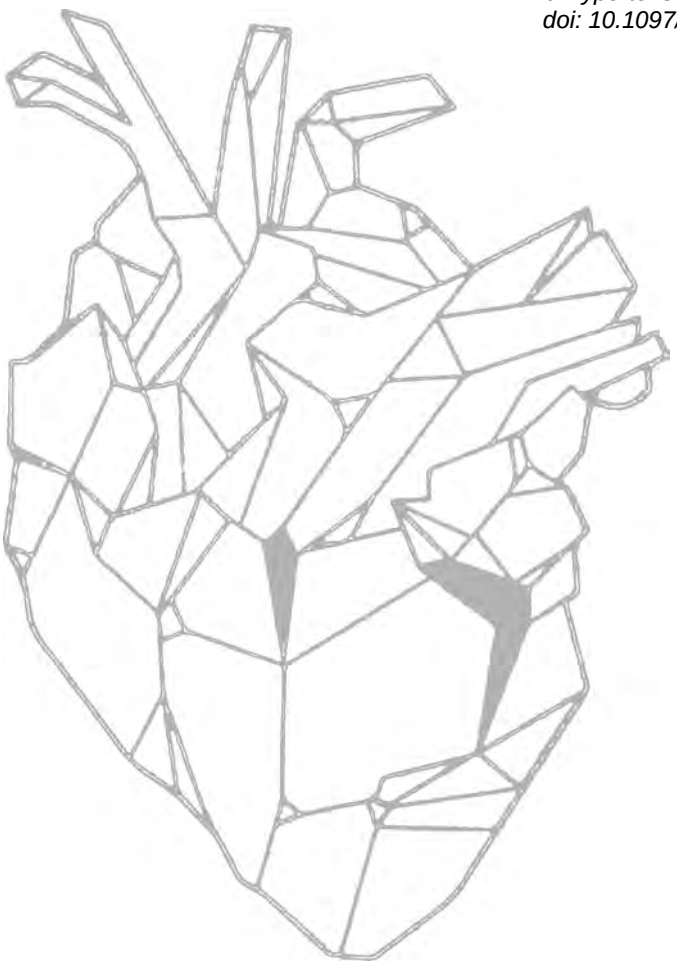


ABSTRACT

Objective: Low birth weight is associated with cardiovascular disease. We examined the effects of fetal and infant growth patterns on cardiovascular outcomes in children.
Methods: In a population-based prospective cohort study among 6,239 children, we estimated fetal femur length and weight by 20 and 30 weeks ultrasound, and child length and weight at birth, 0.5, 1, 2 and 6 years. We measured blood pressure, carotid-femoral pulse wave velocity, aortic root diameter, left ventricular mass and fractional shortening at 6 years. We used regression analyses to identify longitudinal growth patterns associated with height standardized vascular outcomes and body surface area standardized cardiac outcomes.
Results: Younger gestational age and lower birth weight were associated with higher blood pressure, smaller aortic root diameter and lower left ventricular mass in childhood (all p-values <0.05). Children with decelerated or normal fetal growth followed by accelerated infant growth had higher blood pressure, whereas those with decelerated growth during both fetal life and infancy had a relatively larger left ventricular mass. Longitudinal growth analyses showed that children with increased blood pressure tended to be smaller during third trimester of fetal life, but of normal size during infancy, than children with normal blood pressure. Children with increased aortic root diameter or left ventricular mass tended to be larger during fetal life, but of similar size during infancy.
Conclusion: Specific fetal and infant growth patterns are associated with different cardiovascular outcomes in children. Further studies are needed to identify the underlying mechanisms and the long-term cardiovascular consequences.

INTRODUCTION

Previous follow-up studies have shown associations of low birth weight with cardiovascular disease in later life.[8, 9] More recent follow-up studies showed that specifically children with low birth weight followed by high rates of childhood weight gain have increased risks of cardiovascular disease.[3] It has been hypothesized that a mismatch between a suboptimal fetal environment and affluent postnatal environment, characterized by fetal growth restriction followed by rapid childhood weight gain, leads to adverse cardiovascular adaptions, which subsequently predisposes individuals to cardiovascular disease.[4]

Most studies use birth weight as a proxy measure for fetal growth. Studies with directly measured fetal growth are scarce.[10, 77] Results from a prospective study suggested that increased fetal growth between 18 and at 38 weeks of gestation was associated with lower systolic blood pressure in childhood.[10] We have previously reported that smaller size in mid-pregnancy and a larger infant size is related to a higher systolic blood pressure in infancy.[77] Thus far, the specific early growth patterns associated with cardiovascular health and disease outcomes in later life are unknown. Detailed studies focused on the associations of specific fetal and infant growth patterns with cardiovascular adaptations in children might extend our knowledge on the critical periods in the earliest phase of life for cardiovascular disease development.[3]

In a population-based prospective cohort study among 6,239 children, we examined the associations of longitudinal fetal and infant growth patterns and critical periods with cardiovascular outcomes at 6 years. Cardiovascular outcomes included height adjusted blood pressure and carotid-femoral pulse wave velocity and body surface area adjusted aortic root diameter, left ventricular mass and fractional shortening. All outcomes are known to track from childhood onwards and may predict disease and mortality.[15, 17, 61, 78, 79]

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, The Netherlands.[64, 80] Response rate at birth was 61% (2002 - 2006).[64] Fetal and childhood growth were repeatedly assessed by ultrasounds and physical examinations. In total 8,305 children participated in these studies, of whom 6,239 (75%) attended the research center at 6 years for

cardiovascular measurements (flow chart given in **Figure S2.2.1**). Written informed consent was obtained from all parents of participants. The study has been approved by the local Medical Ethics Committee.

Fetal, infant and childhood growth

Fetal ultrasound examinations were carried out in each trimester of pregnancy.[81] First trimester ultrasounds were mainly used for establishing gestational age.[81] Second trimester (median 20.5 weeks, 95% range 18.6 – 23.4) and third trimester (median 30.4 weeks, 95% range 28.4 – 33.1) fetal head circumference, abdominal circumference, and femur length were measured to the nearest millimeter using standardized ultrasound procedures.[82] Estimated fetal weight was calculated using the Hadlock formula.[83] Gestational age adjusted standard deviation scores (SDS) for all fetal growth characteristics were constructed on data from the total study group.[81] These were based on reference growth curves and represent the equivalent of z-scores.[82] In line with previous studies, we defined fetal growth deceleration and acceleration as a decrease or increase of >0.67 standard deviation of weight from 20 weeks of gestational age to birth. The group between these two markers is considered as having normal growth.[75] At birth, information on infant sex, date of birth and weight was obtained from community midwife and hospital registries. We created gestational age- and sex-adjusted birth length and weight SDS within the total study population by using Growth Analyzer 3.5 (Dutch Growth Research Foundation, Rotterdam, the Netherlands) based on North-European reference standards.[84, 85] We defined small size for gestational age at birth as being <5th sex specific percentile for weight and large size for gestational age at birth as being >95th sex specific percentile for weight. Preterm birth was defined as birth <37.0 weeks of gestation.

Infant length and weight were repeatedly measured at the Community Health Centers according to standardized procedures by well-trained staff at the median ages of 6.2 months (95% range 5.2 – 8.3), 11.1 months (95% range 10.1 - 15.5) and 24.8 months (95% range 23.4 – 28.1). Sex and age adjusted SDS for infant growth characteristics were obtained using Dutch reference growth curves.[86] Similarly as for fetal growth, weight growth deceleration and acceleration were defined as a decrease or increase of >0.67 standard deviation (SD) of weight from birth to 24 months of age.[75]

At the median age of 6.2 years (95% range 5.6 – 7.9) years, we measured child height and weight without shoes and heavy clothing, and calculated body mass index.

Childhood cardiovascular outcomes

At the median age of 6.2 years (95% range 5.6 – 7.9) years, we measured blood pressure at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor PlusTM (Paramus, NJ, USA).[87]We calculated the mean value by using the last three blood pressure measurement of each participant. Carotid-femoral pulse wave velocity was assessed using the automatic Complior SP device (Complior; Artech Medical, Pantin, France) with participants in the supine position.[88] Carotid-femoral pulse wave velocity was calculated as the ratio of the distance travelled by the pulse wave and the time delay between the waveforms, as expressed in meters per second.[89] To cover a complete respiratory cycle, the mean of at least 10 consecutive pressure waveforms was used in the analyses. Carotid-femoral pulse wave velocity can be measured reliably, with good reproducibility, in large pediatric population-based cohorts.[90] We performed M-mode echocardiographic measurements using methods recommended by the American Society of Echocardiography. Our sonographers are experienced and worked under supervision of a pediatric cardiologist, who also performed regular quality checks. We measured aortic root diameter, left ventricular diastolic diameter, left ventricular posterior wall thickness and interventricular septum thickness and calculated fractional shortening and left ventricular mass.[65, 67] Intraobserver and interobserver intraclass correlation coefficients were calculated previously in 28 children with a median age 7.5 years, (interquartile range 3.0 - 11.0) and varied between 0.91 to 0.99 and 0.78 to 0.96, respectively.[66]

Covariates

We obtained information about maternal age, pre-pregnancy weight, parity, educational level, household income, smoking status and folic acid use during pregnancy by questionnaires. Maternal height was measured without shoes and pre-pregnancy body mass index was calculated. Information about gestational hypertension and preeclampsia was obtained from midwife and hospital registries. The following criteria were used to identify women with gestational hypertension: systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24 h urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia.[91] Maternal blood pressure was measured at intake during pregnancy, with the validated Omron 907 automated digital

oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands).[92, 93] Infant ethnicity was classified by the countries of birth of the parents and was categorized as European or non-European. The largest non-European groups were the Turkish, Surinamese and Moroccan groups.[64] Breastfeeding was assessed using questionnaires and categorized as 4 months exclusive breastfeeding; 4 months partial breastfeeding; and never breastfeeding.

Statistical analysis

First, we used linear regression models to assess the associations of birth characteristics, both continuously and in clinical categories, with cardiovascular outcomes (systolic blood pressure, diastolic blood pressure, carotid-femoral pulse wave velocity, aortic root diameter, left ventricular mass and fractional shortening). We tested for non-linearity, but no significant non-linear associations were present. Second, we used stratified linear regression models to assess whether the associations of fetal growth deceleration and acceleration, based on the difference in weight SD score between 20 weeks and birth, with cardiovascular outcomes were modified by infant growth acceleration or deceleration based on the difference in weight SD score between birth and 2 years. We calculated the population attributable risk percent (PAR%) for increased systolic blood pressure (upper 15%) for the fetal growth deceleration group and for the infant growth acceleration group (compared to the normal growth groups), by using this formula: PAR=(risk in population) – (risk in non-exposed) and PAR%=PAR/risk in population. Third, we performed conditional regression analyses to identify independent critical early life growth periods associated with cardiovascular outcomes. Conditional regression analyses take into account the correlations between early life growth measures at different ages.[75] These analyses are described in detail in the **Methods S2.2.1**. Briefly, we constructed length, weight and body mass index gain variables, which are statistically independent from each other, using standardized residuals resulting from the linear regression model of length, weight and body mass index regressed on the prior corresponding growth measurements. This allows simultaneous inclusion of all growth measures in a regression model to assess the most critical periods of growth.[71] Fourth, we compared fetal and infant growth patterns between children with and without high-risk cardiovascular outcomes. High-risk cardiovascular outcomes were defined as a high (highest 15%) blood pressure, carotid-femoral pulse wave velocity, aorta root diameter or left ventricle mass or a low (lowest 15%) fractional shortening. For these analyses we used repeated measurement regression models, which take into account the correlation

between repeated growth measurements of the same participant.[94] For all analyses, we constructed height adjusted standard deviation scores for the vascular outcomes (blood pressure, pulse wave velocity) and body surface area adjusted SDS for the cardiac outcomes (aortic root diameter, left ventricular mass) using Generalized Additive Models for Location, Size and Shape (GAMLSS) using R, version 3.2.0 (R Core Team, Vienna, Austria).[95-97] These models enable flexible modelling, taking into account the distribution of the response variable.[70] All models were adjusted for relevant covariates (maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding, and current age), selected on the basis of their associations with the outcomes of interest based on previous studies or change in effect estimate >10%.[98] We did not observe significant statistical interaction terms between fetal and infant growth measures and child sex or ethnicity in relation to cardiovascular outcomes. Missing covariates were imputed using the multiple imputations procedure to reduce potential bias associated with missing data, twenty-five datasets were created and analyzed together (**Methods S2.2.1**).[99, 100] The repeated measurement analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Subject characteristics

Maternal and child characteristics of study participants are shown in **Table 2.2.1** (Characteristics based on original, and not imputed data are shown in **Table S2.2.1**)**.** The participating mothers had a median body mass index of 23.0 kg/m^2. Of the participating children, 50.2% was a boy and 63.9% had the European ethnicity. Non-response analyses show that children who did not participate in the cardiovascular follow-up studies at the age of 6 years, had lower gestational age at birth and lower birth weight and had mothers who were younger and lower educated, than children who did participate (**Table S2.2.2**). All fetal and infant growth measures are given in **Table S2.2.3**.

Table 2.2.1. Subject characteristics (N=6,239)

Subject characteristics	N	
Maternal characteristics		
Age, y	6239	31.1 (19.8 – 39.9)
Height, cm	6239	167.6 (7.3)
Weight, kg	6239	65.0 (49.9 – 95.1)
Body mass index pre-pregnancy, kg/m^2	6239	23.0 (18.2 – 33.6)
Parity, n (%)	6239	
0		3508 (56.2)
≥1		2731 (43.8)
Maternal education, n (%)	6239	
Low/middle		3413 (54.7)
Higher		2826 (45.3)
Folic acid intake during pregnancy, n (%)	6239	
No use		1648 (26.4)
Start in the first 10 weeks		1974 (31.6)
Start periconceptional		2617 (41.9)
Alcohol consumption during pregnancy, n (%)	6239	
No		2865 (45.9)
Yes		3374 (54.1)
Smoking during pregnancy, n(%)	6239	
No		4630 (74.2)
Yes		1609 (25.8)
Systolic blood pressure, mmHg	6239	115.6 (12.2)
Diastolic blood pressure, mmHg	6239	68.1 (9.5)
Gestational hypertensive disorders*, n (%)	6239	397 (6.4)
Birth and infant characteristics		
Sex, boys, n (%)	6239	
Boys		3132 (50.2)
Girls		3107 (49.8)
Ethnicity, European, n(%)	6239	
European		3988 (63.9)
Non-European		2251 (36.1)
Gestational age at birth unit, weeks	6239	40.1 (35.9 – 42.3)
Birth weight, grams	6239	3429 (551)
Preterm birth <37 weeks at delivery (%)	6239	309 (5.0)
Small for gestational age†, n (%)	6239	291 (4.7)
Large for gestational age‡, n (%)	6239	310 (5.0)
Breastfeeding, n (%)	6239	
4 months exclusive		1456 (23.3)
4 months partial		4138 (66.3)
Never		645 (10.3)

Table 2.2.1 (continued)

Child characteristics at 6 years		
Age, y	6239	6.0 (5.6 – 7.9)
Height, cm	6239	119.5 (6.0)
Weight, kg	6239	22.6 (17.6 – 34.2)
Body mass index, kg/m^2	6239	15.9 (13.6 – 21.3)
Systolic blood pressure, mmHg	6196	102.8 (8.3)
Diastolic blood pressure, mmHg	6196	60.8 (6.9)
Carotid-femoral pulse wave velocity, m/s	5078	5.5 (0.9)
Aortic root diameter, mm	5925	19.3 (1.8)
Left ventricular mass, grams	5813	53.4 (11.6)
Fractional shortening, %	5899	35.2 (4.5)

Values are means (SD), medians (95% range) or numbers (%). The values represent the pooled results after multiple imputations (N=25). *Pre-eclampsia or pregnancy induced hypertension. †Sex specific gestational age adjusted birth weight <5th percentile in total cohort. ‡Sex specific gestational age adjusted birth weight >95th percentile in total cohort. Characteristics based on observed, not imputed, data are given in **Table S2.2.1**.

Birth outcomes and childhood cardiovascular outcomes

Table 2.2.2 shows that younger gestational age at birth was associated with a higher systolic blood pressure, smaller aortic root diameter and lower left ventricular mass in childhood (all p-values for trend <0.05). Similarly, a lower birth weight was associated with a higher systolic and diastolic blood pressure and with a smaller aortic root diameter and left ventricular mass in childhood (all p-values for trend <0.05). A lower gestational age adjusted birth weight was associated with a higher systolic and diastolic blood pressure and smaller aortic root diameter and left ventricular mass in childhood (all p-values for trend <0.05). We did not observe consistent associations of birth measurements and gestational age with childhood carotid-femoral pulse wave velocity or fractional shortening. Similar results were present in the models adjusted for age and gender of the child only (data not shown). P-values are shown in **Table S2.2.4.**

Table 2.2.2 Birth characteristics and cardiovascular outcomes at the age of 6 years (N = 6,239)

		Difference in cardiovascular outcomes standard deviation scores (95% Confidence Interval)		
Birth characteristics	**N**	**Systolic blood pressure**	**Diastolic blood pressure**	**Carotid-femoral pulse wave velocity**
Gestational age	**6239**			
<37.0 weeks	309	0.07 (-0.04, 0.19)	-0.02 (-0.13, 0.10)	-0.06 (-0.19, 0.06)
37.0-41.9 weeks	5503	Reference	Reference	Reference
≥42 weeks	427	-0.06 (-0.16, 0.04)	-0.05 (-0.15, 0.04)	-0.06 (-0.17, 0.06)
Trend		*-0.05 (-0.07, -0.02)*†	*-0.01 (-0.04, 0.01)*	*0.00 (-0.03, 0.03)*
Birth weight	**6239**			
<2000 grams	71	0.22 (-0.01, 0.45)	0.11 (-0.12, 0.35)	-0.30 (-0.56, -0.03)*
2000-2499 g	202	-0.01 (-0.15, 0.14)	-0.05 (-0.19, 0.10)	-0.10 (-0.26, 0.07)
2500-2999 g	914	0.01 (-0.07, 0.09)	0.04 (-0.04, 0.12)	-0.06 (-0.15, 0.02)
3000-3499 g	2164	Reference	Reference	Reference
3500-3999 g	2010	-0.11 (-0.17, -0.05)†	-0.09 (-0.15, -0.03)†	-0.03 (-0.10, 0.03)
4000-4499 g	734	-0.15 (-0.23, -0.06)†	-0.11 (-0.19, -0.02)*	-0.03 (-0.16, 0.06)
≥4500 grams	144	-0.20 (-0.37, -0.03)*	-0.15 (-0.32, 0.02)	0.03 (-0.16, 0.21)
Trend		*-0.06 (-0.09,-0.04)*†	*-0.05 (-0.07, -0.02)*†	*0.02 (-0.01, 0.05)*
Birth weight for gestational age	**6239**			
Small	291	0.16 (0.04, 0.28)†	0.12 (0.01, 0.24)*	-0.08 (-0.21, 0.06)
Normal	5638	Reference	Reference	Reference
Large	310	-0.10 (-0.22, 0.01)	-0.15 (-0.27, -0.04) †	0.01 (-0.13, 0.13)
Trend		*-0.05 (-0.07, -0.02)*†	*-0.05 (-0.07, -0.02)*†	*0.02 (-0.01, 0.05)*

		Difference in cardiovascular outcomes standard deviation scores (95% Confidence Interval)		
Birth characteristics	**N**	**Aortic root diameter**	**Left ventricular mass**	**Fractional shortening**
Gestational age	**6239**			
<37.0 weeks	309	-0.10 (-0.22,0.01)	-0.02 (-0.14, 0.09)	0.05 (-0.07, 0.17)
37.0-41.9 weeks	5503	Reference	Reference	Reference
≥42 weeks	427	0.03 (-0.07, 0.13)	0.02 (-0.08, 0.12)	-0.10 (-0.20, 0.01)
Trend		*0.04 (0.02, 0.07)*†	*0.03 (0.00, 0.05)**	*-0.02 (-0.05, 0.01)*
Birth weight	**6239**			
<2000 grams	71	-0.25 (-0.48,-0.01)*	-0.03 (-0.27, 0.21)	0.03 (-0.21, 0.28)
2000-2499 g	202	-0.09 (-0.24, 0.05)	-0.07 (-0.22, 0.07)	0.01 (-0.14, 0.16)
2500-2999 g	914	-0.09 (-0.17, -0.01)*	-0.07 (-0.14, 0.01)	-0.04 (-0.12, 0.04)
3000-3499 g	2164	Reference	Reference	Reference
3500-3999 g	2010	0.12 (0.06, 0.18)†	0.04 (-0.02, 0.10)	-0.04 (-0.10, 0.02)
4000-4499 g	734	0.12 (0.03, 0.20)†	0.09 (0.00, 0.17)*	-0.05 (-0.14, 0.03)
≥4500 grams	144	0.27 (0.11, 0.44)†	0.20 (0.03, 0.37)*	-0.14 (-0.31, 0.04)
Trend		*0.09 (0.06, 0.12)*†	*0.06 (0.03, 0.08)*†	*-0.02 (-0.05, 0.01)*
Birth weight for gestational age	**6239**			
Small	291	-0.16 (-0.27, -0.04)†	-0.05 (-0.17, 0.07)	0.00 (-0.13, 0.12)
Normal	5638	Reference	Reference	Reference
Large	310	0.07 (-0.05, 0.18)	0.13 (0.01, 0.25)*	-0.04 (-0.16, 0.08)
Trend		*0.08 (0.05, 0.10)*†	*0.05 (0.03, 0.08)**	*-0.01 (-0.04, 0.02)*

Values are regression coefficients (95% confidence interval) and reflect the change in standard deviation (SDS) of each cardiovascular outcome for each birth weight or gestational age group, compared to the reference group. Trend estimates represent the effect estimates for the continuous associations per SDS change in birth characteristic. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding and current age.**P < 0.05;* † *P< 0.01* Exact P-values are shown in **Table S2.2.4**.

Fetal and infant growth measures and childhood cardiovascular outcomes

Figure 2.2.1 shows that as compared to children with normal growth during fetal life and infancy, those with fetal weight growth deceleration followed by infant weight growth acceleration had a higher systolic and diastolic blood pressure (differences 0.12 SDS (95% Confidence Interval (CI) 0, 0.24) and 0.13 SDS (95% CI 0.01, 0.25), respectively), and smaller aortic root diameter (difference -0.15 SDS (95% CI -0.27, -0.03). Children with growth deceleration during both fetal life and infancy had the highest left ventricular mass. We observed the highest systolic blood pressure among children with normal fetal growth followed by accelerated growth. The PAR% for the fetal growth deceleration group (compared to normal growth) for increased systolic blood pressure was 3.7%, while the PAR% for infant growth acceleration on systolic blood pressure was 14.9%. Among children with normal fetal growth, those with infant growth acceleration had a smaller aortic root diameter (difference -0.15 SDS (95% CI -0.27, -0.03) and higher fractional shortening (difference 0.15 SDS (95% CI 0.03, 0.28). Fetal growth acceleration followed by infant growth deceleration was associated with lower systolic and diastolic blood pressure (differences -0.17 SDS (95% Confidence Interval (CI) -0.29, -0.05) and -0.16 SDS (95% CI -0.28, -0.04), respectively, compared to children with normal fetal and infant growth). Infant growth was not associated with carotid-femoral pulse wave velocity. Exact results are shown in **Table S2.2.5**.

To further identify critical periods for the different cardiovascular outcomes, we performed conditional regression analyses. Results are given in **Figure S2.2.2** and suggest that not fetal size, but low weight at birth, and high weight and body mass index during infancy are associated with higher systolic blood pressure in childhood, conditional on respective weight and body mass index at earlier ages (**Figure S2.2.2A-B**). Length, but not weight, in late infancy, conditional on earlier measurements, was positively associated with carotid-femoral pulse wave velocity in childhood (**Figure S2.2.2C**). Weight in fetal life and at birth, but not at older ages was positively associated with aortic root diameter and left ventricular mass in childhood (**Figure S2.2.2D-E**). Fetal femur length at 30 weeks was inversely, and length at birth was positively associated with left ventricular mass in childhood (**Figure S2.2.2E**). Body mass index in late infancy was inversely associated with fractional shortening, conditional on respective weight and body mass index at earlier ages (**Figure S2.2.2F**).

Figure 2.2.1 Associations of fetal and infant growth with cardiovascular structures and function at the age of 6 years (N = 3,396)

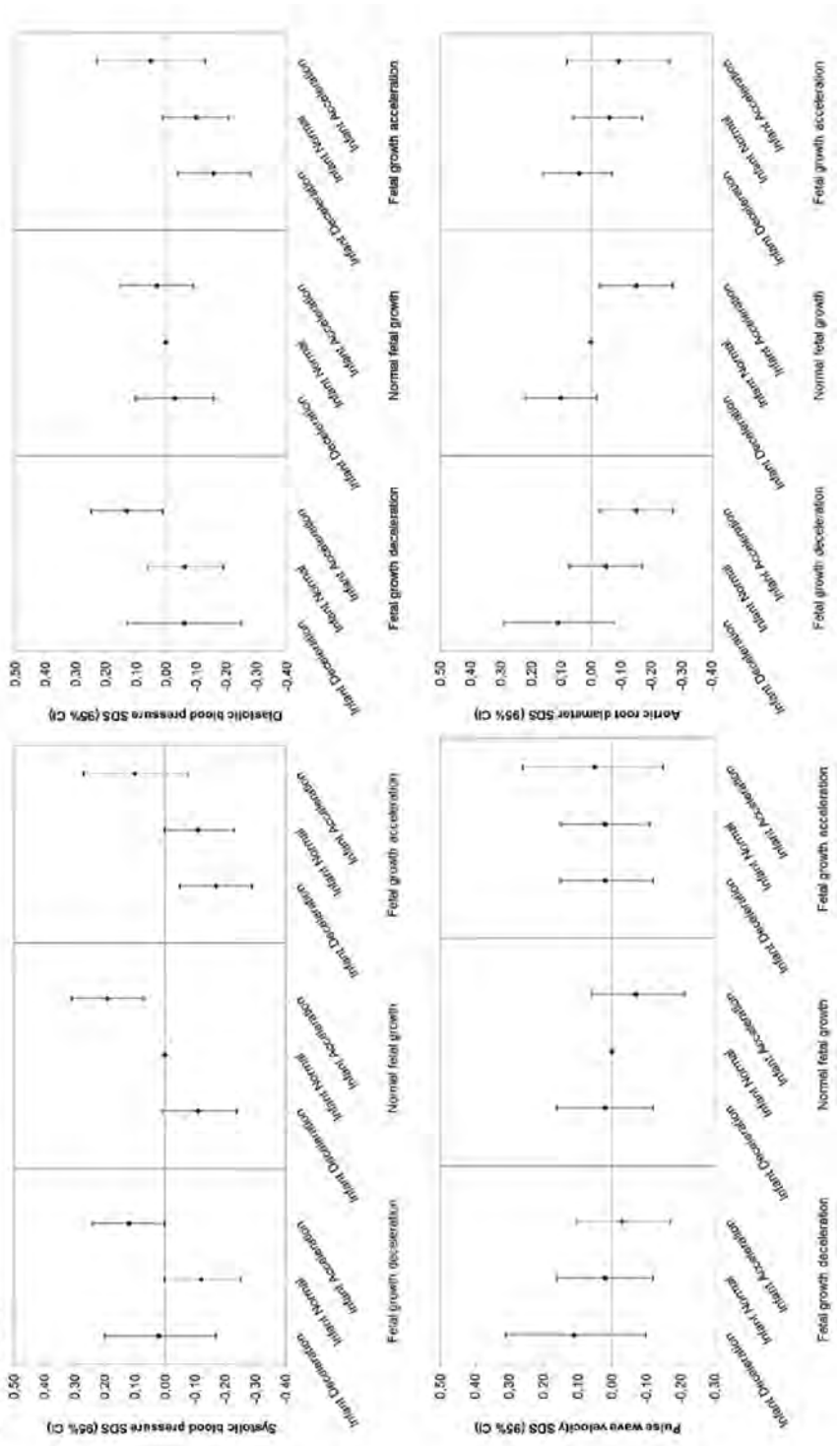
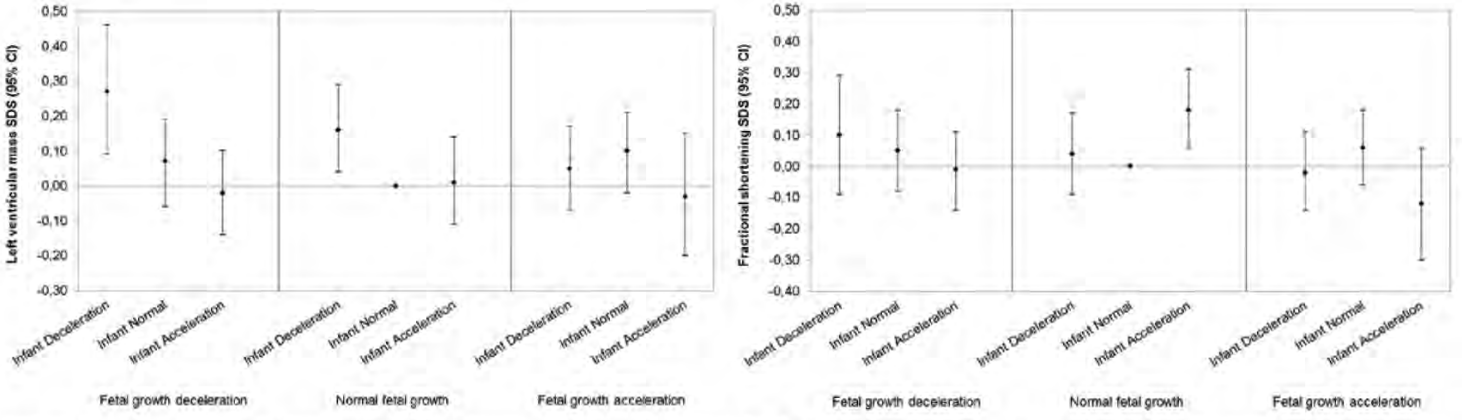


Figure 2.2.1 (continued)



Values are regression coefficients (95% confidence interval) and reflect the difference for each cardiovascular outcome compared to children with normal fetal and infant growth. Fetal growth is defined as weight SDS gain from mid-pregnancy (20 weeks gestational age) to birth. Infant growth was defined as the period between birth to 2 years. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding and current age. Trend tests represent the effect estimates of continuous infant growth (SDS) within each fetal growth group. Interaction terms between fetal growth categories and infant growth were not significant. Exact values are given in T**able S2.2.5.**

Longitudinal growth patterns associated with high-risk childhood cardiovascular outcomes

Figure 2.2.2 shows that as compared to children who had a normal blood pressure at 6 years, those who had an increased blood pressure tended to be smaller during third trimester of fetal life, but of similar size during infancy (p-values <0.05). We observed stronger effect estimates for systolic blood pressure than diastolic blood pressure. Children with increased carotid-femoral pulse wave velocity showed similar fetal growth patterns as children without increased carotid-femoral pulse wave velocity, but they had lower weights during infancy. As compared to children with a normal aortic root diameter and left ventricular mass, those with increased aortic root diameter and left ventricular mass, respectively, tended to be larger during fetal life, but of similar size during infancy (p-values <0.05). We observed a tendency for children with decreased fractional shortening to be taller and heavier during both fetal and infant life as compared to those with normal fractional shortening.

Figure 2.2.2 Fetal and infant growth patterns of children with increased levels of cardiovascular risk factors (N = 6,239)

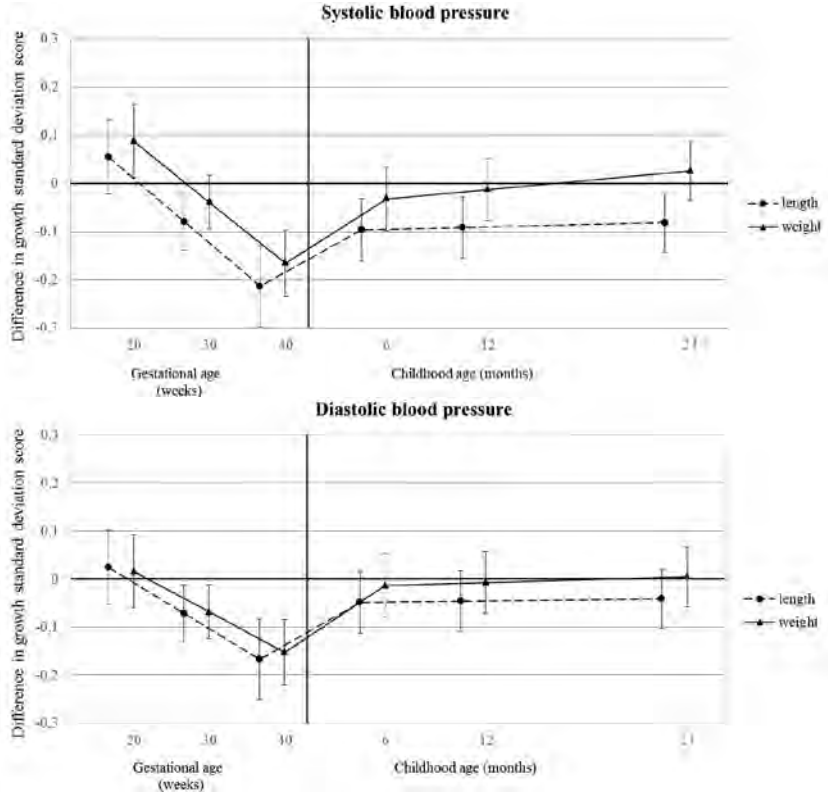
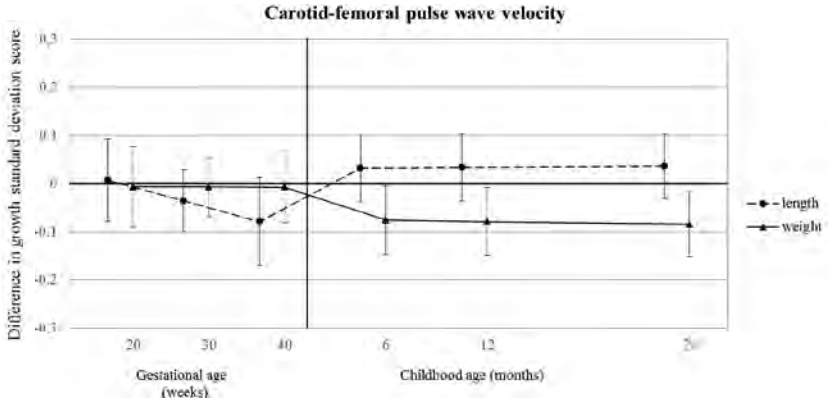
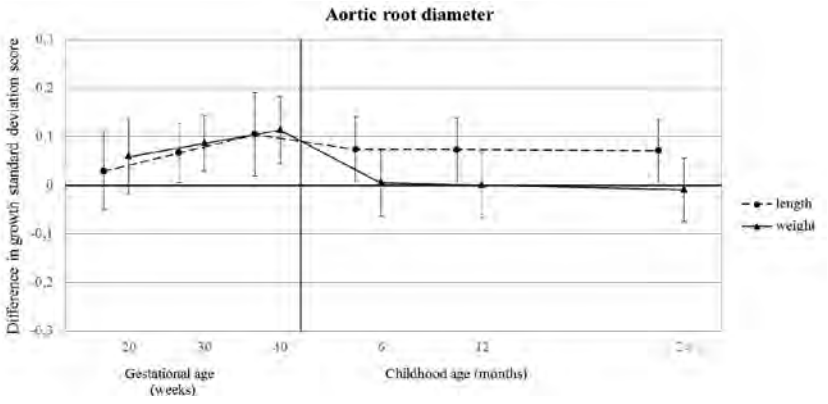


Figure 2.2.2 (continued)





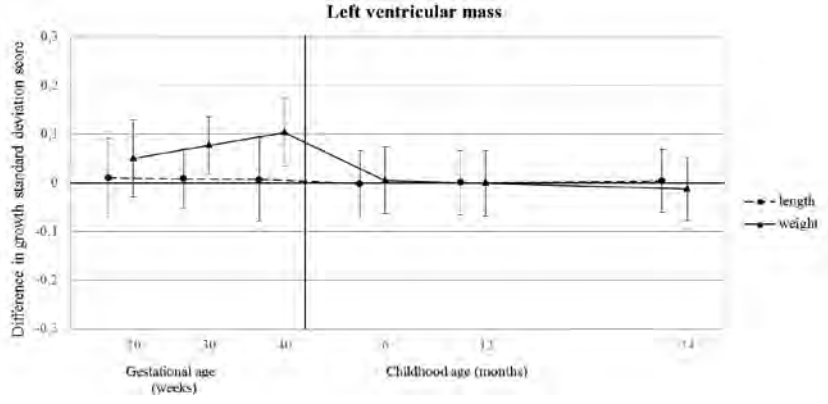
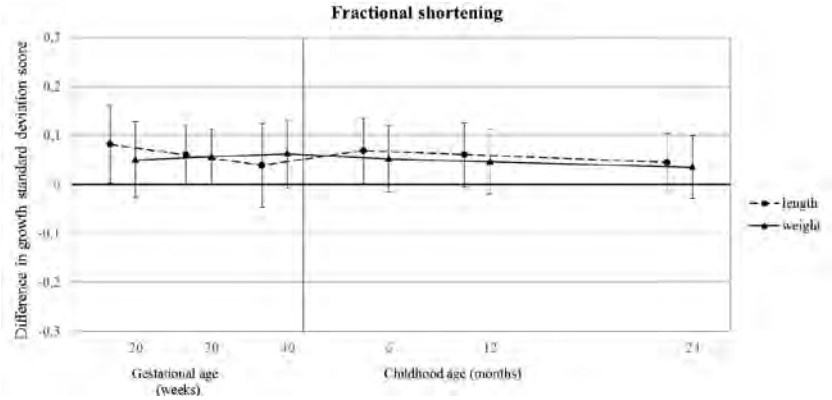


Figure 2.2.2 (continued)



Values are based on repeated linear regression models. Regression coefficients (95% confidence interval) reflect the difference in (femur)length and weight standard deviation score from second trimester onwards for children with increased cardiovascular risk compared to children without this risk. Having increased risk is defined as having systolic and diastolic blood pressure, carotid-femoral pulse wave velocity, aortic root diameter or left ventricular mass ≥85th or having fractional shortening ≤15th centile. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass we used body surface area adjusted standard deviation scores. All models were adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding, and current age.

DISCUSSION

In this population-based, prospective cohort study, we identified fetal and infant growth patterns and critical periods associated with adverse cardiovascular outcomes in childhood. Younger gestational age at birth and lower weight at birth were both associated with higher blood pressure, smaller aortic root diameter and lower left ventricular mass. Also, children who had an increased blood pressure at 6 years tended to be smaller during late fetal life followed by higher growth rates during infancy. Children, who had an increased aortic root diameter or left ventricular mass at 6 years, had higher growth rates during both fetal life and infancy.

Methodological considerations

Main strengths of this study are its population-based prospective design starting in fetal life, and the large number of fetal and infant growth measurements and cardiovascular outcomes available. We investigated the influence of prospectively measured fetal and childhood growth patterns,

in addition to birth size, on cardiovascular outcomes using different statistical approaches. The repeated fetal and infant growth measurements provided the opportunity to assess the effects of both fetal and infant growth on cardiovascular outcomes, to identify critical periods during fetal life and infancy and to discover specific growth patterns associated with an adverse cardiovascular profile. Some limitations need to be discussed. Response rate at baseline in the Generation R Study cohort was 61%. Biased estimates in large cohort studies mainly arise from loss to follow-up, rather than from nonresponse at baseline.[101] Of the children available for follow-up, 75% participated in the cardiovascular follow-up measurements. This could lead to bias if the associations between early growth characteristics with cardiovascular outcomes differ between those included and those not included in the analyses. Lower gestational age at birth and lower birth weight were more prevalent among children who were not included in the study, as compared to those included. The effect of selective loss to follow-up is difficult to estimate. Ethnicity is associated with fetal growth patterns and birth weight.[102, 103] However, in the current study, we did not find significant interaction terms between ethnicity and fetal and infant growth measures in relation to cardiovascular outcomes. We have adjusted the analyses for ethnicity and our results do not suggest that the differences between ethnic groups influence the results. Although we used several cardiovascular outcome measurements, more detailed information can be collected by advanced cardiovascular Magnetic Resonance Imaging (MRI). Previous studies reported specific effects of preterm birth on cardiac shape and morphology, rather than on left ventricular mass.[104] For the repeated measurement regression models, we dichotomized our outcomes into increased versus normal risk, using a 15% cut-off. However, results were not dependent on this cut-off, because we observed similar results when we used 10% or 25% as cut-off. Participants in this study have a median age of 6.0 years. Whether the observed associations are clinically important later in life needs to be further studied. Although the potential confounding effect of factors related to the risk of cardiovascular disease is expected to be restricted in young children, and we did take many potential confounders into account, the influence of residual confounding should be considered, as in all observational studies.

Interpretation of main findings

Fetal and infant life seem to be critical periods for development of cardiovascular health in adulthood.[4] It has been hypothesized that a mismatch between suboptimal fetal environment and affluent postnatal environment leads to adverse cardiovascular adaptations, which

subsequently predisposes individuals to cardiovascular disease.[10] Previous studies have shown that children born with a small size at birth and childhood growth acceleration have a greater risk of cardiovascular disease in later life.[3, 14] In the current study, we examined the associations of fetal and infant growth with cardiovascular outcomes and aimed to identify critical periods and longitudinal growth patterns associated with adverse cardiovascular profiles in childhood.

The associations of low birth weight with blood pressure in later life are well established but the effect estimates seem to be small.[105] Results from a systematic review suggest that especially children with a low birth weight followed by high childhood growth rates have an increased blood pressure.[14] By using conditional regression analyses, another study showed that low birth weight and accelerated growth in infancy are associated with higher blood pressure, independent from each other.[106] However, the effects of adiposity in childhood on blood pressure were much stronger that the effects of birth outcomes.[106] Another study using adiposity growth trajectories on repeatedly measures blood pressure, shows that adiposity trajectories in childhood are associated with higher blood pressure, independent from birth weight.[107] In line with these studies, we observed associations of lower gestational age, birth weight and gestational age adjusted birth weight with higher systolic and diastolic blood pressure in childhood. Furthermore, we observed that body mass index increase in infancy, independent from fetal growth or birth weight, was positively associated with blood pressure. Our results suggest that both lower fetal weight gain and high infant weight gain are associated higher blood pressure in childhood, with stronger effect estimates for infant weight gain.

A suboptimal fetal development may lead to impaired elastin synthesis, and subsequently stiffer arteries and increased blood pressure.[108] A study amongst 337 adults aged 50 showed that lower birth weight was associated with higher femoro-popliteal-tibial pulse wave velocity, but not with the pulse wave velocity on the aorto-iliac segment.[109] Another study in 86 children aged 8.2 years showed that children who were born preterm and small for gestational age had increased arterial stiffness.[110] We did not observe consistent associations of gestational age, birth weight or gestational age adjusted birth weight with aortic-femoral pulse wave velocity. We could not identify critical periods of growth associated with arterial stiffness. Our results are in line with a study among 951 adults aged 25 years, in which no relation was found between birth weight and pulse wave velocity.[111] Further studies are needed to examine whether the associations of birth outcomes with pulse wave velocity become present at older ages.

Previous studies suggested that early life growth characteristics might influence cardiac structure in children and adults.[11, 46] We observed that fetal growth rates were positively associated with aortic root diameter and left ventricular mass. In line with our findings, a study among 216 children reported a positive association of birth weight with aortic root diameter.[112] The Framingham Heart Study reported height and weight to be positively associated with aortic root diameter in adults aged 20 to 89 (mean 48 years).[113] In the same study, systolic blood pressure was inversely associated with aortic root diameter.[113] This is in line with our results. We observed that both gestational age adjusted birth weight and fetal growth deceleration followed by growth acceleration were associated with higher systolic blood pressure and with smaller aortic root diameter.

A study in 200 children aged 5 years showed that children with growth restriction in fetal life had a different cardiac shape, with increased transversal diameters and more globular cardiac ventricles.[11] Another study among 102 young adults, who were born preterm, showed that left ventricle geometry was altered and ventricular mass was increased.[104] We observed that birth weight was positively associated with body surface area standardized left ventricular mass. In childhood, muscle mass is an important determinant of left ventricular mass.[22, 74] Lean mass is also positively associated with birth weight.[114] It is possible that children with a higher birth weight have both higher lean mass and thus cardiac mass. Early cardiac growth is determined by myocardial cell hyperplasia, while later in life cardiac growth is determined by hypertrophy.[7] Children with normal or decelerated fetal growth, followed by infant growth deceleration had the highest left ventricular mass, relative to current body size. This is in line with another study, which found low weight at the age of one year to be associated with left ventricular hypertrophy in adulthood.[46] Further studies are needed to assess geometry of the cardiac structures of children with fetal growth restriction, and the possible associations with an adverse cardiovascular profile in adulthood. The observation that a lower gestational age and fetal growth deceleration followed by infant weight growth acceleration with higher blood pressure were not paired with left ventricular hypertrophy or aortic root diameter, might also be explained by the association of lean mass with left ventricular mass in children.[74]

Although the observed effect estimates of fetal and infant growth on cardiovascular outcomes are small and without clinical relevance for individuals, the results from this study may be important on a population based level. The results are an important contribution to the field of developmental origins of cardiovascular disease. They suggest that both fetal and infant growth, and especially the combination of both, influence

cardiovascular development and might predispose individuals to cardiovascular disease in adulthood. The critical periods and growth patterns for vascular development may differ from those for cardiac development. Although previous studies indicated tracking of a range of structural and functional properties of the cardiovascular system from childhood to adulthood, the consequences of the modest effect estimates on the individual risk of cardiovascular disease in later life need to be further studied. [15, 36, 60]

SUPPLEMENTAL MATERIAL

Methods S2.2.1 Statistical analysis

To take into account the correlation between fetal and infant growth characteristics, we used conditional regression analyses to examine the associations of these measurers with childhood outcomes.[75] We constructed length, weight and body mass index gain variables, which are statistically independent from each other, using standardized residuals resulting from the linear regression model of length, weight and body mass index regressed on the prior corresponding growth measurements. This allows simultaneous inclusion of all growth measures in a regression model.[75]

The percentages of missing covariate values within the population for analysis were lower than 15.0%, except for folic acid use (30.6%) maternal pre-pregnancy BMI (24.8%) and breastfeeding (21.7%). Data were imputed according to the Markov Chain Monte Carlo method, assuming no monotone missing pattern. Data were analyzed in each set separately, and pooled estimates from the 25 imputed datasets were used to report the effect estimates and their 95% confidence intervals (95% CI).[100]

Figure S2.2.1 Flow chart of participants included in the analysis

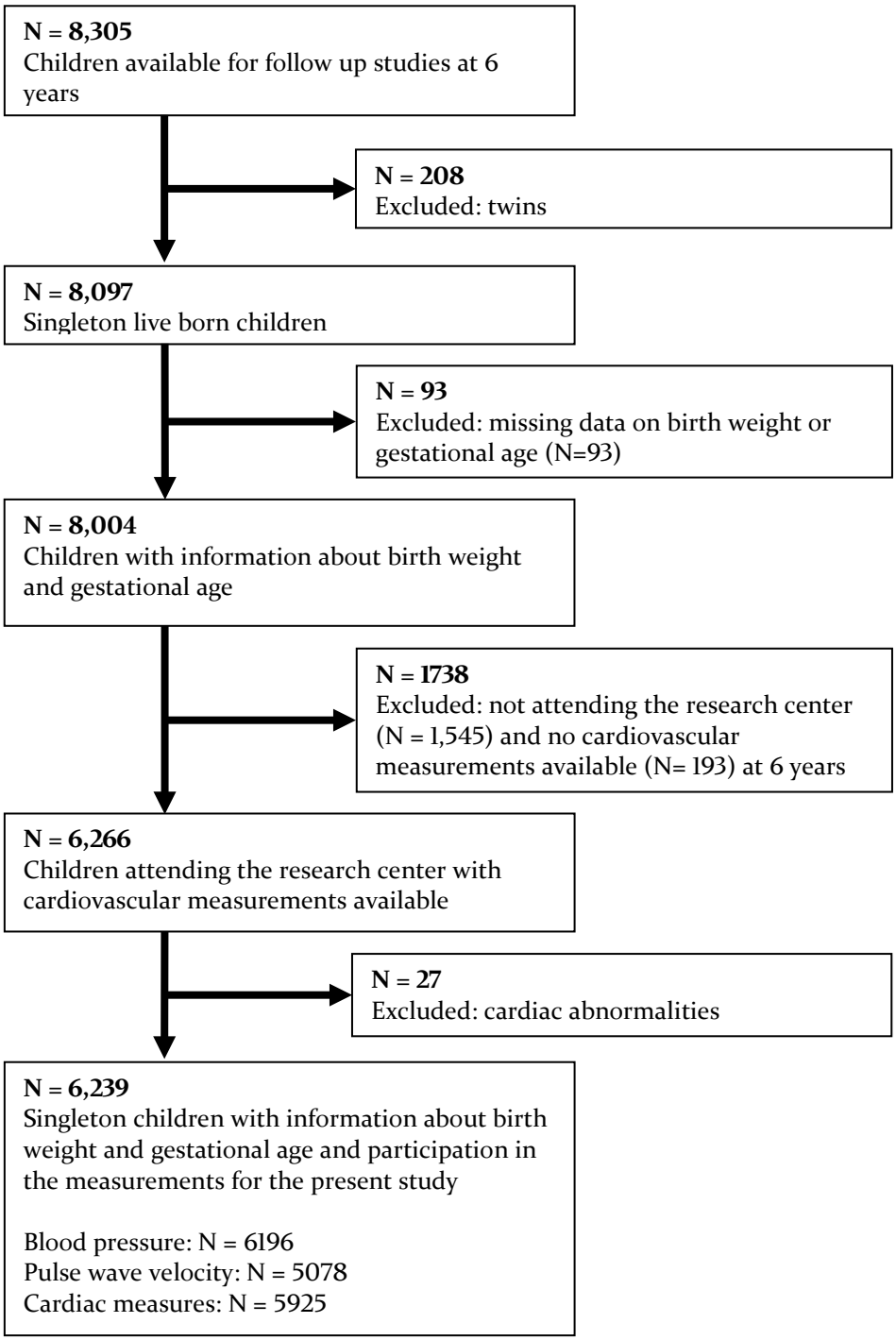
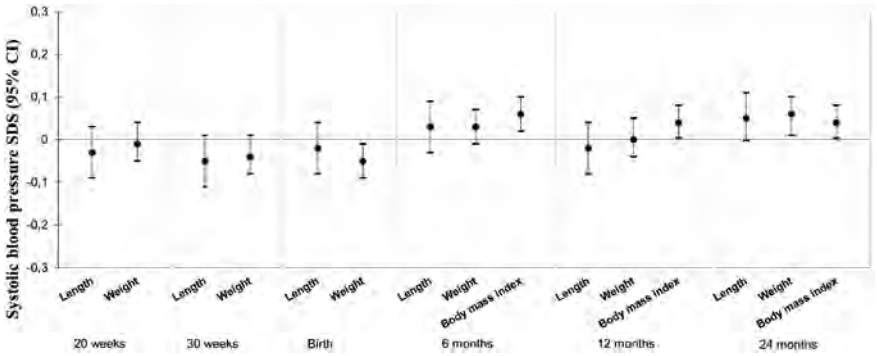
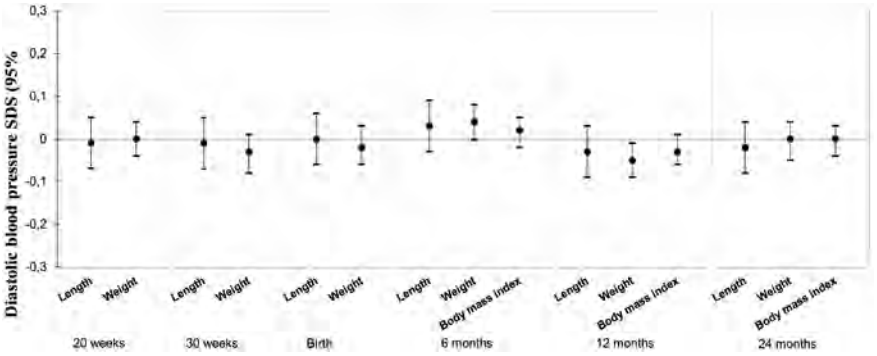


Figure S2.2.2 Associations of fetal and infant growth measures with childhood cardiovascular outcomes from conditional analyses (N = 6,239)

A.



B.



C.

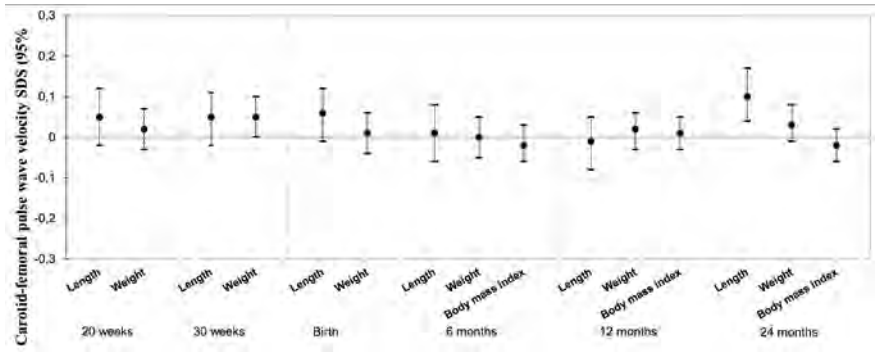
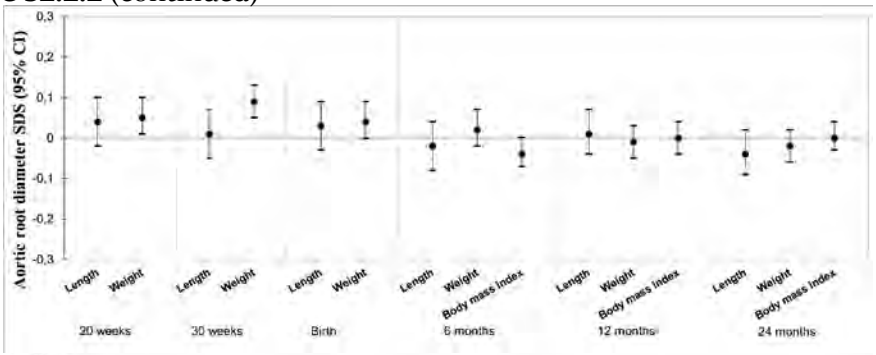
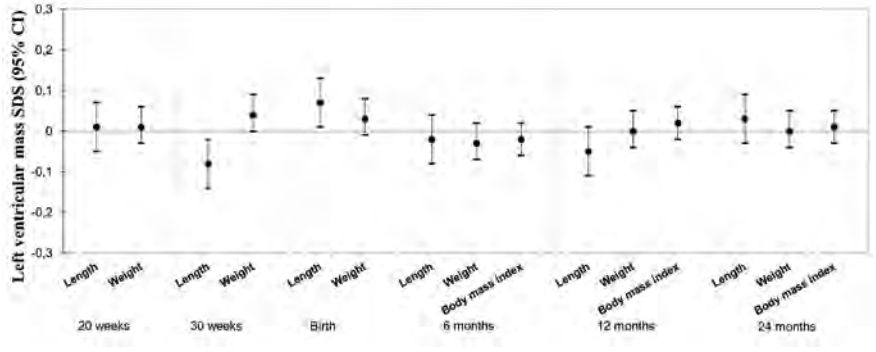


Figure S2.2.2 (continued)

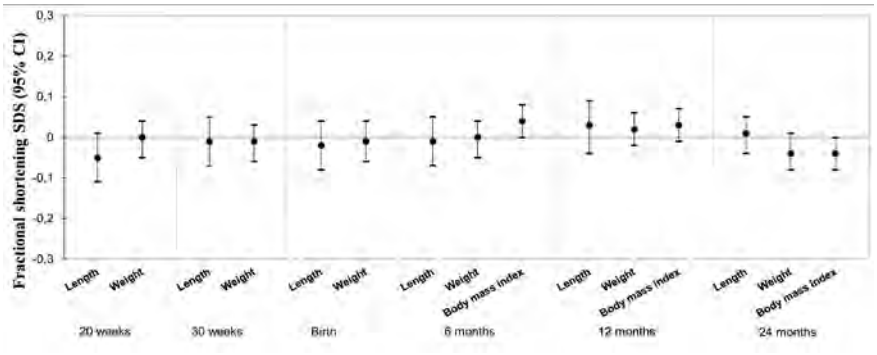
D.



E.



F.



Abbreviations: SDS, Standard deviation scores; CI, confidence interval. Values are regression coefficients (95% CI) from conditional analyses. Exact numbers corresponding to the regression coefficients are presented in **Table S2.2.6.** Length in fetal life represents estimated femur length. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. The estimates represent differences in cardiovascular measures per standardized residual change of fetal and infant growth measures. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding, and current age.

Table S2.2.1 Subject characteristics based on original data (N = 6,239)

Subject characteristics	N	Missing N (%)	
Maternal characteristics			
Age, y	6239	0 (0)	30.5 (5.2)
Height, cm	5677	562 (9.0)	167.5 (7.4)
Weight, kg	4700	1539 (24.7)	64.0 (49.0–98.0)
Body mass index pre-pregnancy, kg/m^2	4691	1548 (24.8)	22.7 (18.1–34.7)
Parity, n (%)	6073	166 (2.7)	
0			3425 (56.4)
≥1			2648 (43.6)
Maternal education, n (%)	5709	530 (8.5)	
Low/middle			3042 (53.3)
Higher			2667 (46.7)
Folic acid intake during pregnancy, n (%)	4330	1909 (30.6)	
No use			1090 (25.2)
Start in the first 10 weeks			1380 (31.9)
Start periconceptional			1860 (43.0)
Alcohol consumption during pregnancy, n (%)	4992	1247 (20.0)	
No			2287 (45.8)
Yes			2705 (54.2)
Smoking during pregnancy, n(%)	5053	1186 (19.0)	
No			3738 (74.0)
Yes			1315 (26.0)
Systolic blood pressure, mmHg	5643	596 (9.6)	115.6 (12.2)
Diastolic blood pressure, mmHg	5643	596 (9.6)	68.1 (9.5)
Gestational hypertensive disorders[a], n (%)	5530	709 (11.4)	335 (6.1)
Birth and infant characteristics			
Sex, boys, n(%)	6239	0 (0)	
Boys			3132 (50.2)
Girls			3107 (49.8)
Ethnicity, European, n(%)	6087	152 (2.4)	
European			3914 (64.3)
Non-European			2173 (35.7)
Gestational age at birth unit, weeks	6239	0 (0)	40.1 (35.9 –42.3)
Birth weight, grams	6239	0 (0)	3429 (551)
Preterm birth <37 weeks at delivery (%)	6239	0 (0)	309 (5.0)
Small for gestational age[b], n (%)	6239	0 (0)	291 (4.7)
Large for gestational age[c], n (%)	6239	0 (0)	310 (5.0)
Breastfeeding, n (%)	3940	2299 (36.8)	
4 months exclusive			1003 (16.1)
4 months partial			2564 (41.1)
Never			373 (6.0)

Table S2.2.1 (continued)

Child characteristics at 6 years			
Age, y	6239	0 (0)	6.0 (5.6–7.9)
Height, cm	6239	0 (0)	119.5 (6.0)
Weight, kg	6239	0 (0)	22.6 (17.6–34.2)
Body mass index, kg/m^2	6239	0 (0)	15.9 (13.6–21.3)
Systolic blood pressure, mmHg	6196	43 (0.7)	102.8 (8.3)
Diastolic blood pressure, mmHg	6196	43 (0.7)	60.8 (6.9)
Carotid-femoral pulse wave velocity, m/s	5078	1161 (18.6)	5.5 (0.9)
Aortic root diameter, mm	5925	314 (5.0)	19.3 (1.8)
Left ventricular mass, grams	5813	426 (6.8)	53.4 (11.6)
Fractional shortening, %	5899	340 (5.4)	35.2 (4.5)

Values are means (SD), medians (95% range) or numbers (%). Values represent the results based on the original, non-imputed data. [a] Pre-eclampsia or pregnancy induced hypertension. [b]Sex specific gestational age adjusted birth weight <5th percentile in total cohort. [c]Sex specific gestational age adjusted birth weight >95th percentile in total cohort.

Table S2.2.2 Non-response analysis for childhood follow-up data at 6 years (N=8,305)

Subject characteristics	Follow-up at 6 years N=6,239	Lost to follow-up at 6 years N=2,066	P-value
Maternal characteristics			
Age, y	30.5 (5.2)	29.5 (5.4)	<0.01
Height, cm	167.5 (7.4)	167.3 (7.4)	0.20
Weight, kg	64.0 (49.0-98.0)	64.0 (48.0-98.9)	0.33
Body mass index pre-pregnancy, kg/m^2	22.7 (18.1-34.7)	22.6 (17.8-35.0)	0.28
Primiparity, n (%)	3425 (56.4)	1022 (52.4)	0.00
Maternal education, higher, n (%)	2667 (46.7)	708 (40.3)	<0.01
Folic acid intake during pregnancy, never, n (%)	1090 (25.2)	416 (31.3)	<0.01
Alcohol consumption during pregnancy, never, n (%)	2287 (45.8)	862 (54.7)	<0.01
Smoking during pregnancy, never, n(%)	3738 (74.0)	1134 (70.8)	0.01
Systolic blood pressure, mmHg	115.6 (12.2)	115.2 (12.4)	0.28
Diastolic blood pressure, mmHg	68.1 (9.5)	67.5 (9.7)	0.03
Gestational hypertensive disorders[a], n (%)	335 (6.1)	110 (6.3)	0.69
Birth and infant characteristics			
Sex, boys, n(%)	3132 (50.2)	1057 (51.2)	0.44
Ethnicity, European, n(%)	3914 (64.3)	1195 (63.4)	0.47
Gestational age at birth unit, weeks	40.1 (35.9-42.3)	39.9 (33.7-42.3)	<0.01
Birth weight, grams	3429 (551)	3307 (643)	<0.01
Preterm birth <37 weeks at delivery (%)	309 (5.0)	215 (10.8)	<0.01
Small for gestational age[b], n (%)	291 (4.7)	119 (6.1)	0.01
Large for gestational age[c], n (%)	310 (5.0)	100 (5.1)	0.81
Breastfeeding, n (%)			<0.01
4 months exclusive	1003 (25.5)	210 (20.6)	
4 months partial	2564 (65.1)	675 (66.1)	
Never	373 (9.5)	136 (13.3)	

Values are means (SD), medians (95% range) or numbers (%). Values represent the results based on the original, non-imputed data. [a] Pre-eclampsia or pregnancy induced hypertension. [b]Sex specific gestational age adjusted birth weight <5th percentile in total cohort. [c]Sex specific gestational age adjusted birth weight >95th percentile in total cohort.

Table S2.2.3 Fetal and infant length, weight and body mass index and cardiovascular outcomes at the age of 6 years (N = 6,239)

		Difference in cardiovascular outcomes standard deviation score (95% Confidence Interval)					
	N	Systolic blood pressure	Diastolic blood pressure	Pulse wave velocity	Aortic root diameter	Left ventricular mass	Fractional shortening
Length (SDS)							
20 weeks	5354	-0.01 (-0.04, 0.01) P=0.286	-0.02 (-0.05, 0.01) P=0.164	0.02 (-0.01, 0.05) P=0.137	0.02 (-0.00, 0.05) P=0.085	0.02 (-0.01, 0.04) P=0.246	-0.03 (-0.05, 0.00) P=0.057
30 weeks	5496	-0.06 (-0.09, -0.04)** P=0.000	-0.04 (-0.07, -0.01)** P=0.004	0.02 (-0.01, 0.05) P=0.183	0.05 (0.02, 0.07)** P=0.000	0.01 (-0.02, 0.04) P=0.552	-0.04 (-0.07, -0.01)** P=0.004
Birth	3816	-0.05 (-0.08, -0.02)** P=0.001	-0.04 (-0.07, -0.01)** P=0.003	(-0.03, 0.04) P=0.833	-0.00, 0.05) P=0.060	0.00 (-0.02, 0.04) P=0.425	-0.02 (-0.05, 0.01) P=0.159
6 months	4075	-0.07 (-0.10, -0.04)** P=0.000	-0.02 (-0.05, 0.02) P=0.308	0.01 (-0.03, 0.05) P=0.617	0.06 (0.03, 0.09)** P=0.000	0.02 (-0.01, 0.05) P=0.224	-0.02 (-0.05, 0.02) P=0.308
12 months	4194	-0.04 (-0.07, -0.01)* P=0.025	0.00 (-0.04, 0.03) P=0.844	-0.01 (-0.04, 0.03) P=0.733	0.04 (0.01, 0.07)* P=0.015	0.00 (-0.03, 0.03) P=0.969	0.02 (-0.02, 0.05) P=0.292
24 months	3889	-0.02 (-0.05, 0.01) P=0.148	-0.03 (-0.06, 0.00) P=0.085	0.01 (-0.02, 0.05) P=0.492	0.01 (-0.02, 0.04) P=0.403	0.00 (-0.03, 0.03) P=0.925	0.00 (-0.03, 0.04) P=0.820
Weight (SDS)							
20 weeks	5317	0.01 (-0.02, 0.04) P=0.495	-0.01 (-0.04, 0.02) P=0.430	0.02 (-0.01, 0.05) P=0.202	0.04 (0.01, 0.07)** P=0.004	0.05 (0.02, 0.07)** P=0.001	-0.01 (-0.04, 0.02) P=0.525
30 weeks	5479	-0.04 (-0.06, -0.01)** P=0.007	-0.04 (-0.06, -0.01)** P=0.005	0.04 (0.01, 0.07)* P=0.012	0.09 (0.06, 0.11)** P=0.000	0.05 (0.03, 0.08)** P=0.000	-0.02 (-0.04, 0.01) P=0.261
Birth	6196	-0.05 (-0.07, -0.02)** P=0.000	-0.05 (-0.07, -0.02)** P=0.000	0.02 (-0.01, 0.05) P=0.184	0.08 (0.05, 0.10)** P=0.000	0.05 (0.03, 0.08)** P=0.000	-0.01 (-0.04, 0.02) P=0.407
6 months	4549	0.02 (-0.01, 0.05) P=0.173	0.03 (-0.00, 0.06) P=0.059	0.00 (-0.04, 0.03) P=0.805	0.02 (-0.01, 0.05) P=0.287	-0.01 (-0.04, 0.02) P=0.415	0.02 (-0.01, 0.06) P=0.137
12 months	4201	0.04 (0.01, 0.07)* P=0.015	0.00 (-0.03, 0.04) P=0.833	-0.01 (-0.04, 0.03) P=0.789	0.00 (-0.03, 0.03) P=0.900	0.00 (-0.03, 0.03) P=0.902	0.03 (0.00, 0.07)* P=0.042
24 months	3946	0.04 (0.01, 0.07)* P=0.022	-0.01 (-0.04, 0.02) P=0.543	-0.02 (-0.05, 0.02) P=0.365	0.00 (-0.03, 0.03) P=0.885	0.00 (-0.03, 0.03) P=0.972	0.01 (-0.02, 0.04) P=0.515

Table S2.2.3 (continued)

Body mass index (SDS)							
6 months	4049	0.07 (0.04, 0.10)** P=0.000	0.04 (0.01, 0.08)** P=0.004	-0.01 (-0.05, 0.02) P=0.419	-0.01 (-0.04, 0.02) P=0.559	-0.02 (-0.05, 0.01) P=0.252	0.04 (0.00, 0.07)** P=0.003
12 months	4173	0.08 (0.05, 0.11)** P=0.000	0.01 (-0.03, 0.04) P=0.741	0.00 (-0.04, 0.03) P=0.822	-0.03 (-0.06, -0.00)* P=0.047	0.00 (-0.03, 0.03) P=0.954	0.03 (-0.00, 0.06) P=0.058
24 months	3881	0.06 (0.03, 0.09)** P=0.000	0.01 (-0.02, 0.04) P=0.542	-0.03 (-0.07, 0.00) P=0.069	-0.02 (-0.05, 0.02) P=0.326	0.00 (-0.03, 0.03) P=0.982	0.01 (-0.02, 0.04) P=0.432

Values are regression coefficients (95% confidence interval) and reflect the difference for each cardiovascular outcome per SDS increase of fetal and infant anthropometrics at different ages: 20 weeks of gestational age; 30 weeks of gestational age; birth; 6 months; 12 months and 24 months. In fetal life length represents estimated femur length. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding, and current age. *$*P < 0.05; ** P< 0.01$*

Table S2.2.4 P-values of birth characteristics and cardiovascular outcomes at the age of 6 years (N = 6,239)

		Difference in cardiovascular outcomes standard deviation scores (95% Confidence Interval)					
Birth characteristics	**N**	**Systolic blood pressure**	**Diastolic blood pressure**	**Carotid-femoral pulse wave velocity**	**Aortic root diameter**	**Left ventricular mass**	**Fractional shortening**
Gestational age	**6239**						
<37.0 weeks	309	0.202	0.794	0.338	0.072	0.705	0.382
37.0-41.9 wks	5503	Reference	Reference	Reference	Reference	Reference	Reference
≥42 weeks	427	0.227	0.284	0.327	0.541	0.677	0.061
Trend		*0.000*	*0.311*	*0.825*	*0.001*	*0.039*	*0.145*
Birth weight	**6239**						
<2000 grams	71	0.065	0.351	0.028	0.040	0.810	0.793
2000 - 2499 g	202	0.917	0.503	0.252	0.197	0.326	0.922
2500 - 2999 g	914	0.769	0.309	0.153	0.020	0.097	0.290
3000 - 3499 g	2164	Reference	Reference	Reference	Reference	Reference	Reference
3500 - 3999 g	2010	0.000	0.003	0.352	0.000	0.179	0.218
4000 - 4499 g	734	0.001	0.012	0.518	0.006	0.046	0.230
≥4500 grams	144	0.020	0.083	0.798	0.001	0.023	0.132
Trend		*0.000*	*0.000*	*0.156*	*0.000*	*0.000*	*0.195*
Birth weight for gestational age	**6239**						
Small	291	0.008	0.039	0.253	0.009	0.403	0.950
Normal	5638	Reference	Reference	Reference	Reference	Reference	Reference
Large	310	0.080	0.009	0.942	0.233	0.028	0.532
Trend		*0.000*	*0.000*	*0.184*	*0.000*	*0.000*	*0.407*

Values are P-values corresponding to tests in **Table 2.2.2**.

Table S2.2.5. Associations of fetal and infant growth with cardiovascular structures and function at the age of 6y

		Difference in cardiovascular outcomes standard deviation scores (95% Confidence Interval)					
Fetal growth	**Infant growth**	**Systolic blood pressure (N=3370)**	**Diastolic blood pressure (N=3370)**	**Pulse wave velocity (N=2766)**	**Aortic root diameter (N=3228)**	**Left ventricular mass (N=3167)**	**Fractional shortening(N=3213)**
Deceleration	Deceleration	0.02 (-0.17, 0.20) N=127 P=0.874	-0.06 (-0.25, 0.13) N=127 P=0.531	0.11 (-0.10, 0.31) N=107 P=0.299	0.11 (-0.08, 0.29) N=121 P=0.255	0.27 (0.09, 0.46)† N=119 P=0.004	0.10 (-0.09, 0.29) N=121 P=0.292
	Normal	-0.12 (-0.25, 0.00) N=356 P=0.057	-0.06 (-0.19, 0.06) N=356 P=0.311	0.02 (-0.12, 0.16) N=296 P=0.757	-0.05 (-0.17, 0.07) N=339 P=0.413	0.07 (-0.06, 0.19) N=335 P=0.274	0.05 (-0.08, 0.18) N=340 P=0.457
	Acceleration	0.12 (-0.00, 0.24) N=400 P=0.054	0.13 (0.01, 0.25)* N=400 P=0.041	-0.03 (-0.17, 0.10) N=326 P=0.651	-0.15 (-0.27, -0.03)* N=389 P=0.012	-0.02 (-0.14, 0.10) N=379 P=0.741	-0.01 (-0.14, 0.11) N=388 P=0.829
	Trend effect estimates	*0.08 (0.02, 0.15)*† P=0.008	*0.07 (0.02, 0.13)** P=0.013	*-0.06 (-0.13, 0.01)* P=0.091	*-0.09 (-0.15, -0.03)*† P=0.002	*-0.08 (-0.14, -0.02)** P=0.012	*-0.03 (-0.10, 0.03)* P=0.316
Normal	Deceleration	-0.11 (-0.24, 0.01) N=340 P=0.075	-0.03 (-0.16, 0.10) N=340 P=0.628	0.02 (-0.12, 0.16) N=280 P=0.755	0.10 (-0.02, 0.22) N=321 P=0.111	0.16 (0.04, 0.29)* N=315 P=0.014	0.04 (-0.09, 0.17) N=321 P=0.520
	Normal	**Reference** N=762	**Reference** N=762	**Reference** N=624	**Reference** N=733	**Reference** N=718	**Reference** N=728
	Acceleration	0.19 (0.07, 0.31)† N=377 P=0.003	0.03 (-0.09, 0.15) N=377 P=0.041	-0.07 (-0.21, 0.06) N=316 P=0.299	-0.15 (-0.27, -0.03)* N=356 P=0.016	0.01 (-0.11, 0.14) N=348 P=0.848	0.18 (0.06, 0.31)* N=352 P=0.005
	Trend effect estimates	*0.11 (0.06, 0.16)*† P=0.000	*0.01 (-0.04, 0.07)* P=0.612	*-0.03 (-0.09, 0.03)* P=0.294	*-0.09 (-0.14, -0.04)*† P=0.000	*-0.04 (-0.09, 0.02)* P=0.165	*0.06 (0.00, 0.11)* P=0.034
Acceleration	Deceleration	-0.17 (-0.29,-0.05)† N=407 P=0.005	-0.16 (-0.28,-0.04)† N=407 P=0.008	0.02 (-0.12, 0.15) N=330 P=0.821	0.04 (-0.07, 0.16) N=392 P=0.465	0.05 (-0.07, 0.17) N=382 P=0.421	-0.02 (-0.14, 0.11) N=387 P=0.782
	Normal	-0.11 (-0.23, 0.00) N=460 P=0.051	-0.10 (-0.21, 0.01) N=460 P=0.087	0.02 (-0.11, 0.15) N=377 P=0.736	-0.06 (-0.17, 0.06) N=439 P=0.333	0.10 (-0.02, 0.21) N=436 P=0.099	0.06 (-0.06, 0.18) N=440 P=0.315
	Acceleration	0.10 (-0.08, 0.27) N=141 P=0.281	0.05 (-0.13, 0.23) N=141 P=0.570	0.05 (-0.15, 0.26) N=110 P=0.611	-0.09 (-0.26, 0.08) N=137 P=0.319	-0.03 (-0.20, 0.15) N=135 P=0.763	-0.12 (-0.30, 0.06) N=136 P=0.204
	Trend effect estimates	*0.05 (-0.02, 0.10)* P=0.143	*0.02 (-0.04, 0.08)* P=0.488	*0.01 (-0.06, 0.07)* P=0.180	*-0.03 (-0.09, 0.03)* P=0.262	*0.00 (-0.06, 0.06)* P=0.981	*-0.01 (-0.07, 0.06)* P=0.840

Values are regression coefficients (95% confidence interval) and reflect the difference for each cardiovascular outcome compared to children with normal fetal and infant growth. Fetal growth is defined as weight SDS gain from mid-pregnancy (20 weeks gestational age) to birth. Infant growth was defined as the period between birth to 2 years. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding and current age. Trend tests represent the effect estimates of infant growth (SDS) within each fetal growth group. Interaction terms between fetal growth categories and infant growth were not significant. *$P < 0.05$; † $P < 0.01$

Table S2.2.6 Associations of fetal and infant growth measures with childhood cardiovascular outcomes from conditional analyses (N = 6,239)

	Systolic blood pressure	Diastolic blood pressure	Pulse wave velocity	Aortic root diameter	Left ventricular mass	Fractional shortening
Length (SDS)	**N=1110**	**N=1110**	**N=920**	**N=1059**	**N=1037**	**N=1054**
20 weeks	-0.03 (-0.09, 0.03) P=0.381	-0.01 (-0.07, 0.05) P=0.701	0.05 (-0.02, 0.12) P=0.176	0.04 (-0.02, 0.10) P=0.160	0.01 (-0.05, 0.07) P=0.788	-0.05 (-0.11, 0.01) P=0.095
30 weeks	-0.05 (-0.11, 0.01) P=0.085	-0.01 (-0.07, 0.05) P=0.681	0.05 (-0.02, 0.11) P=0.192	0.01 (-0.05, 0.07) P=0.751	-0.08 (-0.14, -0.02)* P=0.015	-0.01 (-0.07, 0.05) P=0.797
Birth	-0.02 (-0.08, 0.04) P=0.456	0.00 (-0.06, 0.06) P=0.922	0.06 (-0.01, 0.12) P=0.098	0.03 (-0.03, 0.09) P=0.287	0.07 (0.01, 0.13)* P=0.016	-0.02 (-0.08, 0.04) P=0.571
6 months	0.03 (-0.03, 0.09) P=0.383	0.03 (-0.03, 0.09) P=0.347	0.01 (-0.06, 0.08) P=0.761	-0.02 (-0.08, 0.04) P=0.563	-0.02 (-0.08, 0.04) P=0.457	-0.01 (-0.07, 0.05) P=0.694
12 months	-0.02 (-0.08, 0.04) P=0.427	-0.03 (-0.09, 0.03) P=0.322	-0.01 (-0.08, 0.05) P=0.675	0.01 (-0.04, 0.07) P=0.655	-0.05 (-0.11, 0.01) P=0.118	0.03 (-0.03, 0.09) P=0.362
24 months	0.05 (-0.00, 0.11) P=0.070	-0.02 (-0.08, 0.04) P=0.499	0.10 (0.04, 0.17)** P=0.002	-0.04 (-0.09, 0.02) P=0.190	0.03 (-0.03, 0.09) P=0.320	0.01 (-0.04, 0.07) P=0.642

Table S2.2.6 (continued)

Weight (SDS)	**N=2074**	**N=2074**	**N=1707**	**N=1975**	**N=1936**	**N=1965**
20 weeks	-0.01 (-0.05, 0.04) P=0.802	0.00 (-0.04, 0.04) P=0.931	0.02 (-0.03, 0.07) P=0.333	0.05 (0.01, 0.10)* P=0.010	0.01 (-0.03, 0.06) P=0.520	0.00 (-0.04, 0.05) P=0.916
30 weeks	-0.04 (-0.08, 0.01) P=0.084	-0.03 (-0.08, 0.01) P=0.130	0.05 (0.00, 0.10)* P=0.041	0.09 (0.05, 0.13)** P=0.000	0.04 (-0.00, 0.09) P=0.053	-0.01 (-0.06, 0.03) P=0.589
Birth	-0.05(-0.09, -0.01)* P=0.029	-0.02 (-0.06, 0.03) P=0.426	0.01 (-0.04, 0.06) P=0.590	0.04 (-0.00, 0.08) P=0.059	0.03 (-0.01, 0.08) P=0.160	-0.01 (-0.06, 0.04) P=0.658
6 months	0.03 (-0.01, 0.07) P=0.193	0.04 (-0.00, 0.08) P=0.064	0.00 (-0.05, 0.05) P=0.688	0.02 (-0.02, 0.07) P=0.260	-0.03 (-0.07, 0.02) P=0.192	0.00 (-0.05, 0.04) P=0.943
12 months	0.00 (-0.04, 0.05) P=0.872	-0.05(-0.09, -0.01)* P=0.023	0.02 (-0.03, 0.06) P=0.524	-0.01 (-0.05, 0.03) P=0.717	0.00 (-0.04, 0.05) P=0.900	0.02 (-0.02, 0.06) P=0.394
24 months	0.06 (0.01, 0.10)** P=0.011	0.00 (-0.05, 0.04) P=0.870	0.03 (-0.01, 0.08) P=0.164	-0.02 (-0.06, 0.02) P=0.295	0.00 (-0.04, 0.05) P=0.891	-0.04 (-0.08, 0.01) P=0.092
Body mass index (SDS)	**N=2742**	**N=2742**	**N=2263**	**N=2620**	**N=2569**	**N=2608**
6 months	0.06 (0.02, 0.10)** P=0.002	0.02 (-0.02, 0.05) P=0.373	-0.02 (-0.06, 0.03) P=0.455	-0.04 (-0.07, 0.00) P=0.058	-0.02 (-0.06, 0.02) P=0.303	0.04 (-0.00, 0.08) P=0.058
12 months	0.04 (0.00, 0.08)* P=0.031	-0.03 (-0.06, 0.01) P=0.170	0.01 (-0.03, 0.05) P=0.699	0.00 (-0.04, 0.04) P=0.966	0.02 (-0.02, 0.06) P=0.332	0.03 (-0.01, 0.07) P=0.100
24 months	0.04 (0.00, 0.08)* P=0.030	0.00 (-0.04, 0.03) P=0.875	-0.02 (-0.06, 0.02) P=0.438	0.00 (-0.03, 0.04) P=0.847	0.01 (-0.03, 0.05) P=0.664	-0.04(-0.08, -0.00)* P=0.043

Values are regression coefficients (95% CI) from conditional analyses. Length in fetal life represents estimated femur length. For blood pressure and carotid femoral pulse wave velocity, we used height adjusted standard deviation scores, whereas for aortic root diameter and left ventricular mass, we used body surface area adjusted standard deviation scores. The estimates represent differences in cardiovascular measures per standardized residual change of fetal and infant growth measures. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, educational level, smoking status and folic acid use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, child's sex, ethnicity, breastfeeding, and current age **P < 0.05; ** P< 0.01*

Chapter 2.3

Early infant growth velocity patterns and cardiovascular and metabolic health in childhood

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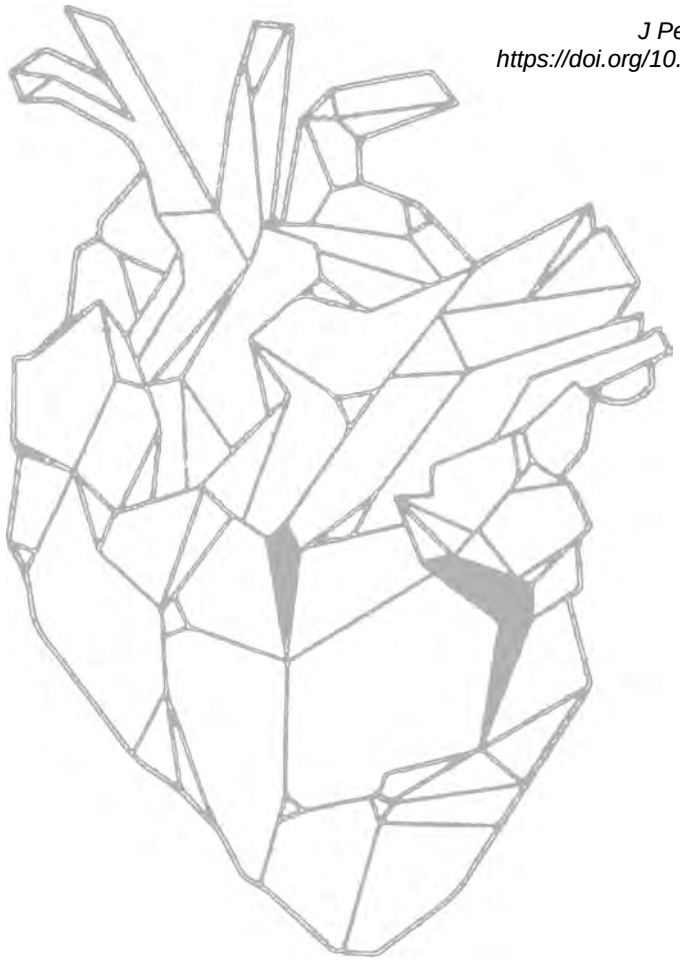


ABSTRACT

Objective: To evaluate the impact of infant growth on childhood health, we examined the associations of detailed longitudinal infant weight velocity patterns with childhood cardiovascular and metabolic outcomes.
Study Design: In a population-based prospective cohort study among 4,649 children, we used repeated growth measurements between 0 and 3 years to derive peak weight velocity (PWV), age at adiposity peak (AGEAP) and body mass index at adiposity peak (BMIAP). At the age of 6 years, we measured blood pressure, left ventricular mass, and cholesterol, triglycerides and insulin concentrations and define children with clustering of risk factors. We assessed the associations using two multivariable linear regression models.
Results: A 1-standard deviation score (SDS) higher infant PWV was associated with higher diastolic blood pressure (0.05 SDS (95% Confidence Interval (CI) 0.02, 0.09)), and lower left ventricular mass (-0.05 SDS (95% CI -0.09, -0.01), independently of body size. A 1-SDS higher BMIAP was associated with higher systolic (0.12 (95% CI 0.09, 0.16) and diastolic blood pressure (0.05 (95% CI 0.01, 0.08)), but these associations were explained by childhood BMI. We did not observe associations of PWV, BMIAP and AGEAP with cholesterol and insulin concentrations. Higher PWV and AGEAP were associated with higher risk of clustering of cardiovascular risk factors in childhood (p-values<0.05).
Conclusion: Infant weight velocity patterns are associated with cardiovascular outcomes. Further studies are needed to explore the associations with metabolic outcomes and long-term consequences.

INTRODUCTION

Rapid growth in early life is associated with an increased cardiovascular risk profile later in life.[3] Previous studies suggest that subjects with higher cardiovascular disease (CVD) risk were small at birth, but had accelerated childhood growth.[2, 115, 116] Especially rapid weight gain in the first 3 months of life is associated with risk factors of CVD in early adulthood.[13, 117] Similarly, excessive weight gain in infancy is associated with increased blood pressure in early adulthood.[44] We have previously observed that specific fetal and infant weight gain were associated with various cardiovascular properties at 6 years.[118]

Early growth velocity patterns can be studied in more detail by deriving specific growth measures from longitudinal data. Repeatedly measured anthropometric data enable construction of infant weight growth indices, such as infant peak weight velocity (PWV), body mass index at adiposity peak (BMIAP) and age at adiposity peak (AGEAP).[119, 120] We have previously reported that these measures are strongly related to childhood adiposity. Higher infant PWV and BMIAP were associated with higher childhood BMI, body fat percentage, android/gynoid fat mass ratio and pre-peritoneal abdominal fat area.[45, 119] An increasing number of studies suggest that growth velocity patterns during early infancy are associated with cardiovascular risk later in life, but studies on the association between more detailed growth indices and cardiovascular and metabolic factors are lacking.[121, 122] We hypothesized that infant growth velocity patterns are associated with cardiovascular risk in school-aged children.[123]

We examined in a population-based prospective cohort study among 4,649 children followed from fetal life onwards, the associations of infant PWV, BMIAP and AGEAP with childhood cardiovascular and metabolic outcomes, including blood pressure, left ventricular mass, and total-, HDL-, and LDL-cholesterol, triglycerides, and insulin concentrations and clustering of cardiovascular risk factors.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[64, 80] Of all eligible children in the study area, 61% participated in the study at birth.[64] The study protocol was approved by the local Medical Ethical Committee of the Erasmus MC (MEC-2007-413). Written informed consent was obtained from all mothers.

Infant growth measures were available in 6,523 children participating in the preschool phase of the study. We excluded 1,797 children who did not have at least 3 infant growth measurements, which were necessary for infant growth modeling. Of the remaining 4,726 children, 4,681 participated in the follow-up studies at the age of 6 years. Cardiovascular and metabolic outcomes were measured in 4,649 children (**Figure S2.3.1**).

Longitudinal infant growth velocity patterns

Gestational age and sex adjusted standard deviation scores for birth weight and length were calculated using North-European growth charts.[84] Childhood length and weight were measured according to standardized procedures at birth, at the ages of 1, 2, 3, 4, 6, 11, 14, 18, 24 and 36 months. The median number of postnatal growth measurements was 5 (full range: 3-11).[64] Age- and sex-adjusted SDS for all growth characteristics were obtained with Dutch reference growth charts.[86] As previously described, these growth measures were used to construct longitudinal weight and body mass index growth patterns, and derive infant PWV, AGEAP and BMIAP.[119, 120] Briefly, infant PWV was derived using the Reed1 model for boys and girls separately.[124] The model was fitted by sex on all weight measurements taken at 0-3 years of age, including birth weight. The first derivative of the fitted distance curve was taken to obtain the weight velocity curve. To obtain the infant PWV, the maximum of this curve was taken. This value reflects the maximum rate of growth in infancy. For infant BMIAP, a cubic mixed effects model was fitted on log(BMI) from 14 days to 1.5 years, using sex as a covariate. Modeling of BMI growth was performed from the age of 14 days onwards, because children may lose up to 10% of their body weight in the first 2 weeks of life. When fitting the model, age was centralized to 0.75 years. In addition to fixed effects, we included random effects for the constant and the slope in the model. Subsequently, BMI was derived for each individual at the point where the curve reaches its maximum, which gives BMIAP and AGEAP.

Childhood cardiovascular and metabolic properties

Children visited our research center for follow up measurements at the median age of 6 years (95% range 5.6 – 7.3). We measured blood pressure at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmonanometer Datascope Accutor PlusTM(Paramus, NJ, USA).[87] We calculated the mean value by using the last three blood pressure measurement of each participant. M-mode echocardiographic measurements were performed and left ventricular

mass was computed using the formula derived by Devereux.[65, 67] Intraobserver and interobserver intraclass correlation coefficients were calculated previously and varied between 0.91 to 0.99 and 0.78 to 0.96, respectively.[66] Thirty-minutes fasting blood samples were collected to measure total-, HDL-, and LDL-cholesterol, triglycerides, and insulin concentrations, using Cobas 8000 analyser (Roche, Almere, the Netherlands). Quality control samples demonstrated intra and inter assay coefficients of variation ranging from 0.77-1.39%, and 0.87-2.40%, respectively.

We defined children with clustering of cardiovascular risk factors, using the previously described definition of childhood metabolic syndrome phenotype, which means having three or more of the following components: android fat mass % =>75th percentile; systolic or diastolic blood pressure =>75th percentile; HDL-cholesterol <=25th percentile or triglycerides =>75th percentile; and insulin level =>75th percentile.[94] Percentiles were derived from the study population. We used android fat mass as percentage of total body fat mass, which was used as proxy for waist circumference, because waist circumference was not available. Total body fat mass and android fat mass were measured using a Dual-energy X-ray absorptiometry (DXA) scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA) and analyzed with the enCORE software v.12.6.[125]

Covariates

Maternal age, and pre-pregnancy BMI were assessed at enrollment in the study. Information on maternal educational level, smoking, alcohol consumption, and folic acid supplement use during pregnancy was obtained by questionnaires.[64] Information on gestational hypertensive disorders was obtained from midwife and hospital registries.[126] Child´s ethnicity (European, Non-European) was classified by the countries of birth of the parents. At the age of 6 years, we measured height and weight and calculated BMI. We obtained sex- and age-specific SDS based on Dutch reference growth curves.[86]

Statistical analyses

First, we compared characteristics between boys and girls using One-Way ANOVA, Kruskal-Wallice and Chi-square tests. Also, we explored correlations between early growth measures and cardiovascular properties using Pearson correlation coefficients. Second, we assessed the associations of infant PWV, AGEAP and BMIAP with childhood cardiovascular and metabolic outcomes using two multivariable linear regression models, respectively. The basic model was adjusted for child age and sex, while the

confounder model was additionally adjusted for covariates selected on their associations with the outcome of interest based on previous studies or a change in effect estimate of >10%. For blood pressure and metabolic outcomes, we additionally created a third model controlling for current childhood BMI. Finally, we used logistic regression models to examine the associations of infancy PWV, AGEAP and BMIAP with the risk of clustering of cardiovascular risk factors. We did not adjust these analyses for multiple testing, because of the strong correlation between the different exposures and outcomes. Metabolic risk factors that were not normally distributed were log-transformed or root-transformed. We constructed standard deviation scores (SDS)(SDS=(observed value-mean/SD)) of determinants and metabolic outcomes. We constructed height adjusted SDS for the blood pressure and body surface area adjusted SDS for the left ventricular mass using Generalized Additive Models for Location, Size and Shape (GAMLSS) using R, version 3.2.0 (R Core Team, Vienna, Austria).[95-97, 127] We tested the interaction of PWV, AGEAP and BMIAP with sex, ethnicity and gestational age and weight at birth, in relation to cardiovascular variables. Because interaction between AGEAP and sex on systolic and diastolic blood pressure was significant, we performed the regression analyses stratified by sex. To reduce the possibility of potential bias associated with missing data, missing values in covariates were imputed using the multiple imputations procedure with five imputations and these datasets were analyzed together.[99] Statistical analyses were performed using SPSS version 21.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp).

RESULTS

Subject characteristics

Table 2.3.1 shows the subject characteristics according to sex. Girls had lower infant PWV and BMIAP (all p values <0.01) than boys. At the age of 6 years, boys had higher height, weight and left ventricular mass, whereas girls had higher blood pressure, android fat mass percentages, total-cholesterol and triglycerides concentrations and higher percentage of clustering of cardiovascular risk factors (all p-values <0.05). Subject characteristics before imputation are shown online in **Table S2.3.1,** while **Table S2.3.2** and **Table S2.3.3** show Pearson correlation coefficients between infant PWV, AGEAP and BMIAP and childhood cardiovascular and metabolic properties, respectively.

Infant growth velocity patterns and cardiovascular outcomes
Table 2.3.2 shows that a 1-SDS increase of infant PWV was associated with higher diastolic blood pressure (0.06 SDS (95% Confidence Interval (CI) 0.02, 0.09)), and lower left ventricular mass (-0.05 SDS (95% CI -0.09, -0.01), independently of current body size. The positive association of PWV with systolic blood pressure was explained by current childhood BMI. A 1-SDS higher infant BMIAP was associated with higher systolic (0.12 (95% CI 0.09, 0.16), and diastolic blood pressure (0.05 (95% CI 0.01, 0.08), but these associations were explained by current childhood BMI. The associations of infant PWV and BMIAP with childhood blood pressure tended to be stronger in girls. Infant AGEAP was not associated with cardiovascular outcomes. **Table S2.3.4** shows results from the basic models adjusted for child's age and sex only.

Infant growth velocity patterns and metabolic outcomes
Table 2.3.3 shows in the models adjusted for current BMI that a 1-SDS higher AGEAP was associated with lower triglycerides concentrations (-0.05 SDS (95% CI -0.10, -0.01)), and this association tended to be stronger in boys. Infant PWV and BMIAP were not associated with any of the metabolic outcomes. None of the infant growth patterns were associated with childhood LDL and HDL cholesterol concentrations or with LDL/total cholesterol ratio (results are not shown). We did not observe any association of infant growth velocity patterns with metabolic properties in the basic models adjusted for child's age and sex (**Table S2.3.5).**

Infant growth velocity patterns and risk of clustering of cardiovascular risk factors
Table 2.3.4 shows that, after adjusting for covariates, higher infant PWV and AGEAP were associated with higher risk of clustering of cardiovascular risk factors (Odds Ratio (OR) 1.13 (95% CI 1.01, 1.26) and OR 1.11 (95% CI 1.00, 1.22), respectively). BMIAP was with borderline significance associated with the risk of clustering of cardiovascular risk factors (OR 1.11 (95% CI 1.00, 1.22)).

Table 2.3.1 Subject characteristics (N = 4,649)

Subject characteristics	Total (N=4,649)	Boys (N=2,323)	Girls (N=2,326)	*P value*
Maternal characteristics				
Age, y	31.0 (4.9)	31.1 (4.9)	31.0 (4.8)	0.31
Body mass before pregnancy, kg/m^2	22.6 (18.1, 34.2)	22.7 (18.0, 33.9)	22. 6 (18.1, 34.6)	0.51
Maternal education, n (%)				0.70
Low/middle	2275 (48.9)	1130 (48.6)	1145 (49.2)	
Higher	2380 (51.1)	1197 (51.4)	1183 (50.8)	
Folic acid intake during pregnancy, n (%)				0.18
No use	1056 (22.7)	548 (23.5)	508 (21.8)	
Start in the first 10 weeks	1453 (31.2)	739 (31.8)	714 (30.7)	
Start periconceptional	2146 (46.1)	1040 (44.7)	1107 (47.6)	
Alcohol consumption during pregnancy, n				0.51
No	1983 (42.6)	980 (42.1)	1004 (43.1)	
Yes	2672 (57.4)	1347 (57.9)	1324 (56.9)	
Smoking during pregnancy, n(%)				0.22
No	3555 (76.4)	1768 (76.0)	1787 (76.8)	
Yes	1100 (23.6)	559 (24.01)	541 (23.2)	
Systolic blood pressure, mmHg	115.3 (12.0)	115.7 (12.04)	115.8 (11.9)	0.80
Diastolic blood pressure, mmHg	68.2 (9.4)	68.1 (9.3)	68.3 (9.5)	0.61
Gestational hypertensive disorders[a]	282 (6.1)	127 (5.5)	155 (6.7)	0.13
Birth and infant characteristics				
Ethnicity, n (%)				0.49
European	3518 (75.7)	1751 (75.4)	1769 (76.1)	
Non-European	1131 (24.3)	572 (24.6)	557 (23.9)	
Gestational age at birth unit, weeks	40.1 (36.0, 42.3)	40.1 (36.0, 42.3)	40.1 (36.0, 42.1)	0.23

Table 2.3.1 (continued)

Birth weight, grams	3459 (535)	3523 (545)	3390 (516)	**<0.01**
Preterm birth <37 weeks at delivery, n (%)	193 (4.1)	98 (4.2)	95 (4.1)	0.88
Peak weight velocity (kg/year)	12.2 (2.1)	13.2 (2.0)	11.3 (1.77)	**<0.01**
Age at adiposity peak (months)	8.4 (7.8, 9.6)	8.4 (7.8,9.6)	8.6 (7.8,9.6)	0.99
Body mass index at adiposity peak (kg/m^2)	17.6 (16.1, 19.2)	17.8 (16.3, 19.4)	17.3 (15.9,19.0)	**<0.01**
Child characteristics at 6 years				
Age, y	6.0 (5.6, 7.3)	6.0 (5.6, 7.4)	6.0 (5.6, 7.2)	0.29
Height, cm	118.9 (0.1)	119.3 (5.6)	118.4 (5.6)	**<0.01**
Weight, kg	22.2 (17.4, 32.4)	22.6 (17.6, 32.2)	22.0 (17.2, 32.4)	**0.01**
Body mass index, kg/m^2	15.8 (13.6, 20.7)	15.8 (13.7, 20.6)	15.8 (13.5, 20.8)	0.70
Android fat mass (%)	3.7 (0.9)	3.6 (0.8)	4.0 (1.0)	**<0.01**
Systolic blood pressure, mmHg	102.5 (8.2)	101.9 (7.7)	103.0 (8.5)	**<0.01**
Diastolic blood pressure, mmHg	60.6 (6.8)	59.9 (6.6)	61.2 (6.8)	**<0.01**
Left ventricular mass, grams	52.8 (11.2)	55.2 (11.4)	50.4 (10.3)	**<0.01**
Total cholesterol, mmol/l	4.2 (0.6)	4.1 (0.6)	4.3 (0.6)	**<0.01**
Triglycerides, mmol/l	1.0 (0.4, 2.4)	1.0 (0.4, 2.3)	1.1 (10.4, 2.5)	**<0.01**
Insulin, U/l	114.0 (17.8, 398.3)	114.7 (16.2, 385.5)	112.6 (19.1, 422.2)	0.05
Clustering of risk factors, N (%)	743 (24.8)	355 (22.9)	388 (27)	**0.01**

Values are means (SD), medians (95% range). Values represent the results based on imputed data. Characteristics based on original data are shown in **Table S2.3.1.**

[a] Pre-eclampsia or pregnancy induced hypertension.

Table 2.3.2 Associations of infant growth velocity measures with childhood cardiovascular outcomes (N=4,649)

SDS difference in childhood cardiovascular outcomes (95% Confidence Interval)			
	Systolic blood pressure	**Diastolic blood pressure**	**Left ventricular mass**
Total group			
PWV (1 SDS = 2.1 kg/year)	0.09 (0.05, 0.12)**	0.06 (0.02, 0.09)**	-0.05 (-0.09,-0.01)**
BMI model - PWV	0.01 (-0.02, 0.05)	0.05 (0.01, 0.09)*	NA
AGEAP (1 SDS = 0.5 months)	0.02 (-0.02, 0.05)	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.05)
BMI model - AGEAP	0.00 (-0.03, 0.03)	0.00 (-0.03, 0.03)	NA
BMIAP (1 SDS = 0.8 kg/m^2)	0.12 (0.09, 0.16)**	0.05 (0.01, 0.08)*	-0.02 (-0.06, 0.01)
BMI model – BMIAP	0.04 (0.00, 0.08)	0.04 (0.00, 0.08)	NA
Boys			
PWV (1 SDS = 2.0 kg/year)	0.07 (0.03, 0.12)**	0.04 (-0.01, 0.09)	-0.05 (-0.10, -0.00)*
BMI model - PWV	0.00 (-0.05, 0.05)	0.02 (-0.03, 0.07)	NA
AGEAP (1 SDS = 0.5 months)	-0.03 (-0.07, 0.02)	-0.03 (-0.08, 0.01)	0.03 (-0.01, 0.08)
BMI model - AGEAP	-0.04 (-0.09, 0.00)	-0.04 (-0.08, 0.01)	NA
BMIAP (1 SDS = 0.8 kg/m^2)	0.14 (0.09, 0.19)**	0.03 (-0.02, 0.08)	-0.03 (-0.08, 0.03)
BMI model – BMIAP	0.05 (0.00, 0.11)	0.00 (-0.06, 0.06)	NA
Girls			
PWV (1 SDS = 1.8 kg/year)	0.10 (0.04, 0.16)**	0.08 (0.02, 0.14)**	-0.05 (-0.10, 0.01)
BMI model - PWV	0.03 (-0.04, 0.09)	0.09 (0.02, 0.15)**	NA
AGEAP (1 SDS = 0.5 months)	0.06 (0.01, 0.11)*	0.04 (-0.01, 0.09)	-0.01 (-0.06, 0.04)
BMI model - AGEAP	0.04 (-0.01, 0.09)	0.04 (-0.01, 0.09)	NA
BMIAP (1 SDS = 0.8 kg/m^2)	0.11 (0.05, 0.16)**	0.06 (0.01,0.12)*	-0.02 (-0.07, 0.03)
BMI model – BMIAP	0.03 (-0.03, 0.09)	0.07 (0.01, 0.13)*	NA

Abbreviations: N: number, SDS: standard deviation scores, BMI: body mass index, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak, NA not applicable. Values are linear regression coefficients (95% CI) based on multiple linear regression models and reflect the change in outcome per SDS increase in each infant growth characteristics. Confounder model is adjusted for maternal factors: pre-pregnancy body mass index, educational level, smoking during pregnancy, use of alcohol during pregnancy, folic acid supplement use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, and child factors: age, sex (total group), ethnicity, birth weight and gestational age at birth. BMI model for systolic and diastolic blood pressure was additionally adjusted for childhood BMI. For blood pressure we used height adjusted standard deviation scores, whereas for left ventricular mass, we used body surface area adjusted standard deviation scores. P<0.05 for interaction between AGEAP and sex on systolic and diastolic blood pressure.

* P value <0.05, ** P value <0.01.

Table 2.3.3 Associations of infant growth velocity patterns with childhood metabolic outcomes (N=3,136)

SDS difference in childhood cardiovascular outcomes (95% Confidence Interval)			
	Total cholesterol	**Triglycerides**	**Insulin**
Total group			
PWV (1 SDS = 2.1 kg/year)	0.03 (-0.02, 0.07)	-0.00 (-0.05, 0.04)	0.04 (-0.01, 0.08)
BMI model - PWV	-0.00 (-0.05, 0.05)	-0.03 (-0.07, 0.02)	-0.03 (-0.08, 0.02)
AGEAP (1 SDS = 0.5 months)	-0.03 (-0.07, 0.01)	-0.05 (-0.09, -0.01)*	-0.00 (-0.04, 0.04)
BMI model - AGEAP	-0.04 (-0.08, 0.00)	-0.05 (-0.10, -0.01)**	-0.02 (-0.06, 0.02)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.06)	0.04 (-0.01, 0.08)
BMI model - BMIAP	-0.02 (-0.07, 0.03)	-0.01 (-0.06, 0.04)	-0.05 (-0.10, -0.00)
Boys			
PWV (1 SDS = 2.0 kg/year)	0.04 (-0.02, 0.09)	0.00 (-0.05, 0.07)	0.01 (-0.04, 0.07)
BMI model - PWV	0.01 (-0.06, 0.07)	-0.02 (-0.08, 0.05)	-0.03 (-0.09, 0.03)
AGEAP (1 SDS = 0.5 months)	-0.02 (-0.08, 0.03)	-0.06 (-0.11, -0.00)	0.00 (-0.05, 0.06)
BMI model - AGEAP	-0.03 (-0.08, 0.02)	-0.07 (-0.12, -0.01)*	-0.01 (-0.06, 0.05)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.04, 0.08)	0.01 (-0.05, 0.07)	0.02 (-0.05, 0.08)
BMI model - BMIAP	-0.03 (-0.09, 0.05)	-0.03 (-0.10, 0.05)	-0.05 (-0.12, 0.02)
Girls			
PWV (1 SDS = 1.8 kg/year)	0.02 (-0.06, 0.09)	-0.02 (-0.09, 0.05)	0.07 (0.00, 0.15)
BMI model - PWV	-0.02 (-0.10, 0.06)	-0.05 (-0.07, -0.02)	-0.03 (0.11, 0.05)
AGEAP (1 SDS = 0.5 months)	-0.04 (-0.10, 0.02)	-0.04 (-0.10, 0.02)	-0.01 (-0.08, 0.05)
BMI model - AGEAP	-0.05 (-0.11, 0.02)	-0.04 (-0.10, 0.04)	-0.04 (-0.10, 0.03)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.04, 0.09)	0.02 (-0.04, 0.09)	0.06 (-0.01, 0.13)
BMI model - BMIAP	-0.01 (-0.09, 0.07)	0.01 (-0.06, 0.08)	-0.06 (-0.13, 0.02)

Abbreviations: N: number, SDS: standard deviation scores, BMI: body mass index, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Values are linear regression coefficients (95% CI) based on multiple linear regression models and reflect the change in outcome per SDS increase in each infant growth characteristics. Model adjusted for maternal factors: pre-pregnancy body mass index, educational level, smoking during pregnancy, use of alcohol during pregnancy, folic acid supplement use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, and child factors: age, sex, ethnicity, birth weight and gestational age at birth. All models were additionally adjusted for childhood BMI. P<0.05 for interaction between AGEAP and sex on systolic and diastolic blood pressure.
* P value <0.05, ** P value <0.01.

Table 2.3.4 Associations of infant growth velocity patterns with clustering of cardiovascular risk factor (N=2,990)

	Odds Ratio for clustering for cardiovascular risk factors (95% CI)
PWV (1 SDS = 2.1 kg/year)	1.13 (1.01 – 1.26)*
AGEAP (1 SDS = 0.5 months)	1.11 (1.00 - 1.22)*
BMIAP (1 SDS = 0.8 kg/m^2)	1.11 (1.00-1.24)

Abbreviations: N: number, SDS: standard deviation scores, BMI: body mass index PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Models are adjusted for maternal factors: pre-pregnancy body mass index, educational level, income, smoking during pregnancy, use of alcohol during pregnancy, folic acid supplement use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, and child factors: age, sex, ethnicity, birth weight and gestational age; * P value <0.05;

DISCUSSION

We observed in a large population-based prospective cohort study that early infant growth velocity patterns are associated with certain cardiovascular outcomes, but not consistently with metabolic outcomes. The observed associations of infant growth patterns with childhood blood pressure tended to be stronger in girls than in boys. Higher infant growth velocity patterns were also associated with an increased risk of clustering of cardiovascular risk factors.

Methodological considerations

The main strength of our study is the population-based prospective cohort design, including a large number of subjects whom we studied from early fetal life onwards. The repeated infant growth measures enabled us to study the effects of infant growth velocity patterns on the cardiovascular and metabolic properties. However, some limitations need to be discussed. Of the total group of singleton live born children with information on growth, follow-up measurements were available in 71%. Loss to follow-up would lead to selection bias if the associations of early infant growth measures with childhood cardiovascular and metabolic measures would be different between those included and those not included in the final analyses.[101] A limitation is that blood lipids and insulin concentrations were measured in blood samples that were collected in 30 minutes-fasting states. According to studies in adults, fasting time has little influence on cholesterol levels, but concentrations of triglyceride and insulin vary more substantially with differences in fasting time.[128, 129] The measurement error for triglycerides and insulin levels in our study

population could have led to non-differential misclassification, and this may have resulted in an underestimation of our effect estimates for associations with triglycerides and insulin levels. Finally, although we performed adjustments for a large number of potential maternal and childhood confounders, residual confounding still might have occurred, as in any observational study.

Interpretation of main findings

A pattern of rapid growth in early life is associated with a higher risk of CVD in later life.[3] A study among 2,285 Japanese adolescents aged 13 to 14 years shows that rapid weight gain during early childhood predicts high blood pressure and unfavorable lipid concentrations.[123] Moreover, data from five birth cohort studies showed that faster relative weight gain during early infant life was associated with an increased risk of elevated blood pressure in adulthood.[130] In line with these studies, we showed that early infant growth patterns are associated with cardiovascular variables in childhood. However, we did not find an association with metabolic variables.

Most previous studies used change in weight or length between two time points as indicator of growth. More detailed infant growth patterns can be derived from the longitudinally collected anthropometric measures, such as PWV, BMIAP and AGEAP. Previously, a study in Germany among 1127 children up to the age of 10 years, reported that higher PWV in infancy is associated with an increase in systolic and diastolic blood pressure after adjustment for confounders, including BMI.[131] However, another cohort study among 2822 children in the Netherlands showed that the association of BMIAP with blood pressure at the age of 6 years was mediated by current BMI.[132] In the same study, it has been shown that timing of BMI peak seemed not to be associated as strongly as magnitude of BMI peak with blood pressure.[132] In line with these studies, we observed an association of infant PWV and BMIAP with childhood blood pressure, which could be explained by current BMI. The association of infant PWV and BMIAP and diastolic blood pressure in our study was independent from current BMI in girls. Also, in the present study, infant AGEAP was not associated with childhood blood pressure.

Fetal and early life growth patterns may also influence cardiac structures in children and adults.[11, 46] A previous study examined the effect of early infant weight on left ventricular mass among 290 men born in East Hertfordshire, England. They reported that low weight at 1 year was associated with higher left ventricular mass in adulthood.[46] In line with this study, we observed that higher PWV leads to lower left ventricular mass,

relative to current body size. Recent study suggests that fat mass is a minor predictor of left ventricular mass, while lean body mass is a much stronger predictor.[22] Therefore, the association between higher PWV and lower left ventricular mass might be explained by a different body composition of children with and without increased PWV, relative to current body surface area (BSA).

Several studies have been performed focused on the associations of infant weight gain with metabolic outcomes. A study among 1,999 subjects showed that a slower increase in BMI during the first 6 months after birth was associated with an atherogenic lipid profile in adult life.[133] An additional study among 396 men aged 58 years reported that the combination of being born small followed by a rapid weight gain was associated with the occurrence of risk factors included in the metabolic syndrome, and that growth patterns during infancy and childhood correlate with insulin levels later in life.[134] In our study, we only observed an inverse association of infant AGEAP with triglycerides concentrations after adjusting for current BMI. This observation was not consistent with other results and may be a chance finding. Also, our observation may be explained by the strong positive association of BMI with triglycerides. Results from a study among 128 subjects suggest that rapid weight gain during infancy predicted clustering of metabolic risk factors at age 17 years.[135]. Consistently with these results, our results showed higher risk of clustering of cardiovascular risk factors in children with higher PWV and AGEAP. Thus, the increased risk for clustering seemed to be mainly driven by the increased blood pressure and adiposity, and not by the metabolic risk factors.

Although the observed effect estimates infant growth on cardiovascular and metabolic outcomes are small and without clinical relevance for individuals, the results from this study may be important on a population based level. These results are especially important from an etiological perspective and give an important contribution to the field of developmental origins of cardiovascular disease. They suggest that infant growth influence cardiovascular development and might predispose individuals to cardiovascular disease in adulthood. We have shown that infant weight gain patterns may most importantly affect blood pressure and adiposity in childhood. Future research should focus on whether these changes in early childhood are associated with cardiovascular morbidity and mortality at later ages.

SUPPLEMENTAL MATERIAL

Table S2.3.1 Subject characteristics based on original data (N = 4,649)

Subject characteristics	Total (N=4,649)	Boys (N=2,323)	Girls (N=2,326)	*P value*
Maternal characteristics				
Age, y	31.0 (4.9)	31.1 (4.9)	31.0 (4.8)	0.31
Body mass before pregnancy, kg/m^2	22.6 (18.1, 34.3)	22.5 (18.0, 34.0)	22.7 (18.3, 35.0)	0.24
Maternal education, n (%)				0.41
Low/middle	2076 (47.7)	1023 (47.1)	1053 (48.3)	
Higher	2275 (52.3)	1150 (52.9)	1125 (51.7)	
Folic acid intake during pregnancy, n (%)				0.36
No use	680 (20.7)	347 (21.3)	333 (20.1)	
Start in the first 10 weeks	1033 (31.5)	523 (32.1)	510 (30.8)	
Start periconceptional	1571 (47.8)	759 (46.6)	812 (49.1)	
Alcohol consumption during pregnancy, n (%)				0.39
No	1567 (41.7)	764 (41.0)	803 (42.4)	
Yes	2188 (58.3)	1099 (59.0)	1089 (57.6)	
Smoking during pregnancy, n(%)				0.16
No	3129 (76.3)	1555 (76.1)	1574 (76.4)	
Yes	974 (23.7)	489 (23.9)	485 (23.6)	
Systolic blood pressure, mmHg	115.7 (12.0)	115.7 (12.04)	115.8 (11.9)	0.80
Diastolic blood pressure, mmHg	68.2 (9.4)	68.1 (9.3)	68.3 (9.5)	0.61
Gestational hypertensive disorders[a]	243 (5.8)	107 (5.1)	136 (6.5)	0.06
Birth and infant characteristics				
Ethnicity, European, n				0.50
European	3482	1730	1752	
Non-European	1110	565	545	

Table S2.3.1 (continued)

Gestational age at birth unit, weeks	40.1 (36.0, 42.3)	40.1 (36.0, 42.3)	40.1 (36.0, 42.1)	0.27
Birth weight, grams	3457.2 (536.0)	3523.7 (545.1)	3390.6 (516.5)	**<0.01**
Preterm birth <37 weeks at delivery, n (%)	193 (4.2)	98 (4.2)	95 (4.1)	0.88
Peak weight velocity (kg/year)	12.2 (2.1)	13.2 (2.0)	11.3 (1.77)	**<0.01**
Age at adiposity peak (months)	8.4 (7.8, 9.6)	8.4 (7.8–9.6)	8.6 (7.8–9.6)	0.99
Body mass index at adiposity peak (kg/m^2)	17.6 (16.1, 19.2)	17.8 (16.3, 19.4)	17.3 (15.9, 19.0)	**<0.01**
Child characteristics at 6 years				
Age, y	6.0 (5.6, 7.3)	6.0 (5.6, 7.4)	6.0 (5.6, 7.2)	0.29
Height, cm	118.9 (0.1)	119.3 (5.6)	118.4 (5.6)	**<0.01**
Weight, kg	22.2 (17.4, 32.4)	22.6 (17.6, 32.2)	22.0 (17.2, 32.4)	**0.01**
Body mass index, kg/m^2	15.8 (13.6, 20.7)	15.8 (13.7, 20.6)	15.8 (13.5, 20.8)	0.70
Android fat mass (%)	3.7 (0.9)	3.6 (0.8)	4.0 (1.0)	**<0.01**
Systolic blood pressure, mmHg	102.5 (8.2)	101.9 (7.7)	103.0 (8.5)	**<0.01**
Diastolic blood pressure, mmHg	60.6 (6.8)	59.9 (6.6)	61.2 (6.8)	**<0.01**
Left ventricular mass, grams	52.8 (11.2)	55.2 (11.4)	50.4 (10.3)	**<0.01**
Total cholesterol, mmol/l	4.2 (0.6)	4.1 (0.6)	4.3 (0.6)	**<0.01**
Triglycerides, mmol/l	1.0 (0.4, 2.3)	0.9 (0.4, 2.3)	1.0 (0.4, 2.5)	**<0.01**
Insulin, U/l	114.0 (17.8, 398.3)	114.7 (16.2, 385.5)	112.6 (19.1, 422.2)	0.05
Clustering of cardiovascular risk factors, %	743 (24.8)	355 (22.9)	388 (27)	**0.01**

Values are means (SD) or medians (95% range). Values represent the results based on the original, non-imputed data. [a] Pre-eclampsia or pregnancy induced hypertension.

Table S2.3.2 Correlation coefficients between early growth and childhood cardiovascular properties (N=4,649)

	Infant growth measures (SDS)			**Childhood cardiovascular measures (SDS)**		
	PWV (kg/year)	AGEAP (months)	BMIAP (kg/m2))	Systolic blood pressure	Diastolic blood pressure	Left ventricular mass
PWV (kg/year)	1	-	-	-	-	-
AGEAP (months)	-0.02	1	-	-	-	-
BMIAP (kg/m^2)	0.69**	-0.21**	1	-	-	-
Systolic blood pressure	0.05**	0.02	0.06**	1	-	-
Diastolic blood pressure	0.03	0.01	-0.01	0.62**	1	-
Left ventricular mass	0.04**	0.00	0.06**	-0.05**	-0.06**	1

Abbreviations; N: number, SDS: standard deviation score, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Values are Pearson's correlation coefficients. For blood pressure we used height adjusted standard deviation scores, whereas for left ventricular mass we used body surface area adjusted standard deviation scores.
*P value <0.05, ** P value <0.01.

Table S2.3.3 Correlation coefficients between early growth and childhood metabolic properties(N=3,136)

	Infant growth measures (SDS)			Childhood metabolic measures (SDS)				
	PWV (kg/year)	AGEAP (months)	BMIAP (kg/m2))	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Tri-glycerides	Insulin
Total-cholesterol	0.05**	-0.02	-0.02	1	-	-	-	-
HDL-cholesterol	0.02	0.02	-0.02	0.30**	1	-	-	-
LDL-cholesterol	-0.04*	-0.04*	0.00	0.85**	-0.06**	1	-	-
Triglycerides	-0.03	-0.01	-0.02	0.18**	-0.39**	0.14**	1	-
Insulin	0.01	0.00	0.01	-0.02	-0.08**	-0.04*	0.22**	1

Abbreviations: N: number, SDS: standard deviation scores, BMI: body mass index, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Values are Pearson's correlation coefficients.We constructed standard deviation scores (SDS)(SDS=(observed value-mean/SD)) of growth measures and metabolic outcomes.

* P value <0.05, ** P value <0.01

Table S2.3.4 Associations of infant growth measures with childhood cardiovascular properties; basic model (N=4,649)

	SDS difference in childhood cardiovascular outcomes (95% Confidence Interval)		
	Systolic blood pressure	Diastolic blood pressure	Left ventricular mass
Total group			
PWV (1 SDS = 2.1 kg/year)	0.1 (0.07, 0.13)**	0.08 (0.05, 0.10)**	-0.07 (-0.10, -0.03)**
AGEAP (1 SDS = 0.5 months)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.00 (-0.02, 0.03)
BMIAP (1 SDS = 0.8 kg/m^2)	0.08 (0.05, 0.11)**	0.02 (-0.02, 0.05)	-0.01 (-0.04, 0.02)
Boys			
PWV (1 SDS = 2.0 kg/year)	0.08 (0.04, 0.12)**	0.06 (0.02, 0.10)**	-0.05 (-0.10, -0.01)*
AGEAP (1 SDS = 0.5 months)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	0.02 (-0.02, 0.06)
BMIAP (1 SDS = 0.8 kg/m^2)	0.10 (0.06, 0.14)**	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)
Girls			
PWV (1 SDS = 1.8 kg/year)	0.12 (0.07, 0.17)**	0.10 (0.05, 0.15)**	-0.08 (-0.13, -0.03)**
AGEAP (1 SDS = 0.5 months)	0.08 (0.03, 0.12)	0.05 (0.01, 0.09)*	-0.01 (-0.06, 0.03)
BMIAP (1 SDS = 0.8 kg/m^2)	0.07 (0.02, 0.11)**	0.02 (-0.02, 0.06)	-0.02 (-0.06, 0.03)

Abbreviations: N: number, SDS: standard deviation scores, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Values are linear regression coefficients (95% CI) based on multiple linear regression models and reflect the change in outcome per SDS increase in each infant growth characteristics. Models adjusted for child's age and sex only.For blood pressure we used height adjusted standard deviation scores, whereas left ventricular mass, we used body surface area adjusted standard deviation scores. P<0.05 for interaction between AGEAP and sex on systolic and diastolic blood pressure.

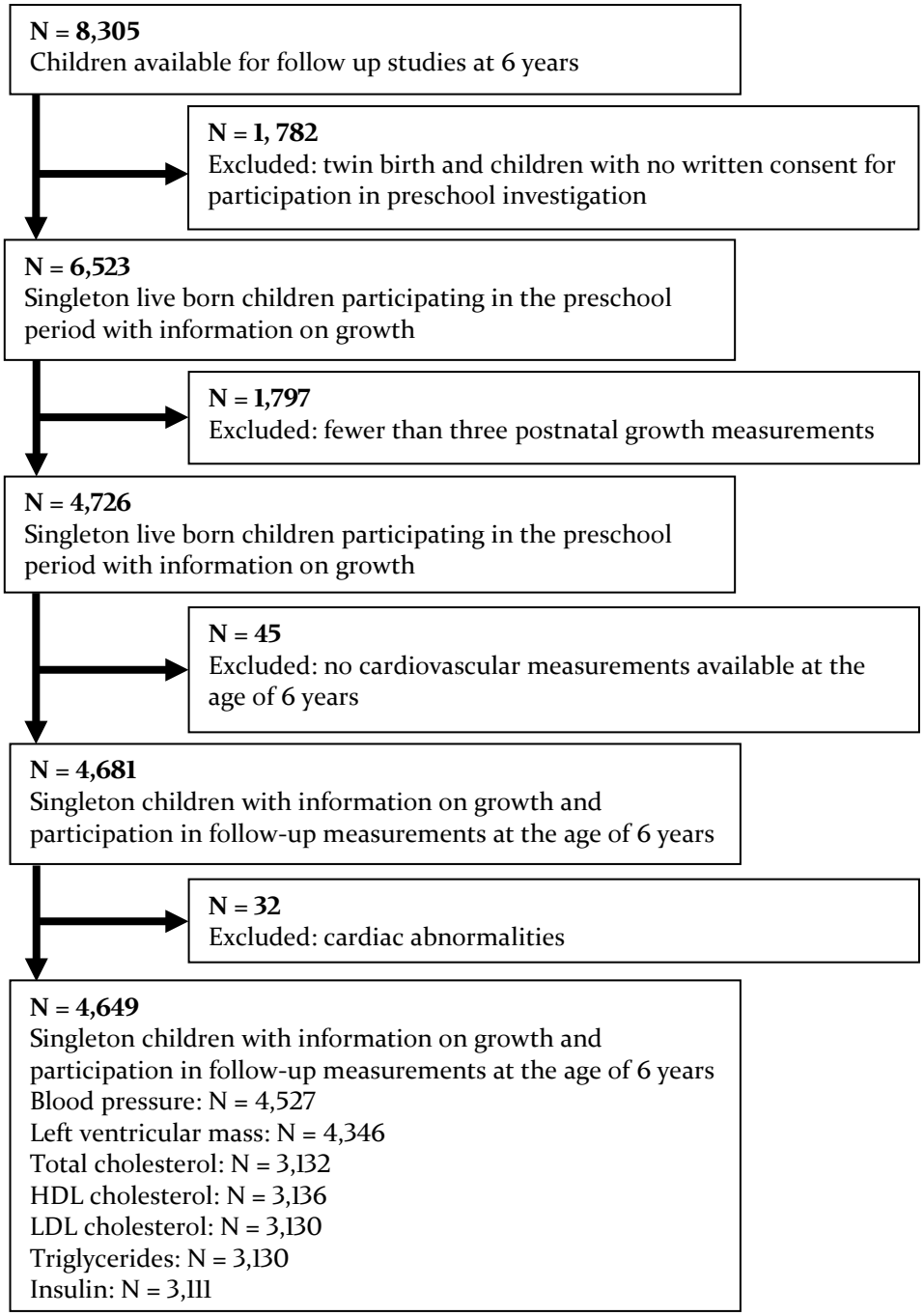
* P value <0.05, ** P value <0.01

Table S2.3.5 Associations of infant growth measures with childhood metabolic properties; basic model(N=3,136).

	SDS difference in childhood metabolic outcomes (95% Confidence Interval)		
	Total cholesterol	Triglycerides	Insulin
Total group			
PWV (1 SDS = 2.1 kg/year)	0.03 (-0.02, 0.07)	0.00 (-0.05, 0.04)	0.04 (-0.01, 0.08)
AGEAP (1 SDS = 0.5 months)	-0.03 (-0.07, 0.01)	-0.05 (-0.09, 0.01)	0.00 (-0.04, 0.04)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.06)	0.04 (-0.01, 0.08)
Boys			
PWV (1 SDS = 2.0 kg/year)	0.04 (-0.02, 0.09)	0.01 (-0.05, 0.07)	0.01 (-0.04, 0.07)
AGEAP (1 SDS = 0.5 months)	-0.02 (-0.08, 0.03)	-0.06 (-0.12, 0.00)	0.00 (-0.05, 0.06)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.04, 0.08)	0.01 (-0.05, 0.07)	0.02 (-0.05, 0.07)
Girls			
PWV (1 SDS = 1.8 kg/year)	0.02 (-0.06, 0.09)	-0.02 (-0.09, 0.05)	0.07 (0.00, 0.15)
AGEAP (1 SDS = 0.5 months)	-0.04 (-0.10, 0.02)	-0.04 (-0.10, 0.02)	-0.01 (-0.08, 0.05)
BMIAP (1 SDS = 0.8 kg/m^2)	0.02 (-0.04, 0.09)	0.02 (-0.04, 0.09)	0.06 (0.00, 0.13)

Abbreviations: N: number, SDS: standard deviation scores, PWV: peak weight velocity, AGEAP: age at adiposity peak, BMIAP: body mass index at adiposity peak. Values are linear regression coefficients (95% CI) based on multiple linear regression models and reflect the change in outcome per SDS increase in each infant growth characteristics. Model adjusted for maternal factors: pre-pregnancy body mass index, educational level, income, smoking during pregnancy, use of alcohol during pregnancy, folic acid supplement use during pregnancy, systolic and diastolic blood pressure at intake and gestational hypertensive disorders, and child factors: age, sex, ethnicity, birth weight and gestational age at birth.
All p-values > 0.05.

Figure S2.3.1 Flow chart of participants included in the analysis.



Chapter 2.4

Longitudinal fetal and childhood growth patterns and childhood cardiac measures

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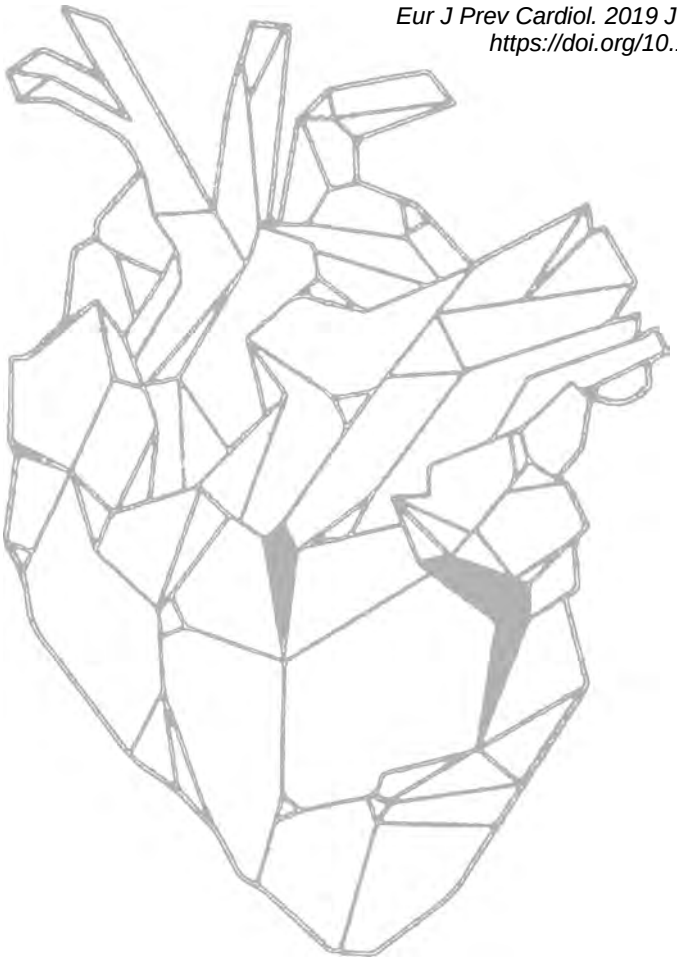


ABSTRACT

Objectives Early-life is critical for cardiac development. We examined the associations of longitudinal fetal and childhood growth patterns with childhood right and left ventricular structures measured by cardiac MRI.
Methods In a population-based prospective cohort study among 2,827 children, we measured growth at 20 and 30 weeks of pregnancy, at birth, 0.5, 1, 2, 6 and 10 years. At 10 years, we measured right ventricular end-diastolic volume (RVEDV), left ventricular end-diastolic volume (LVEDV), left ventricular mass (LVM) and left ventricular mass-to-volume ratio (LMVR) by cardiac MRI.
Results Small size for gestational age at birth was associated with smaller RVEDV and LVEDV, relative to current body surface area (BSA), but with larger LMVR ($p<0.05$). Children in the upper 25% of RVEDV, LVEDV and LVM at age 10 years, were larger at birth and became taller and leaner in childhood ($p<0.05$). In contrast, children in the lower 25% of RVEDV, LVEDV and LVM were smaller at birth and became shorter and heavier in childhood ($p<0.05$). Both fetal and childhood growth were independently of each other associated with childhood RVEDV, LVEDV and LVM.
Conclusion Children who are larger at birth, and grow taller and leaner in childhood have larger hearts, relative to BSA. Small size at birth children, who grow shorter and heavier in childhood have relatively smaller hearts with larger LMVR. Both fetal and childhood growth are important for development of cardiac dimensions.

INTRODUCTION

Fetal exposure to an adverse environment leads to cardiovascular adaptations, which predispose individuals to disease in later life.[3, 4] Evidence suggests that early-life growth patterns directly affect cardiac structure and function.[11, 46, 136] Follow-up studies have shown that individuals with a lower weight in infancy have a higher left ventricular mass (LVM) in adulthood, an independent risk factor for mortality.[46] Recent studies have shown that children with fetal growth restriction have more globular, shorter ventricles, and cardiac dysfunction.[11] Similar changes were observed in preterm born young adults.[104] A major limitation of previous studies is that no information is available on right ventricular measures, while the right ventricle is dominant in fetal life.[137] Cardiac Magnetic Resonance Imaging (cMRI) is a more precise method to assess cardiac measures than echocardiography and enables imaging of both left and right ventricular dimensions.[29] A previous cMRI study in adolescents showed that preterm birth was associated with changes in cardiac geometry, which were more pronounced in the right than in the left ventricle.[31] Although these previous studies strongly suggest that early-life is important for programming of cardiac structure, function and disease in later life, it remains unknown which period in fetal life or infancy is critical.

We examined in a population-based prospective cohort study among 2,827 children the associations of fetal and infant growth with cardiac measures assessed by cMRI in children aged 10 years. Cardiac measures included right ventricular end-diastolic volume (RVEDV), right ventricular ejection fraction (RVEF), left ventricular end-diastolic volume (LVEDV), left ventricular ejection fraction (LVEF), left ventricular mass (LVM) and left ventricular mass-to-volume ratio (LMVR). We hypothesized that both gestational age at birth, and fetal and childhood growth across their full spectrum are associated with right and left cardiac adaptations, independent of current body size. Analyses were focused on longitudinal growth patterns and identification of critical growth periods.

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, The Netherlands.[138] Response rate at birth was 61% (2002 - 2006).[138] Fetal and childhood growth were repeatedly assessed by ultrasounds and physical examinations until the age of 10 years. Good quality cardiac MRI was

obtained in 2,827 children (**Figure S2.4.1**). Written informed consent was obtained from all parents of participants. The study has been approved by the local Medical Ethics Committee.

Fetal and childhood growth measurements

As previously described, fetal ultrasound examinations were carried out in each trimester of pregnancy.[81] Second trimester (median 20.5 weeks, 95% range 18.6 – 23.4) and third trimester (median 30.4 weeks, 95% range 28.4 – 33.1) fetal head circumference, abdominal circumference, and femur length as proxy for fetal length were measured to the nearest millimeter using standardized ultrasound procedures.[82] Estimated fetal weight was calculated using the Hadlock formula.[83] Gestational age adjusted standard deviation scores (SDS) for all fetal growth characteristics were constructed based on reference growth curves.[81, 82] At birth, information on infant sex, date of birth and weight was obtained from community midwife and hospital registries. We created gestational age- and sex-adjusted birth length and weight SDS by using Growth Analyzer 3.5 (Dutch Growth Research Foundation, Rotterdam, the Netherlands) based on North-European reference standards.[84] We defined small or large size for gestational age as being <10th or >90th sex specific percentile for weight. Preterm birth was defined as birth <37.0 weeks of gestation.

Infant length and weight were measured at the Community Health Centers using standardized procedures at the median ages of 6.2 months (95% range 5.2 – 8.3), 11.1 months (95% range 10.1 - 15.5) and 24.8 months (95% range 23.4 – 28.1). Sex and age adjusted SDS were obtained using Dutch reference growth curves.[86]

At the median ages of 5.9 years (95% range 5.7- 7.3) and 9.9 years (95% range 9.5– 11.8), we measured child height and weight without shoes and heavy clothing, and calculated body mass index (BMI) and body surface area (BSA) using the Haycock formula.[69]

Cardiac Magnetic Resonance Imaging

We performed cMRI using a wide-bore GE Discovery MR 750 3T scanner (General Electric, Milwaukee, MI, USA), as described in more detail in **Methods S2.4.1**. Briefly, we acquired localizer images, followed by ECG gated breath-held scans lasting less than 10 seconds per breath-hold. A short-axis SSFP cine stack was then obtained with basal slice alignment and covering the ventricles with contiguous 8-mm thick slices over several end-expiration breath-holds. The scans were stored on a digital archive for post-processing.

Off-line image analyses for right and left ventricular measures on the short-axis cine stack was performed by Precision Image Analysis (Kirkland, WA, USA) using Medis QMASS software (Medis, Leiden, the Netherlands). The guidelines of the Society for Cardiovascular Magnetic Resonance were followed to semi-automatically contour right and left ventricular short-axis endocardial and left ventricular epicardial borders.[139] Papillary muscle was included in the ventricular cavity. Cardiac measurements included RVEDV, RVEF, LVEDV, LVEF, and LVM. We calculated LMVR as LVM/LVEDV.

Covariates

Information about education, household income, height and parity was collected by questionnaires and medical charts. Infant ethnicity was classified by the countries of birth of the parents and was categorized as Dutch or non-Dutch.[138] Largest non-Dutch ethnicities are: European, Turkish, Moroccan, Surinamese, Cape Verdian and Dutch Antilles. Child systolic and diastolic blood pressure were measured on the right brachial artery, using the validated automatic sphygmomanometer Accutorr Plus (Datascope Corporation, Fairfield, New Jersey). Child blood pressure and anthropometric measurements preceded the cMRI visit by 1.1 months (95% range 0-2.8 months).

Statistical analysis

We constructed BSA adjusted SDS for the cardiac measures using Generalized Additive Models for Location, Size and Shape (GAMLSS) in R, version 3.2.0 (R Core Team, Vienna, Austria). This enables flexible modelling, taking into account the distribution of the outcome variable.[70] Since LMVR is a ratio we did not create a BSA adjusted SDS, but standardized this measure as (observed value-mean)/SD. Similarly, we created SDS for all growth measures to enable comparison of effect estimates. First, we used linear regression models to assess the associations of birth characteristics (gestational age, weight and gestational age adjusted size at birth), both continuously and in clinical categories, with cardiac measures (RVEDV, RVEF, LVEDV, LVEF, LVM, LMVR). Second, we compared longitudinal fetal and childhood growth patterns between different quartiles of the cardiac measures. For these analyses, we used repeated measurement regression models, which take into account the correlation between repeated growth measurements of the same participant.[94] Finally, we performed conditional regression analyses to identify independent critical growth periods associated with cardiac measures. Conditional regression analyses take into account the

correlations between growth measures at different ages. This allows simultaneous inclusion of all growth measures in a regression model to assess the most critical periods of growth.[71] All models were adjusted for relevant covariates, selected on the basis of their associations with the outcomes of interest based on previous studies and change in effect estimate >10%. Maternal BMI, blood pressure, smoking during pregnancy or gestational hypertensive disorders did not change the effect estimates and were therefore not included in the final models. We did not observe significant statistical interaction terms with child sex or ethnicity. Missing data of covariates and anthropometric measures for conditional analyses were imputed using multiple imputations in five created datasets, and analyzed together.[99] We performed repeated analyses using SAS software version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA), all other analyses were performed using Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Study participant characteristics

Maternal and child characteristics are shown in **Table S2.4.1**. Non-response analysis is shown in **Table S2.4.2**. Correlation coefficients between growth and cardiac measures are given in **Table S2.4.3**.

Birth outcomes and cardiac measures at school-age

Table 2.4.1 shows that preterm birth was associated with higher LVEF in childhood than term birth (difference 0.25 SDS (95% Confidence Interval (CI) 0.07, 0.43)), but not with other cardiac measures. A 1-SDS higher birth weight was associated with higher RVEDV (0.09 SDS (95% CI 0.06, 0.13)) and LVEDV (0.10 SDS (95% CI 0.06, 0.13)), relative to current body size. Birth weight adjusted for gestational age was also associated with lower LMVR (-0.06 SDS (95% CI -0.10, -0.02)). **Table S2.4.4** shows that additional adjusting for lean body mass percentage did not change the main results.

Table 2.4.1 Birth characteristics and ventricular outcomes at the age of 10 years (N = 2,827)

		Difference in cardiac measures standard deviation scores (95% Confidence Interval)		
Birth characteristics	**N**	**Right ventricular end-diastolic volume**	**Right ventricular ejection fraction**	**Left ventricular end-diastolic volume**
Gestational age				
<37.0 weeks	126	-0.08 (-0.24, 0.07)	0.04 (-0.14, 0.22)	-0.16 (-0.31, 0.00)
37.0-41.9 weeks	2500	Reference	Reference	Reference
≥42 weeks	201	-0.01 (-0.14, 0.12)	-0.05 (-0.19, 0.10)	-0.08 (-0.20, 0.05)
Trend	*2827*	*-0.01 (-0.04, 0.03)*	*0.00 (-0.04, 0.03)*	*0.01 (-0.03, 0.04)*
Birth weight				
<2000 g	28	-0.01 (-0.33, 0.32)	0.00 (-0.37, 0.37)	-0.16 (-0.49, 0.16)
2000-2499 g	88	-0.06 (-0.25, 0.13)	0.11 (-0.11, 0.33)	-0.08 (-0.27, 0.11)
2500-2999 g	401	-0.02 (-0.12, 0.08)	-0.08 (-0.20, 0.04)	-0.09 (-0.19, 0.01)
3000-3499 g	959	Reference	Reference	Reference
3500-3999 g	922	0.13 (0.05, 0.21)**	0.02 (-0.08, 0.11)	0.12 (0.04, 0.20)**
4000-4499g	351	0.20 (0.09, 0.31)**	-0.10 (-0.23, 0.02)	0.17 (0.06, 0.28)**
≥4500 grams	75	0.44 (0.24, 0.65)**	-0.02 (-0.26, 0.22)	0.31 (0.11, 0.52)**
Trend	*2827*	*0.09 (0.06, 0.13)***	*-0.03 (-0.07, 0.01)*	*0.10 (0.06, 0.13)***
Birth weight for gestational age				
Small	255	-0.14 (-0.26, -0.03)**	0.00 (-0.13, 0.13)	-0.18 (-0.29, -0.06)**
Normal	2274	Reference	Reference	Reference
Large	298	0.22 (0.11, 0.32)**	-0.02 (-0.15, 0.09)	0.18 (0.07, 0.28)**
Trend	*2827*	*0.11 (0.08, 0.14)***	*-0.03 (-0.07, 0.11)*	*0.11 (0.08, 0.14)***
Birth characteristics	**N**	**Left ventricular ejection fraction**	**Left ventricular mass**	**Left ventricular mass-to-volume ratio**
Gestational age				
<37.0 weeks	126	0.25 (0.07, 0.43)**	-0.08 (-0.24, 0.08)	0.05 (-0.13, 0.23)
37.0-41.9 weeks	2500	Reference	Reference	Reference
≥42 weeks	201	0.00 (-0.15, 0.14)	0.00 (-0.13, 0.12)	0.06 (-0.09, 0.20)
Trend	*2827*	*-0.05 (-0.09, -0.01)**	*0.01 (-0.02, 0.04)*	*0.01 (-0.03, 0.05)*
Birth weight				
<2000 g	28	0.41 (0.03, 0.78)*	-0.33 (-0.66, -0.00)*	-0.25 (-0.62, 0.12)
2000-2499 g	88	0.16 (-0.06, 0.38)	0.06 (-0.13, 0.25)	0.12 (-0.10, 0.34)
2500-2999 g	401	-0.04 (-0.16, 0.08)	0.03 (-0.08, 0.13)	0.10 (-0.02, 0.22)
3000-3499 g	959	Reference	Reference	Reference
3500-3999 g	922	0.00 (-0.10, 0.09)	0.00 (-0.08, 0.08)	-0.09 (-0.18, 0.00)
4000-4499 g	351	-0.10 (-0.22, 0.03)	0.10 (-0.01, 0.21)	0.00 (-0.13, 0.12)
≥4500 grams	75	0.16 (-0.08, 0.40)	0.13 (-0.08, 0.34)	-0.09 (-0.33, 0.14)
Trend	*2827*	*-0.05 (-0.09, -0.01)**	*0.02 (-0.01, 0.06)*	*-0.05 (-0.08, 0.01)*
Birth weight for gestational age				
Small	255	-0.03 (-0.16, 0.10)	0.04 (-0.08, 0.15)	0.16 (0.03, 0.29)*
Normal	2274	Reference	Reference	Reference
Large	298	0.01 (-0.11, 0.14)	0.12 (0.01, 0.22)*	0.01 (-0.12, 0.13)
Trend	*2827*	*-0.02 (-0.06, 0.02)*	*0.02 (-0.01, 0.05)*	*-0.06 (-0.10, -0.02)***

Values are regression coefficients (95% confidence interval) and reflect the change in standard deviation (SDS) of each cardiac measure for each birth weight or gestational age group, compared to the reference group. Trend estimates represent the effect estimates for

the continuous associations per SDS change in birth characteristic. We used body surface adjusted standard deviation scores, except for left ventricular mass-to-volume ratio. Models are adjusted for maternal height, parity, educational level, income level, child's sex, ethnicity, current age, and time difference between BSA measurement and MRI.
P < 0.05; P< 0.01.* Models additionally adjusted for childhood lean body mass percentage are shown in **Table S2.4.4**.

Longitudinal fetal and infant growth patterns and cardiac measures at school-age

Figure 2.4.1 shows the results of the longitudinal growth analyses. As compared to children in the 25-75% range of RVEDV and LVEDV, those in the upper 25% had higher fetal length and weight growth, whereas those in the lower 25% had lower fetal length and weight. In childhood the children in the upper 25% for RVEDV and LVEDV had normal height and slightly lower weight growth, whereas children in the lower 25% had lower childhood height growth and higher weight gain **(Figure 2.4.1A-B)**. Children in the highest quartile of LVM had higher height and lower weight gain in childhood (**Figure 2.4.1C**). Children in the highest LMVR had higher weight gain **(Figure 2.4.1D)**. No differences in fetal and infant growth patterns were observed for RVEF and LVEF (**Figure 2.4.1E-F**).

Figure 2.4.1 Fetal and childhood growth patterns and cardiac structure and function measures (N = 2,827)

Figure 2.4.1A

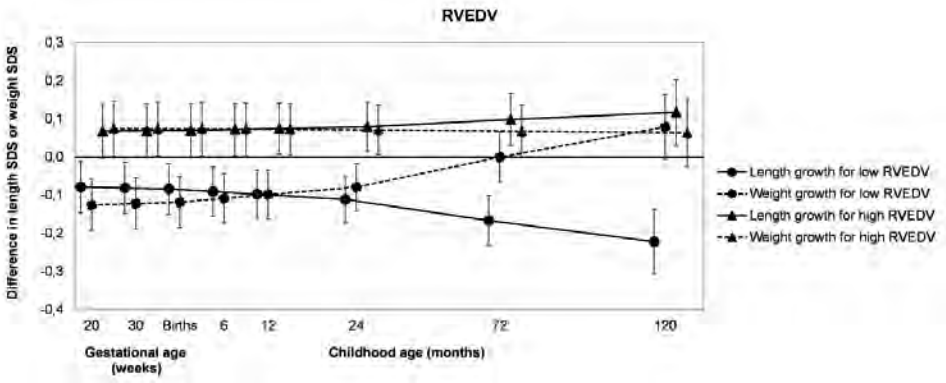


Figure 2.4.1B

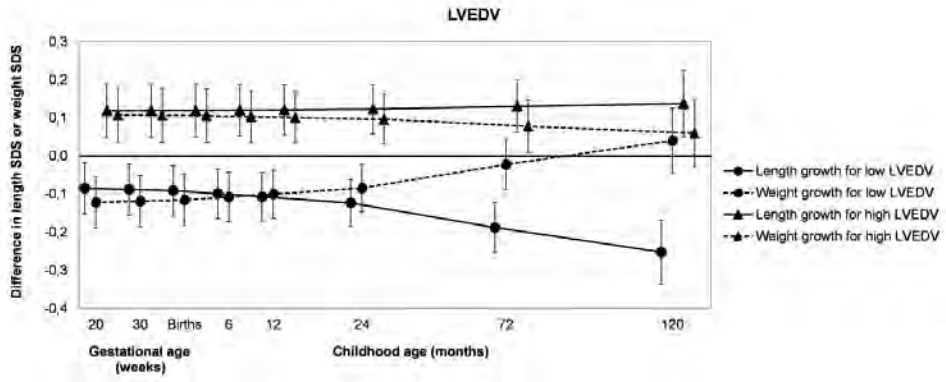


Figure 2.4.1C

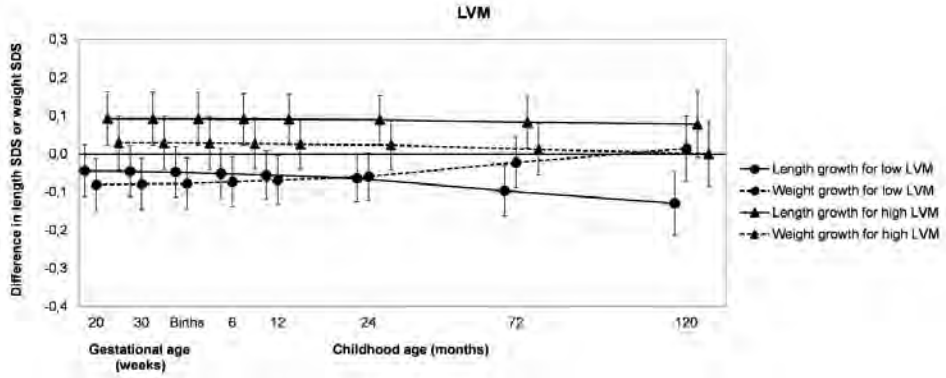


Figure 2.4.1D

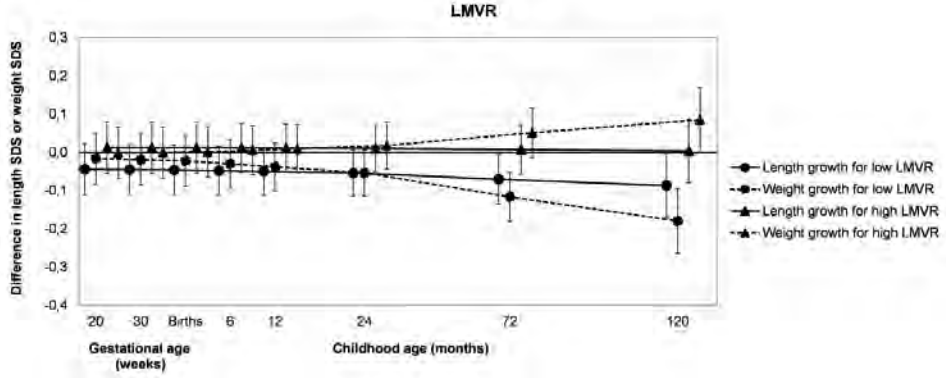


Figure 2.4.1E

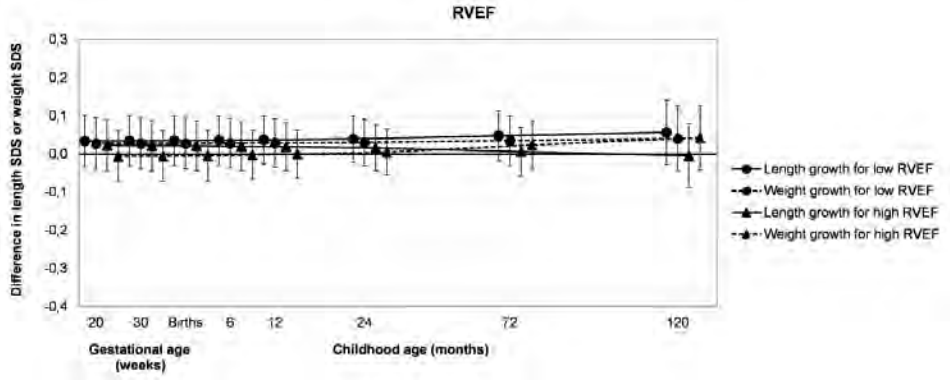
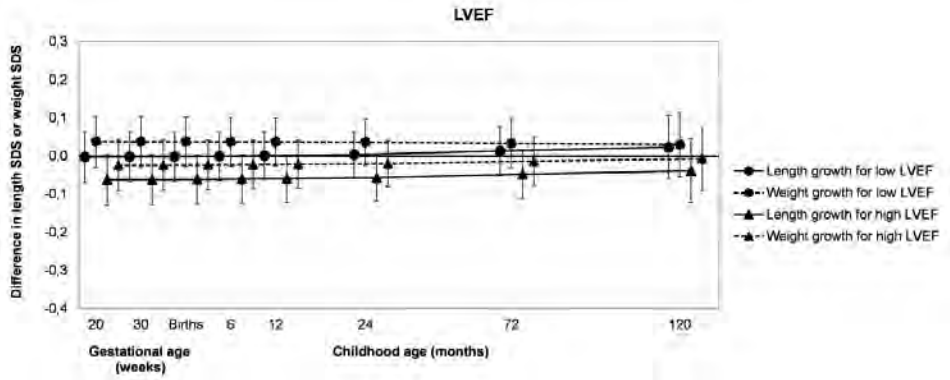


Figure 2.4.1F



Figures show results of repeated measurement regression models. Each point shows the difference in length or weight (± 95% confidence interval), from children in the lowest or highest 25% of the cardiac measure, compared to the children in the middle 25-75%. A parallel line indicates growth rates comparable to the reference group, while an increase or decrease of the slope indicates lower or higher growth rates.
Abbreviations: SDS standard deviation score; RVEDV right ventricular end-diastolic volume; RVEF right ventricular ejection fraction; LVEDV left ventricular end-diastolic volume; LVEF left ventricular ejection fraction; LVM left ventricular mass; LMVR left ventricular mass-to-volume ratio.

Early life critical periods and cardiac measures at school-age

Figure 2.4.2A-B shows that fetal length and weight growth from 20 weeks gestational age until birth were positively associated with RVEDV and LVEDV, independent from growth in other periods, or from current childhood body size (p-values <0.05). The highest effect estimate was observed for height gain from 24 months to 6 years. Higher weight and BMI gain from 6 to 10 years were associated with lower RVEDV and LVEDV. Height gain between 24 months and 6 years was associated with higher

LVM, relative to current body size, while higher weight and BMI gain between 6 and 10 years were associated with lower LVM at the age of 10 years (**Figure 2.4.2C**). Weight gain between 24 months and 6 years was associated with higher LMVR, while weight gain in late pregnancy was associated with lower LMVR (**Figure 2.4.2D**). We did not observe independent associations of fetal or infant growth with RVEF or LVEF at school-age (**Figure 2.4.2E-F**).

Figure 2.4.2 Associations of fetal and childhood growth measures with childhood cardiac measures from conditional analyses (N=2,827)

Figure 2.4.2A

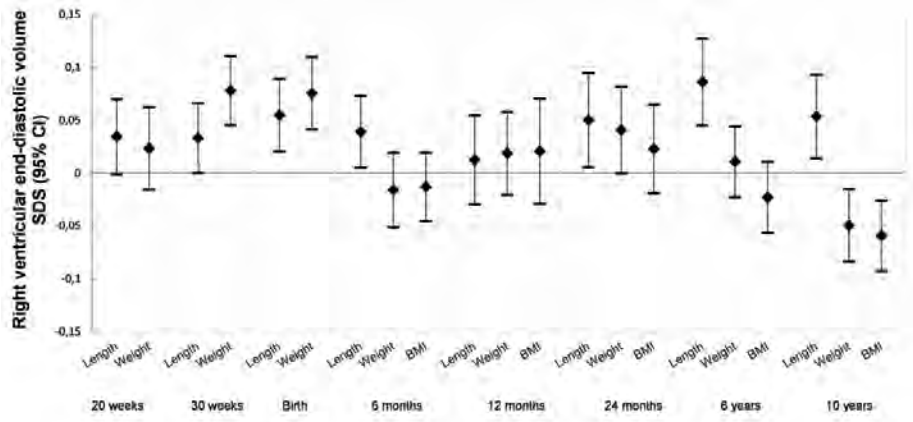


Figure 2.4.2B

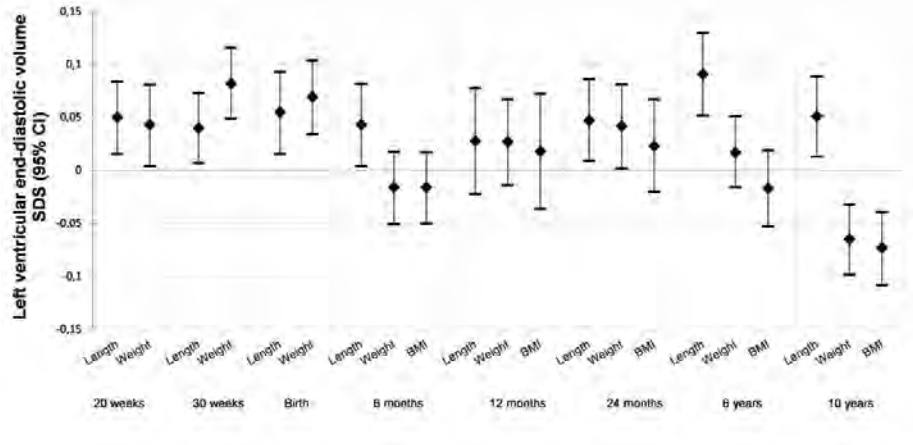


Figure 2.4.2C

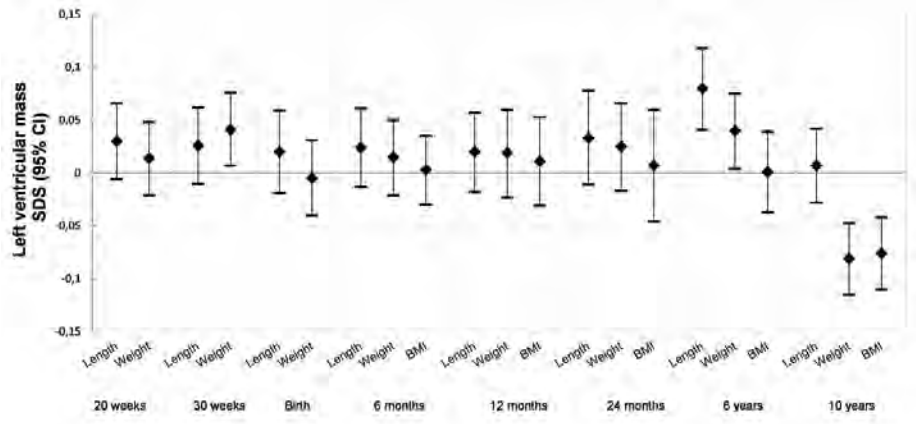


Figure 2.4.2D

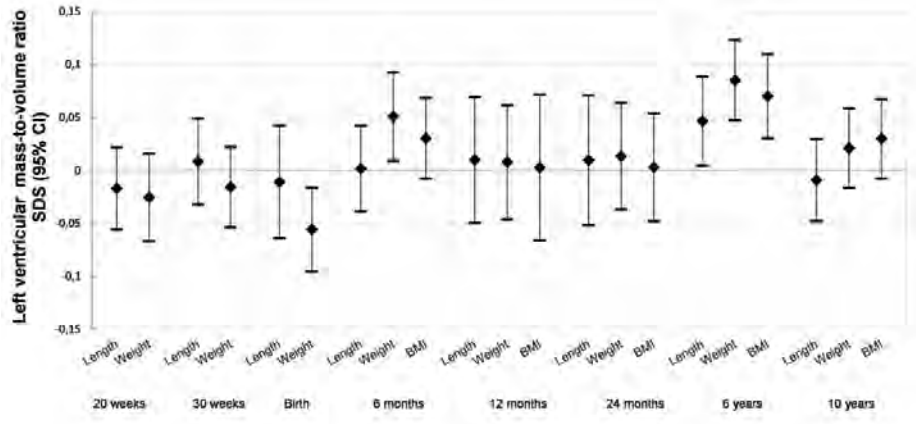


Figure 2.4.2E

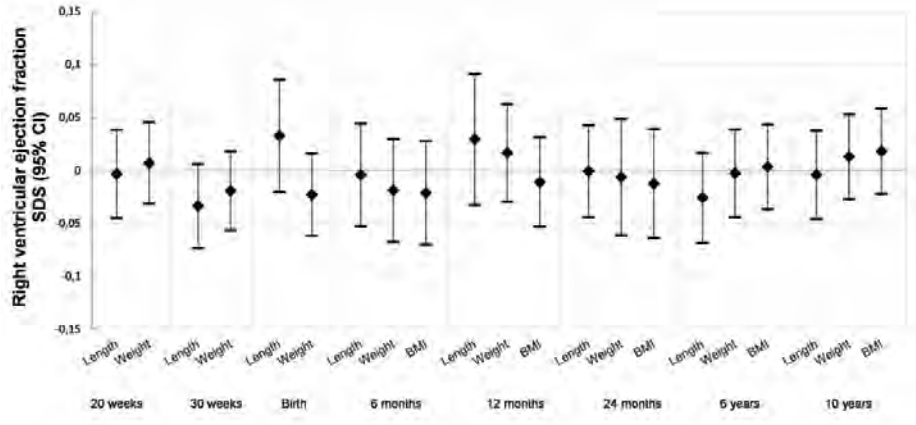
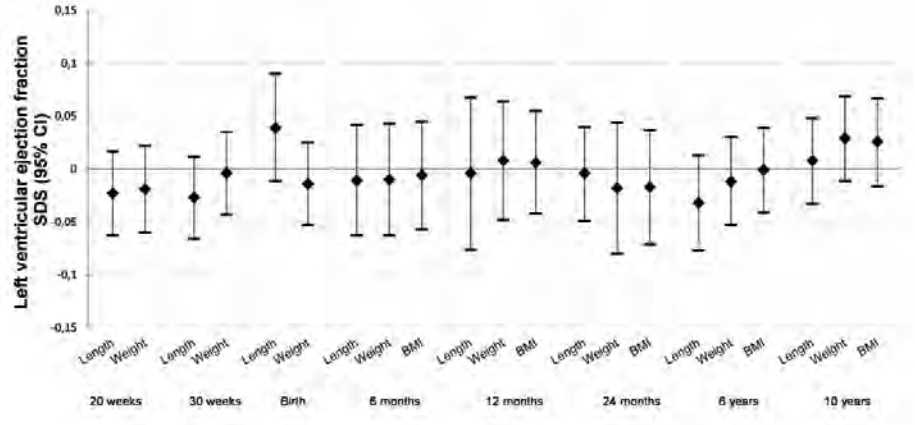


Figure 2.4.2F



Conditional growth models for length, weight, and BMI to cardiac structure at 10 years. Each point shows the strength of association (±95% CI) for the period of growth from the preceding point and represents a difference from normal population growth over that period.

DISCUSSION

In this population-based prospective cohort study, we observed that higher birth weight for gestational age was associated with higher RVEDV and LVEDV, and with lower LMVR. Longitudinal growth analyses suggest that children who are larger at birth, and grow to be taller and leaner in childhood have larger hearts, relative to their body size, whereas children who are smaller at birth and who are shorter and heavier in childhood have smaller hearts with a larger LMVR. Both fetal and childhood growth seem to be independently related to cardiac dimensions in childhood.

Interpretation of main findings

Adults who were born preterm or with low birth weight are at increased cardiovascular risk later in life, especially when followed by increased childhood weight gain.[3] In an adverse fetal environment, fetal blood flow and cardiac adaptations may lead to better short-term survival.[4] However, previous studies suggest that a mismatch reflected by a restricted fetal environment followed by an affluent postnatal environment, leads to an increased risk of cardiovascular disease.[5] Animal and human studies show that fetal growth restriction affects the maturation and sarcomere structure of cardiomyocytes, and causes changes in fetal hemodynamics leading to cardiac pressure and volume overload.[19-21] These changes might affect cardiac structure and shape around the time of birth, and the development

of the heart in later life.[19, 21] During childhood, the heart grows in accordance with the hemodynamic demands of a growing body.[136] A study in adolescents observed that both birthweight and current body size were independently from each other associated with LVM, but infant growth was not.[136] In the current study, we examined the associations of fetal, infant and childhood growth with right and left cardiac structure and function, independent of current body size and aimed to identify critical periods of growth.

Previous studies in young adults have shown that those who were born preterm had higher right ventricular mass and LVM, but lower RVEDV and LVEDV than term born adults.[31, 104] The changes were greater in the right ventricle.[31] In our study, we did not find associations of gestational age with cardiac structure. In our study, preterm birth averaged at 34.4 weeks gestational age, compared to 30.3 weeks in the previous studies.[31, 104] Effects of preterm birth on cardiac structure in later life might only be present in extreme prematurity or changes might become detectable after childhood when the heart has been exposed to more and longer periods of physical stress. Next to preterm birth, fetal growth restriction may affect cardiac development. [21] A study in 11-year old children with fetal growth restriction showed shorter and more global ventricles than in children with normal fetal growth.[21] The Young Finns Study in 784 young adults showed a slightly larger left ventricular diameter in the adults who were born small for gestational age, but no differences in LVEDV, RVEDV or LVM.[43] We observed smaller ventricular volumes and mass in children who were born small for gestational age. Direct comparison with the two previous studies is difficult since they used echocardiography. Also, the indexing methods to account for current body size were different.[21, 43] Altogether, preterm birth and weight at birth might influence cardiac size and cardiac shape in later life. These changes in cardiac size and shape could also relate to subclinical cardiac dysfunction and ultimately to cardiac disease risk in later life.[21]

We have previously observed that higher weight in fetal life, but not infant weight were independently associated with higher LVM at the age of 6 years.[118] One study in 418 adolescents observed that both birth weight and current size were associated with LVM, independently from each other.[136] We observed that children with larger ventricular volumes and mass are taller and heavier from fetal life onwards until childhood. Also, we observed that children with relatively smaller ventricular volumes had lower height gain and higher weight gain than children with normal sized hearts in childhood, relative to current body size. Children with higher LMVR had higher weight gain in childhood than children with lower LMVR. This is in

line with previous studies, that show cardiac remodeling in obese children.[56] The higher LMVR we observed could be the first sign of concentric remodeling, known to be associated with cardiac disease in adulthood.[26]

We used conditional regression analyses to identify critical periods of growth. Both fetal and childhood growth, but not infant growth were associated with cardiac dimensions. Our findings suggest that fetal and childhood growth patterns influence cardiac structure at school-age. We previously observed moderate tracking of cardiac measures between infancy and childhood, which became stronger later in childhood.[140] This tracking also continues until late adolescence and possibly into adulthood.[18] However, it is unclear how the observed cardiac adaptations in childhood relate to adult cardiac disease. In adults, cardiac remodeling resulting in left ventricular hypertrophy is associated with cardiac disease.[26] Obesity in childhood is associated with LVH in later adulthood.[35] However, the larger cardiac dimensions we observed in taller and leaner children might not be a sign of pathological adaptation, but of physiological adaptation. This adaptive remodeling can also be observed in preadolescent athletes.[141] We observed that a growth pattern with higher childhood weight gain was associated not only with lower RVEDV, LVEDV and LVM, but also with higher LMVR. This might be a first sign of adverse cardiac remodeling, leading to increased cardiac risk later in life. Further research is necessary to examine childhood right and left cardiac growth and remodeling and relate these to cardiac disease.

Methodological considerations

Main strengths of this study are its population-based prospective design, and the large number of fetal and child growth measurements and cardiac imaging available. The population is representative for the general Dutch population, but one should be careful when generalizing our results to other countries or ethnicities. Repeated measurements allowed us to examine growth patterns and to identify critical periods of growth. By using cMRI, we were able to study the right ventricle.[29] Some limitations need to be discussed. Of all children, 72% attended the MRI center and 69% of those had good quality cMRI. This loss to follow up could lead to bias if the associations of growth with cardiac measures differ between those included and not included in the analyses (**Table S2.4.2**). However, we deem this unlikely. We used a 3T MRI because this was available and used in our other population-based studies. Use of this scanner could have affected image quality and maybe led to missing data because of artifacts in some scans, we consider it unlikely that this has affected the associations between

growth and cardiac measures.[142] We standardized our measures on BSA to account for current body size. However, height and weight were measured 1.1 months before MRI. The calculated BSA might underestimate the actual BSA at time of cMRI. We adjusted all our analyses for the time difference, but this measurement error could lead to attenuation of the effect estimates we observed. We did not observe statistical interaction between growth measures and sex. The BSA standardized measures possibly already took into account differences in body size between boys and girls. It is also possible that although boys and girls have different cardiac measures, the associations between growth and cardiac measures do not differ. Despite the fact that we adjusted for a significant number of confounders, residual confounding might be of concern, as in any observational study. In childhood, cardiac mass and size is mainly determined by lean body mass, and not by blood pressure.[22] This is in line with our observations, the results did not change after adding childhood blood pressure to our models (results not shown). We do not have detailed information on exercise and fitness levels, which might also influence body composition and cardiac measures.

CONCLUSION

Size for gestational age is related with right and left cardiac measures in mid-childhood. Relative to current body size, children who are larger at birth, and grow to be taller and leaner in childhood have larger hearts, whereas children who are smaller at birth and who are shorter and heavier in childhood have smaller hearts, but a larger LMVR. Both fetal and childhood growth are critical for development of cardiac dimensions. How these differences in cardiac structure in childhood relate to adult cardiovascular disease needs to be further investigated.

SUPPLEMENTAL MATERIAL

Methods S2.4.1 Cardiac Magnetic Resonance Imaging

We performed cardiac magnetic resonance imaging (cMRI) using a wide-bore GE Discovery MR 750 3T scanner (General Electric, Milwaukee, MI, USA). Children were first introduced with the scanning environment through the use of a simulated scanning session. Approximately 60 minutes were reserved for scanning of the brain, lungs, heart, abdomen and hips. Scanning time allocated to the cardiac imaging was 12 minutes. We used multi-phase ungated free-breathing steady-state-free-precession (SSFP) real-time scans to acquire localizer images in all necessary orientations. From these, 2-chamber and 4-chamber views were acquired with ECG gated breath-held scans lasting less than 10 seconds per breath-hold. A short-axis SSFP cine stack was then obtained with basal slice alignment and covering the ventricles and part of the atria with contiguous 8-mm thick slices over several breath-holds. All breath-held cMRI sequences were retrospectively ECG gated with the help of a precordial 4-lead ECG using the vectocardiogram gating option of the patient monitoring unit and acquired at end-expiration. The imaging parameters for the short axis acquisitions were as follow: field of view of 280 x 280 mm^2, scan matrix of 128 x 128; 16 views per segment, repetition time 3.7 ms, echo time 1.7 ms, flip angle 45°. The 2-chamber, 4-chamber and the short axis SSFP scans were stored on a digital archive for post-processing.

Off-line image analyses for right and left ventricular measures on the short-axis cine stack was performed by Precision Image Analysis (Kirkland, WA, USA) under supervision of an experienced radiologist, using Medis QMASS software (Medis, Leiden, the Netherlands). Right and left ventricular short-axis endocardial and left ventricular epicardial borders were semi-automatically contoured at end-diastole and end-systole to allow automated calculation of right and left ventricular volume and left ventricular mass (LVM), following the guidelines of the Society for Cardiovascular Magnetic Resonance (SCMR).[139] Papillary muscle was included in the ventricular cavity. LVM was calculated as (diastolic epicardial volume-diastolic endocardial volume)×1.05. Since the scan spatial resolution was not adequate to contour right ventricular epicardial borders, no right ventricular mass could be calculated. Cardiac measurements included right ventricular end-diastolic volume (RVEDV), right ventricular ejection fraction (RVEF), left ventricular end-diastolic volume (LVEDV), left ventricular ejection fraction (LVEF), and LVM. Coefficients of variation were calculated in a random subset of 25 scans. Intra-observer variability was between 2.5% and 7.2%, while interobserver was between 3.5% and 8.7%. Intra-observer variability for each measurement were as follows: RVEDV 3.9%; RVEF 5.1%; LVEDV 2.5%; LVEF 4.3% and LVM 7.2%. Inter-observer variability was: RVEDV 5.0%; RVEF 5.3%; LVEDV 3.5%; LVEF 5.9% and LVM 8.7%.

Figure S2.4.1 Flow chart of participants included in the analysis

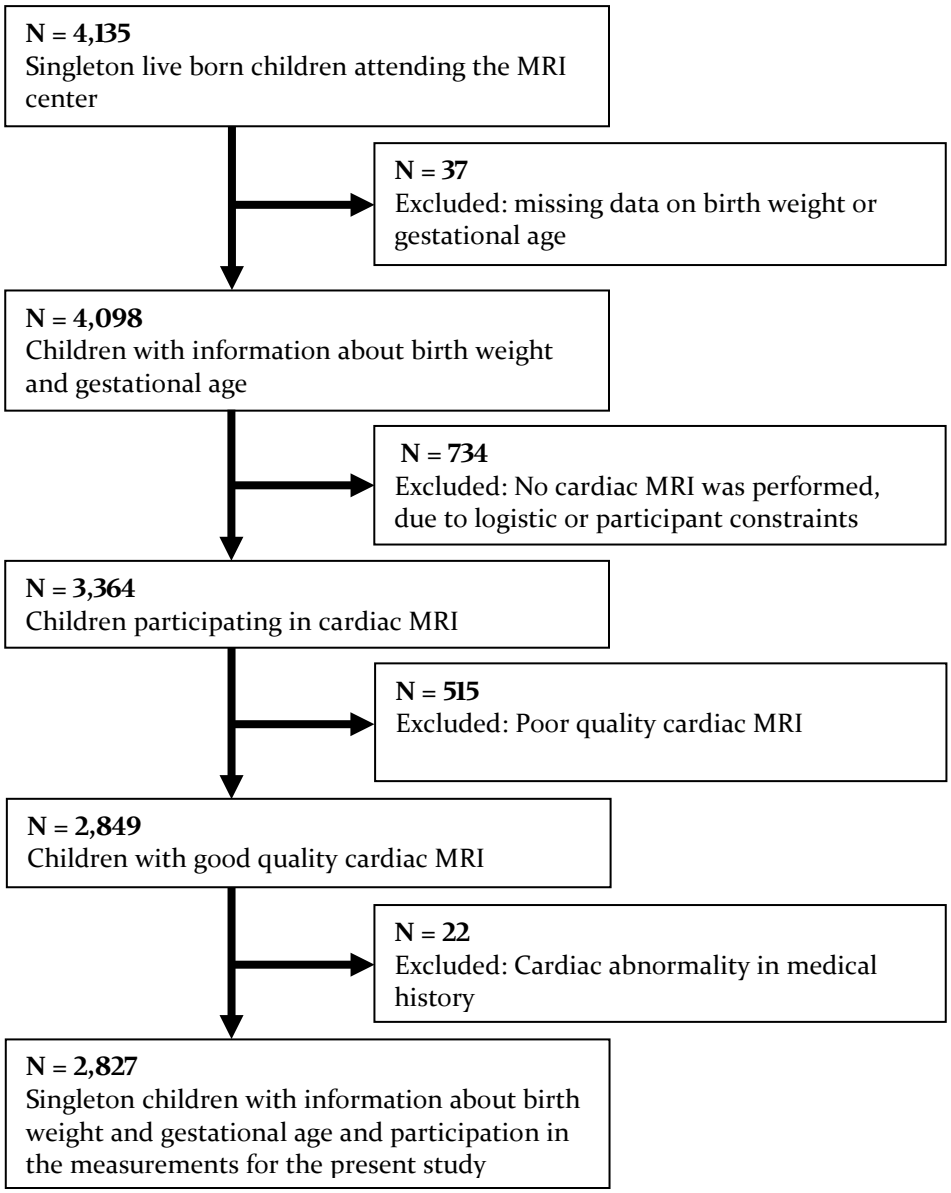


Table S2.4.1 Subject characteristics (N=2,827)

Subject characteristics	N	Values	Imputed values[a]
Maternal characteristics			
Age, y	2827	31.6 (20.6-40.2)	31.5 (20.6-40.2)
Height, cm	2540	167.9 (7.4)	167.9 (7.2)
Weight, kg	2081	64.0 (49.0-99.0)	65.0 (49.9-94.0)
Body mass index pre-pregnancy, kg/m^2	2079	22.5 (18.0-34.6)	22.9(18.1-33.0)
Parity, nulliparous, n (%)	2742	1588 (57.9)	1628(57.6)
Maternal education, higher, n (%)	2620	1349 (53.2)	1454 (51.4)
Household monthly income, <€1600 netto, n (%)	2827	1103 (39.0)	1103 (39.0)
Systolic blood pressure at intake, mmHg	2526	116 (12)	116 (12)
Diastolic blood pressure at intake, mmHg	2526	68 (10)	68 (10)
Birth and infant characteristics			
Sex, boys, n (%)	2827	1369 (48.4)	1369 (48.4)
Ethnicity, Dutch, n (%)	2771	1693 (59.9)	1711 (60.5)
Gestational age at birth unit, weeks	2827	40.1 (36.0-42.3)	40.1 (36.0-42.3)
Preterm birth <37 weeks at delivery (%)	2827	126 (4.5)	126 (4.5)
Birth weight, grams	2827	3447 (555)	3447 (555)
Child characteristics at 10 years			
Age at MRI, y	2827	9.9 (9.5-11.8)	9.9 (9.5-11.8)
Height, cm	2827	141.6 (6.6)	141.6 (6.6)
Weight, kg	2827	33.8 (25.4-53.0)	33.8 (25.4-53.0)
Body mass index, kg/m^2	2827	16.9 (14.0-24.3)	16.9 (14.0-24.3)
Body surface area, m^2	2827	1.15 (0.95-1.50)	1.15 (0.95-1.50)
Lean body mass, %	2808	70.1 (54.6-80.1)	70.1 (54.6-80.1)
Right ventricular end-diastolic volume, ml	2825	98.5 (65.8-144.3)	NA
Right ventricular ejection fraction, %	2825	58.2 (4.9)	NA
Left ventricular end-diastolic volume, ml	2825	98.7 (69.9-138.6)	NA
Left ventricular ejection fraction, %	2826	58.4 (4.6)	NA
Left ventricular mass, g	2825	47.5 (32.9-72.9)	NA
Left ventricular mass-to-volume ratio	2823	0.48 (0.37-0.68)	NA

Values are means (SD), medians (95% range) or numbers (%), based on original, non-imputed data.

NA: not applicable; [a] Values are based on imputed data with N=2827. Cardiac measures were not imputed.

Table S2.4.2 Non-response analysis

Subject characteristics	Children without cardiac MRI N=1,308	Children with cardiac MRI N=2,827	P-Value
Maternal characteristics			
Age, y	31.2 (20.1-39.8)	31.6 (20.6-40.2)	0.04
Height, cm	167.3 (7.4)	167.9 (7.4)	0.02
Weight, kg	64.0 (49.0-100.0)	64.0 (49.0-99.0)	0.83
Body mass index pre-pregnancy, kg/m^2	22.7 (18.1-35.0)	22.5 (18.0-34.6)	0.40
Parity, nulliparous, n (%)	714 (57.3)	1588 (57.9)	0.71
Maternal education, higher, n (%)	533 (45.7)	1394 (53.2)	<0.01
Household income, <€1600 netto, n (%)	603 (46.1)	1103 (39.0)	<0.01
Systolic blood pressure at intake, mmHg	116 (12)	116 (12)	0.87
Diastolic blood pressure at intake, mmHg	68 (9)	68 (10)	0.79
Birth and infant characteristics			
Sex, boys, n (%)	699 (53.4)	1369 (48.4)	<0.01
Ethnicity, Dutch, n (%)	695 (54.5)	1693 (61.1)	<0.01
Gestational age at birth unit, weeks	40.1 (35.6-42.3)	40.1 (36.0-42.3)	0.70
Birth weight, grams	3416 (545)	3447 (555)	0.09
Preterm birth <37 weeks at delivery (%)	64 (5.0)	126 (4.5)	0.67
Child characteristics at 10 years			
Age at MRI, y	9.89 (9.5-12.2)	9.95 (9.5-11.8)	<0.01
Height, cm	141.5 (6.9)	141.6 (6.6)	0.66
Weight, kg	34.2 (25.2-55.6)	33.8 (25.4-53.0)	0.27
Body mass index, kg/m^2	17.1 (14.1-25.3)	16.9 (14.0-24.3)	0.06
Body surface area, m^2	1.16 (0.95-1.53)	1.15 (0.95-1.50)	0.36
Lean body mass, %	70.1 (53.9-79.7)	70.1 (54.6-80.1)	0.37

Values are means (SD), medians (95% range) or numbers (%), based on original, non-imputed data. P-values from ANOVA, Mann-Whitney U or chi-square tests.

Table S2.4.3 Correlation coefficients between growth parameters and cardiac outcomes

	Right ventricular end-diastolic volume	Right ventricular ejection fraction	Left ventricular end-diastolic volume	Left ventricular ejection fraction	Left ventricular mass	Left ventricular mass-to-volume ratio
Length						
20 weeks	0.01	0.00	0.03	-0.02	0.01	-0.02
30 weeks	0.02	-0.02	0.03	-0.04	0.00	-0.02
Birth	0.08**	0.01	0.08**	0.02	0.03	-0.02
6 months	0.11**	-0.02	0.11**	-0.02	0.07**	0.00
12 months	0.06**	0.03	0.08**	-0.01	0.04	0.01
24 months	0.09**	0.01	0.10**	-0.02	0.06**	0.01
6 years	0.10**	0.00	0.11**	-0.03	0.08**	0.03
10 years	0.14**	-0.02	0.15**	-0.03	0.10**	0.02
Weight						
20 weeks	0.04*	0.00	0.06**	-0.02	0.03	-0.02
30 weeks	0.11**	-0.02	0.12**	-0.02	0.06**	-0.04
Birth	0.14**	-0.04	0.13**	-0.03	0.04*	-0.06**
6 months	0.02	-0.03	0.02	-0.02	0.02	0.04
12 months	0.04	0.01	0.06*	0.00	0.03	0.02
24 months	0.06*	-0.01	0.06**	-0.03	0.03	0.03
6 years	0.03	-0.01	0.04	-0.02	0.04	0.08**
10 years	0.01	-0.01	0.01	0.00	0.01	0.09**
BMI						
6 months	-0.05*	-0.02	-0.05	0.00	-0.02	0.05*
12 months	0.01	-0.01	0.01	0.01	0.00	0.02
24 months	0.01	-0.02	0.00	-0.01	0.00	0.03
6 years	-0.04*	-0.01	-0.03	0.00	-0.01	0.09**
10 years	-0.07**	0.01	-0.08**	0.02	-0.05**	0.10**

Values are Pearson correlation coefficients. Growth variables are standardized on sex and age. Cardiac outcomes are standardized on body surface area.
*P-value <0.05; **P-value <0.01.

Table S2.4.4 Birth characteristics and ventricular outcomes at the age of 10 years (N = 2,827), lean body mass adjusted

		Difference in cardiac measures standard deviation scores (95% Confidence Interval)					
Birth characteristics	**N**	**Right ventricular end-diastolic volume**	**Right ventricular ejection fraction**	**Left ventricular end-diastolic volume**	**Left ventricular ejection fraction**	**Left ventricular mass**	**Left ventricular mass-to-volume ratio**
Gestational age							
<37.0 weeks	129	-0.05 (-0.20, 0.10)	0.03 (-0.15, 0.20)	-0.13 (-0.28, -0.03)	0.25 (0.07, 0.43)**	-0.05 (-0.21, 0.10)	0.05 (-0.13, 0.23)
37.0-41.9	2554	Reference	Reference	Reference	Reference	Reference	Reference
≥42 weeks	205	0.01 (-0.11, 0.13)	-0.04 (-0.19, 0.10)	-0.06 (-0.18, 0.06)	0.00 (-0.14, 0.15)	0.00 (-0.12, 0.13)	0.05 (-0.10, 0.19)
Trend	*2827*	*0.00 (-0.04, 0.03)*	*0.00 (-0.04, 0.03)*	*0.01 (-0.02, 0.04)*	*-0.05 (-0.09, -0.01)**	*0.01 (-0.02, 0.04)*	*0.01 (-0.03, 0.04)*
Birth weight							
<2000 grams	29	0.00 (-0.32, 0.32)	-0.03 (-0.40, 0.35)	-0.18 (-0.50, 0.13)	0.42 (-0.36, 0.81)	-0.35(-0.67, -0.02)*	-0.24 (-0.62, 0.14)
2000 - 2499 g	89	-0.10 (-0.28, 0.08)	0.12 (-0.10, 0.34)	-0.12 (-0.30, 0.06)	0.17 (-0.05, 0.39)	0.03 (-0.16, 0.22)	0.13 (-0.09, 0.35)
2500 - 2999 g	417	-0.03 (-0.13, 0.06)	-0.08 (-0.19, 0.04)	-0.11 (-0.21, -0.01)*	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.12)	0.11 (-0.01, 0.22)
3000 - 3499 g	980	Reference	Reference	Reference	Reference	Reference	Reference
3500 - 3999 g	938	0.13 (0.06, 0.21)**	0.02 (-0.07, 0.11)	0.12 (0.05, 0.20)**	-0.01 (-0.10, 0.09)	0.01 (-0.07, 0.09)	-0.09 (-0.18, 0.00)*
4000 - 4499 g	359	0.21 (0.11, 0.31)**	-0.10 (-0.23, 0.02)	0.18 (0.08, 0.29)**	-0.10 (-0.22, 0.03)	0.11 (0.01, 0.22)*	0.00 (-0.12, 0.12)
≥4500 grams	76	0.47 (0.27, 0.67)**	-0.03 (-0.26, 0.21)	0.34 (0.14, 0.53)**	0.15 (-0.09, 0.39)	0.15 (-0.05, 0.35)	-0.10 (-0.33, 0.14)
Trend	*2827*	*0.10 (0.07, 0.13)***	*-0.03 (-0.07, 0.01)*	*0.11 (0.08, 0.14)***	*-0.05 (-0.09, -0.01)**	*0.03 (0.00, 0.07)**	*-0.05 (-0.09, -0.01)**
Birth weight for gestational age							
Small	262	-0.17 (-0.28, -0.06)**	0.00 (-0.13, 0.13)	-0.21 (-0.32, -0.10)**	-0.03 (-0.16, 0.10)	0.02 (-0.09, 0.13)	0.18 (0.05, 0.31)**
Normal	2325	Reference	Reference	Reference	Reference	Reference	Reference
Large	301	0.24 (0.14, 0.34)**	-0.03 (-0.16, 0.09)	0.20 (0.10, 0.30)**	0.01 (-0.11, 0.14)	0.14 (0.04, 0.25)**	0.01 (-0.11, 0.13)
Trend	*2827*	*0.12 (0.09, 0.15)***	*-0.03 (-0.07, 0.01)*	*0.12 (0.09, 0.16)***	*-0.02 (-0.06, 0.02)*	*0.03 (0.00, 0.07)*	*-0.06 (-0.10, -0.02)***

Values are regression coefficients (95% confidence interval) and reflect the change in standard deviation (SDS) of each cardiac measure for each birth weight or gestational age group, compared to the reference group. Trend estimates represent the effect estimates for the continuous associations per SDS change in birth characteristic. We used body surface adjusted standard deviation scores, except for left ventricular mass-to-volume ratio. Models are adjusted for maternal height, parity, educational level, income level, child's sex, ethnicity, current age, lean body mass percentage, and time difference between BSA measurement and MRI. *$P < 0.05$;** $P< 0.01$.

Chapter 2.5

Third trimester fetal cardiac blood flow and cardiac structures in school-age children

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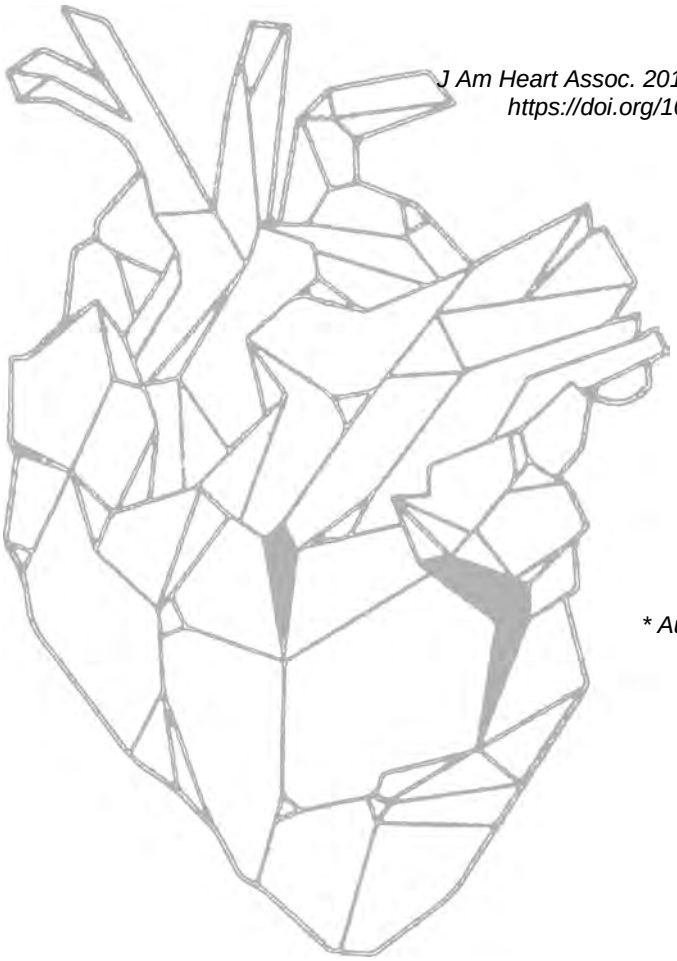


** Authors contributed equally*

ABSTRACT

Background: An adverse fetal environment leads to fetal hemodynamic adaptations with cardiac flow alterations that may subsequently affect cardiac development. We examined the associations of third trimester placental and fetal cardiac hemodynamics with cardiac outcomes in school-age children.

Methods and Results: We performed a population-based prospective cohort study among 547 mothers and their children. At a gestational age of 30.4 (95% range 28.4, 32.7) weeks, we measured umbilical and cerebral artery resistance, cardiac output and tricuspid and mitral E/A waves with Doppler. At the median age of 10.0 years (95% range 9.4, 11.7) we measured cardiac outcomes with cardiac Magnetic Resonance Imaging (cMRI). Cardiac outcomes included right ventricular end-diastolic volume (RVEDV) and ejection fraction (RVEF), left ventricular end diastolic volume (LVEDV) and ejection fraction (LVEF), left ventricular mass (LVM), and left ventricular mass-to-volume ratio (LMVR) as LVM/LVEDV. Higher third-trimester umbilical artery resistance was associated with higher childhood RVEF (p-value<0.05), but not with other cardiac outcomes. The third-trimester umbilical / cerebral artery pulsatility index ratio was not associated with childhood cardiac outcomes. Higher third-trimester fetal left cardiac output was associated with lower childhood LVEF and higher LMVR (p-value<0.05). Third-trimester fetal right cardiac output was not associated with childhood cardiac outcomes. A higher third-trimester fetal tricuspid valve E/A ratio was associated with higher childhood RVEF (p-value<0.05).

Conclusions: Our findings suggest that fetal cardiac blood flow redistribution may have long term effects on cardiac structure and function. These results should be considered as hypothesis generating and need further replication.

INTRODUCTION

Cardiovascular disease is at least partially established in the earliest phase of life.[5] Changes in fetal hemodynamics may be a mechanism linking an adverse fetal environment with cardiovascular adaptations and subsequent risk of cardiovascular disease in later life.[11] An adverse fetal environment leads to fetal blood flow redistribution in favor of the upper parts of the body at expense of the trunk, a phenomenon known as "brain sparing".[143] This fetal blood flow redistribution also leads to intra-cardiac flow changes with cardiac output switched in favor of the left ventricle, which provides blood for the brain circulation.[144] A rise in the placental vascular resistance and peripheral arterial vasoconstriction in the trunk causes increased right ventricular afterload, and a drop in right cardiac output.[145] The rise in ventricular afterload leads to reduced peak systolic velocities in the aorta and pulmonary artery.[146] These changes in intra-cardiac blood flow patterns may affect right and left ventricular structure and function in later life through alterations in shear stress and wall tension, and through the effects on cardiomyocyte maturation and apoptosis.[146] Previously, we reported that lower third-trimester fetal growth was associated with specific fetal hemodynamic changes, such as reduction of cardiac output, stroke volume, and pulmonary artery and cardiac compliance.[147] The long-term consequences of these fetal flow adaptations are not known. A previous study among 200 children reported that fetal growth restriction is associated with cardiac shape and stroke volume alterations in children aged 5 years.[11] In line with these findings, we previously observed that fetal cardiac hemodynamics, mainly uterine artery resistance (UA PI) and left cardiac output in the third trimester, were associated with cardiac dimensions in the first six years.[60, 148]

Therefore, we examined in a population-based prospective cohort study among 547 children the associations of placenta and fetal cardiac blood flow patterns with childhood right and left cardiac outcomes, assessed by cardiac Magnetic Resonance Imaging (cMRI).

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[58] Details of this study have been described previously.[58] Detailed assessments of fetal growth and blood flow patterns were conducted in a subgroup of 1,216 Dutch mothers and their children.[58]

Third-trimester fetal hemodynamics were available in 1,179 singletons live born children, of whom 547 visited the Generation R MRI research center at the age of 10 years and did not have cardiac abnormalities (**Figure S2.5.1**). Written permission was received from parents. The study has been approved by the local Medical Ethics Committee.

Third trimester fetal growth and hemodynamics characteristics

In the third trimester, we measured head circumference, abdominal circumference and femur length and estimated fetal weight using the formula by Hadlock et al.[83] Fetal hemodynamics were assessed by pulsed-wave Doppler at a median gestational age of 30.2 weeks (range 28.8 to 32.3 weeks), as described previously.[147, 148] For each measurement, three consecutive uniform waveforms were recorded and the mean was used for analyses. Feto-placental vascular resistance was evaluated with recorded flow-velocity waveforms from the umbilical artery. An increase in umbilical artery pulsatility index (UA PI) indicates increased umbilical artery resistance.[147] UA PI was determined in a free-floating loop of the umbilical cord. Color Doppler visualization was used to obtain flow-velocity waveforms of the proximal part of the cerebral arteries. An increased ratio between the UA PI and the middle cerebral artery (MCA) PI is an indicator of brain sparing. This umbilical artery-cerebral artery ratio (U/C ratio) is calculated as UA PI/ MCA PI.[146, 148]

Cardiac flow-velocity waveforms at the level of the mitral and tricuspid valves were recorded from the apical four-chamber view of the fetal heart. Peak velocities of the E wave and the A wave were recorded. The E/A ratio is an index for ventricular diastolic function and expresses both cardiac compliance and preload conditions.[147] A higher E/A ration reflects less stiff and more compliant ventricles. Cardiac outflow flow-velocity waveforms from the aorta and pulmonary artery were recorded from the five-chamber view and the short-axis view of the fetal heart just above the semi-lunar valves. Peak systolic velocities in the aorta and pulmonary artery, time-velocity integral, fetal heart rate and the inner diameters during systole were recorded. Left and right cardiac output were calculated in milliliters per minute by multiplying the vessel area of the aorta or pulmonary artery by the time-velocity integral by fetal heart rate.

Cardiac Magnetic Resonance Imaging

MRI scanning was performed on a wide-bore GE Discovery MR 750 3T scanner (General Electric, Milwaukee, MI, USA). The scanning environment was introduced to the children in a 30-minute simulated scanning session. Total brain and body scanning session lasted

approximately 1-hour, of which 12 minutes was reserved for the cardiac imaging. Briefly, we acquired localizer images, followed by ECG gated breath-held scans lasting less than 10 seconds per breath-hold (**Methods S2.4.1**). A short-axis SSFP cine stack was then obtained with basal slice alignment and covering the ventricles and part of the atria with contiguous 8-mm thick slices over several end expiration breath-holds. The scans were stored on a digital archive for post-processing. Off-line image analyses for right and left ventricular measures on the short-axis cine stack was performed by Precision Image Analysis (Kirkland, WA, USA) under supervision of an experienced radiologist, using Medis QMASS software (Medis, Leiden, the Netherlands). The guidelines of the Society for Cardiovascular Magnetic Resonance (SCMR) were followed to semi-automatically contour right and left ventricular short-axis endocardial and left ventricular epicardial borders.[139] Papillary muscle was included in the ventricular cavity. Cardiac measurements included right ventricular end-diastolic volume (RVEDV), right ventricular ejection fraction (RVEF), left ventricular end diastolic volume (LVEDV), left ventricular ejection fraction (LVEF), and left ventricular mass (LVM). We calculated left ventricular mass-to-volume ratio (LMVR) as LVM/LVEDV.

Covariates

We obtained information about gestational age at birth, birth weight and sex from midwife and hospital records. Information about maternal age, pre-pregnancy body mass index, folic acid use and smoking during pregnancy, maternal education, and infant breastfeeding was collected by questionnaires and medical charts. At the age of 10 years, child height and weight were measured without shoes and heavy clothing, and body mass index (BMI) and body surface area (BSA) were calculated. BSA was computed using the Haycock formula (BSA (m^2)=0.024265 x weight $(kg)^{0.5378}$ x height $(cm)^{0.3964}$).[69] Systolic and diastolic blood pressures were measured on the right brachial artery, using the validated automatic sphygmomanometer Accutorr Plus (Datascope Corporation, Fairfield, New Jersey). These measurements preceded the cMRI by a median of 1.1 months (95% range 0 - 24.8 months).

Statistical analyses

First, we assessed the differences in subject characteristics between boys and girls using ANOVA for continuous variables, Kruskal-Wallis tests for non-parametric variables, and chi-square tests for categorical variables. Similarly, we compared subject characteristics for children with and without successful cardiac MRI in a non-response analysis. Second, we

explored the Pearson correlation coefficients between fetal hemodynamics in third trimester and all childhood cardiac outcomes. Main hemodynamic exposure measures included fetal blood flow redistribution measures (UA PI, U/C ratio), right cardiac measures (right cardiac output, pulmonary artery PSV, tricuspid valve E/A ratio) and left cardiac measures (left cardiac output, aorta ascendens PSV and mitral valve E/A ratio). Childhood cardiac outcomes include RVEDV, RVEF, LVEDV, LVEF, LVM and LMVR. Third, we used linear regression models to analyze the associations of third trimester fetal hemodynamics and cardiac outcomes at the age of 10 years. Models were adjusted for child sex, gestational age at 3rd trimester measurement, current age and time difference between measurement of BSA and cardiac MRI. Covariates (estimated third trimester fetal weight, maternal age, folic acid intake and smoking status during pregnancy, maternal education level, breast feeding, and childhood blood pressure) were selected on the basis of their associations with the outcomes of interest based on previous study results, but they were not included in the models, because they did not cause a change in effect estimate >10%. We did not observe sex-specific interaction with fetal hemodynamics in relation to the cardiac outcomes of interest. Therefore, we did not stratify our analysis on sex. We constructed BSA adjusted standard deviation scores (SDS) for the cardiac outcomes using Generalized Additive Models for Location, Size and Shape (GAMLSS) using R, version 3.2.0 (R Core Team, Vienna, Austria).[95-97] These models enable flexible modelling, taking into account the distribution of the response variable.[70] The SDS of the cardiac outcomes are based on the full cohort of all children who had successful cardiac MRI (N=3,018), not only on the sub selection in the current study. Since LMVR is not usually standardized on BSA, we created standard deviation scores as (observed value-mean)/SD. We also created these SDS for all determinants, to enable comparison of effect estimates. In order to reduce potential bias due to missing data, we performed multiple imputations (N=5) of missing covariates. We did not adjust for multiple testing, since the different determinants or outcomes are strongly correlated and adjusting for multiple testing might be too strict. For our analyses we used the Statistical Package of Social Sciences version 21.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject characteristics

Subject characteristics are presented in **Table 2.5.1**. At the age of 10 years RVEDV, LVEDV, and LVM were higher in boys than in girls, whereas RVEF

and LVEF were slightly higher in girls (**Table 2.5.2**). These measures were also different between boys and girls when standardized on BSA (**Table S2.5.1**). The correlations of placental and fetal cardiac hemodynamics with childhood cardiac outcomes (standardized on BSA) are shown in **Table S2.5.2.** The correlation between UA PI and RVEF is visualized in **Figure S2.5.2**. A non-response analysis is shown in **Table S2.5.3**. Children with successful cardiac MRI had mothers who were older, had higher intake of periconceptional folic acid, smoked less frequently during pregnancy and were more often higher educated. There were no differences in fetal hemodynamics and childhood anthropometric measures between children with and without follow up studies.

Table 2.5.1 Subject characteristics (N=547)

Subject characteristics	Boys (n=266)	Girls (n=281)	P value
Maternal characteristics			
Age, years	31.8 (3.8)	32.3 (3.8)	0.18
Prepregnancy BMI, kg/m²	22.6(18.3, 32.9)	23.0(18.8, 34.3)	0.18
Folic acid intake during pregnancy, start periconceptional, n (%)	180 (67.7)	189 (67.2)	0.87
Never smoked during pregnancy, n (%)	222 (80.7)	227 (78.5)	0.20
High education level, n (%)	214 (77.8)	213 (73.7)	0.27
Third trimester fetal measurements			
Gestational age at measurement, weeks	30.5 (28.6, 32.8)	30.4 (28.3, 32.7)	0.22
Estimated fetal weight, grams	1636 (1194, 2236)	1601 (1172, 2232)	0.49
Vascular resistance parameters			
Umbilical artery PI	0.95 (0.15)	0.98 (0.17)	0.05
Umbilical/middle cerebral artery ratio	0.50 (0.11)	0.50 (0.11)	0.51
Fetal cardiac hemodynamics			
Right cardiac output, ml/min	822 (244)	833 (226)	0.62
Pulmonary artery PSV, cm/s	72.5 (9.4)	74.1 (9.5)	0.05
Tricuspid valve E/A ratio	0.77 (0.08)	0.78 (0.09)	0.08
Left cardiac output, ml/min	614 (179)	598 (162)	0.28
Aorta ascendens PSV, cm/s	91.0 (12.3)	91.3 (12.3)	0.74
Mitral valve E/A ratio	0.78 (0.1)	0.79 (0.1)	0.16
Birth characteristics			
Gestational age at birth, weeks	40.4 (37.0, 42.6)	40.1 (37.0, 42.1)	<0.01
Birth weight, grams	3620 (450)	3469 (512)	<0.01
Breastfeeding, never, n (%)	24 (9.0)	26 (9.2)	0.88
Childhood characteristics			
Age at follow up, years	10.0 (9.4, 11.8)	10.0 (9.4, 11.7)	0.41
Height, cm	142.4 (6.4)	142.0 (6.2)	0.53
Weight, kg	33.8(25.8, 50.3)	34.0 (25.1, 47.4)	0.34

Table 2.5.1 (continued)

Body mass index, kg/m^2	16.6 (13.8, 23.0)	17.0 (13.8, 22.5)	0.11
Body surface area, m^2	1.15 (0.96, 1.46)	1.16 (0.95, 1.42)	0.43
Systolic blood pressure, mmHg	102 (7)	104 (7.7)	<0.01
Diastolic blood pressure, mmHg	57 (6)	58 (6)	<0.01

Table 2.5.2 Childhood cardiac outcomes (N=547)

	Boys	**Girls**	**P-value**
Right cardiac outcomes			
Right ventricular end diastolic volume, ml	105.6 (18.7)	95.8 (17.1)	<0.01
Right ventricular ejection fraction, %	58.0 (4.9)	60.0 (5.0)	<0.01
Left cardiac outcomes			
Left ventricular end diastolic volume, ml	104.8 (16.5)	96.7 (14.9)	<0.01
Left ventricular ejection fraction, %	58.3 (4.6)	59.3 (4.5)	0.01
Left ventricular mass, grams	50.6 (9.9)	46.5 (10.0)	<0.01
Left ventricular mass-to-volume ratio, grams/ml	0.49 (0.08)	0.48 (0.08)	0.60

Values represent means (SD). Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests. Cardiac outcomes standardized on body surface area are shown in **Table S2.5.1**

Fetal hemodynamics and left and right cardiac outcomes

Table 2.5.3 shows that a 1-SDS increase in third trimester UA PI was associated with higher childhood RVEF (0.11 SDS (95% CI 0.02, 0.20)). UA PI was not associated with any of the other cardiac outcomes. The U/C ratio was not associated with childhood cardiac outcomes.

Table 2.5.4 shows that right cardiac output and pulmonary artery PSV were not associated with childhood right ventricular outcomes. However, a 1-SDS higher fetal tricuspid valve E/A ratio, reflecting a less stiff and more compliant ventricle, was associated with higher childhood RVEF (0.09 (95% CI 0.00, 0.17)).

Table 2.5.5 shows that a higher fetal left cardiac output was associated with lower childhood LVEF (-0.15 (95% CI -0.24, -0.05)) and higher LMVR (0.11 (95% CI 0.01, 0.20)). Aorta ascendens PSV and mitral valve E/A wave were not associated with childhood left ventricular outcomes.

Table 2.5.3 Associations of fetal blood flow redistribution with childhood right and left cardiac outcomes (N=547)

	Childhood cardiac outcomes (SDS)	
Fetal blood flow redistribution exposures (SDS)	**Umbilical artery PI**	**Umbilical/ cerebral ratio**
Right ventricular end-diastolic volume	-0.05 (-0.13, 0.02)	-0.06 (-0.13, 0.02)
Right ventricular ejection fraction	0.11 (0.02, 0.20)*	0.08 (-0.02, 0.17)
Left ventricular end-diastolic volume	-0.03 (-0.10, 0.04)	-0.05 (-0.13, 0.02)
Left ventricular ejection fraction	0.06 (-0.03, 0.15)	0.09 (-0.00, 0.18)
Left ventricular mass	-0.04 (-0.12, 0.04)	-0.01 (-0.09, 0.07)
Left ventricular mass-to-volume ratio	-0.01 (-0.09, 0.08)	0.04 (-0.06, 0.13)

Abbreviations: SDS standard deviation score; PI pulsatility index.
Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood right and left ventricular outcomes in SDS per SDS change in fetal blood flow redistribution exposures. Cardiac measures at 10 years are standardized on body surface area (except left ventricular mass-to-volume ratio). Model is adjusted for gestational age at 3rd trimester measurement, child sex, current age, and time difference between measurement of BSA and MRI. *p<0.05

Table 2.5.4 Associations of right sided fetal hemodynamics on childhood right ventricular outcomes (N=547)

	Childhood right ventricular outcomes (SDS)	
Fetal right cardiac blood flow exposures (SDS)	**Right ventricular end-diastolic volume**	**Right ventricular ejection fraction**
Right cardiac output	-0.03 (-0.11, 0.06)	0.06 (-0.04, 0.16)
Pulmonary artery PSV	-0.03 (-0.11, 0.04)	0.04 (-0.05, 0.14)
Tricuspid valve E/A wave	-0.02 (-0.09, 0.06)	0.09 (0.00, 0.17)*

Abbreviations: SDS standard deviation score; PSV peak systolic velocity.
Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood right ventricular outcomes in SDS per SDS change in cardiac blood flow exposures. Cardiac measures at 10 years are standardized on body surface area. Model is adjusted for gestational age at 3rd trimester measurement, child sex, current age, and time difference between measurement of BSA and MRI.
*p<0.05

Table 2.5.5 Associations of left sided fetal hemodynamics on childhood left ventricular outcomes (N=547)

	Childhood left ventricular outcomes (SDS)			
Fetal left cardiac blood flow exposures (SDS)	**Left ventricular end-diastolic volume**	**Left ventricular ejection fraction**	**Left ventricular mass**	**Left ventricular mass-to-volume ratio**
Left cardiac output	0.00 (-0.08, 0.08)	-0.15 (-0.24, -0.05)**	0.09 (0.00, 0.17)	0.11 (0.01, 0.20)*
Aorta ascendens PSV	-0.07 (-0.14, 0.01)	-0.05 (-0.14, 0.04)	-0.01 (-0.09, 0.07)	0.04 (-0.05, 0.13)
Mitral valve E/A wave	-0.01 (-0.07, 0.06)	0.02 (-0.06,0.10)	0.06 (-0.02, 0.13)	0.06 (-0.03, 0.14)

Abbreviations: SDS standard deviation score; PSV peak systolic velocity.
Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood left ventricular outcomes in SDS per SDS change in fetal cardiac blood flow exposures. Cardiac measures at 10 years are standardized on body surface area (except left ventricular mass-to-volume ratio).
Model is adjusted for gestational age at 3rd trimester measurement, child sex, current age, and time difference between measurement of BSA and MRI. *p<0.05;**p<0.01

DISCUSSION

In this prospective cohort study, we observed that a higher UA PI, reflecting increased arterial resistance, was associated with higher childhood RVEF. A lower fetal tricuspid valve E/A ratio, indicating a stiffer and less compliant ventricle, was associated with a lower childhood RVEF. Higher fetal left cardiac output was associated with lower childhood LVEF and higher childhood LMVR.

Interpretation of main findings

Changes in fetal hemodynamics in response to an adverse fetal environment may lead to cardiovascular adaptations and subsequent risk of cardiovascular disease in later life.[11] We previously reported that among 1,215 fetuses decreased fetal growth was associated with a higher afterload and lower vascular compliance, even before the apparent stage of fetal growth restriction.[147] These adaptations might lead to cardiac changes in structure and function in later life. Also, in the same cohort it was reported that a higher UA PI was associated with lower aortic root diameter at 2 years and with lower LVM at 6 years.[60, 148] Increased fetal left cardiac output was associated with larger LVM and atrial diameter at 2 years and with larger aortic root diameter at 6 years.[60, 148] Another study among 200 children aged 3-6 years, observed that small for gestational age children have a more globular heart, impaired relaxation and increased blood pressure and intima-media thickness.[149] Thus, previous studies suggest that fetal growth is associated with fetal blood flow alterations and that these changes might be associated with left cardiac structural and functional outcomes in childhood. Another study in preterm born young adults showed that changes in right ventricular structure and function were more pronounced in the right ventricle than in the left.[31] Thus far, no studies on the long term effects of fetal hemodynamics have been performed in children aged older than 6 years. Also, no information is available on structural developmental adaptations of the right ventricle.

In the current study, we did not find associations of fetal UA PI with LVM at the age of 10 years. Possibly, the previously observed association of UA PI with LVM at 6 years is transient and disappears at older age. In the current study, we did observe an association of UA PI with childhood cardiac function. Previous research in children with fetal growth restriction showed reduced longitudinal motion and impaired relaxation, but no difference in ejection fraction.[21] We observed that a higher UA PI was associated with higher RVEF. These findings may suggest that a higher umbilical artery resistance, reflecting a higher fetal right ventricular

afterload, is associated with higher childhood RVEF. A study in growth restricted fetuses observed an increased venous return in the superior vena cava, as a result of increased cerebral flow in brainsparing.[150] This might also affect right ventricular function. However, we did not observe associations of U/C ratio or right cardiac output with childhood RVEF. Further studies are needed to replicate these findings. We also hypothesized that since a higher U/C ratio, reflecting brain sparing, is associated with lower cardiac output in fetal life, it would also be associated with reduced childhood right and left ejection fraction.[143] However, in the current study we did not observe any associations of U/C ratio with childhood cardiac structure or function, independent of current BSA.

Reduced fetal growth across the full range is associated with lower fetal right and left cardiac output, aorta ascendens and pulmonary artery PSV and possibly with lower E/A ratio's.[12, 143, 146, 147] To the best of our knowledge, there are no other studies on the associations of fetal cardiac output with childhood cardiac outcomes. We observed that higher fetal left cardiac output was associated with lower childhood LVEF and higher LMVR, but not with LVEDV or LVM. We also observed that lower fetal tricuspid valve E/A ratio was associated with lower RVEF. A lower E/A ratio indicates a less compliant ventricle and therefore worse diastolic function. Interestingly, in our study the lower diastolic function in fetal life was associated with lower childhood ejection fraction, an indicator of systolic function. Unfortunately, we did not have childhood diastolic cardiac filling measures. We did not observe associations of mitral valve E/A ratio with left cardiac structure or function at the age of 10 years.

Most of the associations we observed could fit the hypothesis that changes in fetal hemodynamics could be associated with cardiac structure and function in later life, putting the individual at risk for cardiovascular disease. The observed associations could reflect chance findings. We did not take into account multiple testing because our different exposures and outcomes are strongly correlated, and adjusting for multiple testing might be too strict. Also, we studied women with relatively healthy pregnancies. It might be possible that in more compromised pregnancies fetal hemodynamic adaptations influence later cardiac structure and function more strongly.

Methodological considerations

The main strength of this study is the prospective design from early fetal life onwards, in a large cohort of children. To our knowledge, this is the first study examining the effects of fetal hemodynamics on cardiac outcomes, measured by cMRI in childhood. MRI is a more precise and

accurate tool than ultrasound and CT to study cardiac structure.[29, 151] For the current sub group study, follow up was available in 46 %. Missing cardiac MRI scans were mostly because of later start of these measurements during the follow up visits, poor quality cardiac MRI scans were often caused by logistical or participant constraints. This loss to follow up could lead to bias if the associations of fetal hemodynamics with cardiac measures differ between those included and not included in the analyses. However, we deem this unlikely since non-response analysis showed no differences in fetal hemodynamics or childhood anthropometrics (**Table S2.5.3**). We have standardized our outcomes on BSA, to take into account current body size. The majority of the children visited the MRI within two months after anthropometrics were taken. A small proportion of children was invited to the MRI at a later age. For these children, the BSA we calculated might underestimate current body size, leading to increased z-scores for the cardiac outcomes. We adjusted all our analyses for the time difference between the BSA measurement and the MRI visit, but this measurement error could still lead to an attenuation of the effect estimates we observed. Despite the fact that we tested and adjusted for confounders, residual confounding might be of concern, as in any other observational study.

CONCLUSION

We observed associations of increased placental resistance and fetal hemodynamics with childhood cardiac structure and function. Our findings suggest that cardiac fetal blood flow redistribution may have long term effects on cardiac structure and function. These results should be considered as hypothesis generating and need further replication.

SUPPLEMENTAL MATERIAL

Figure S2.5.1 Flow chart of the study population

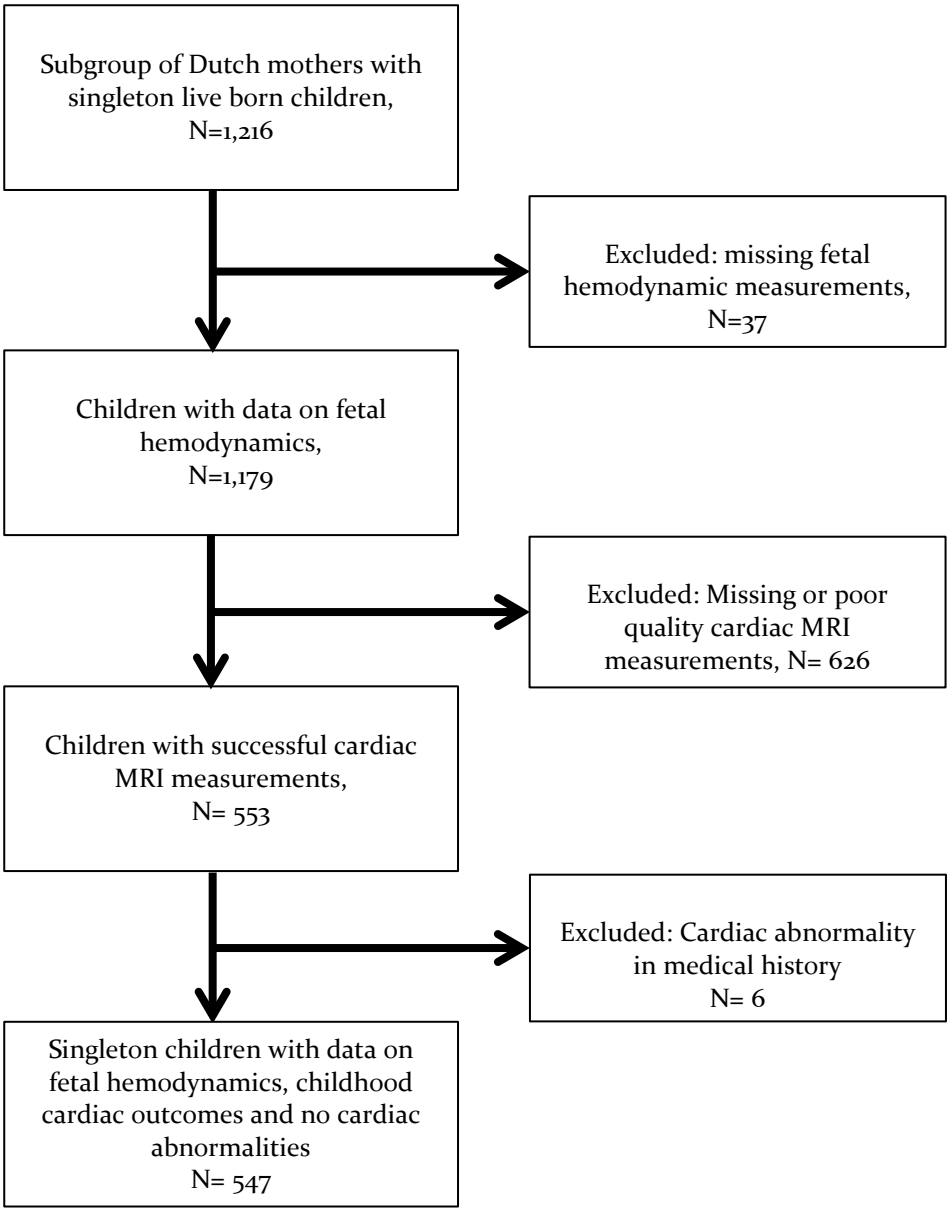
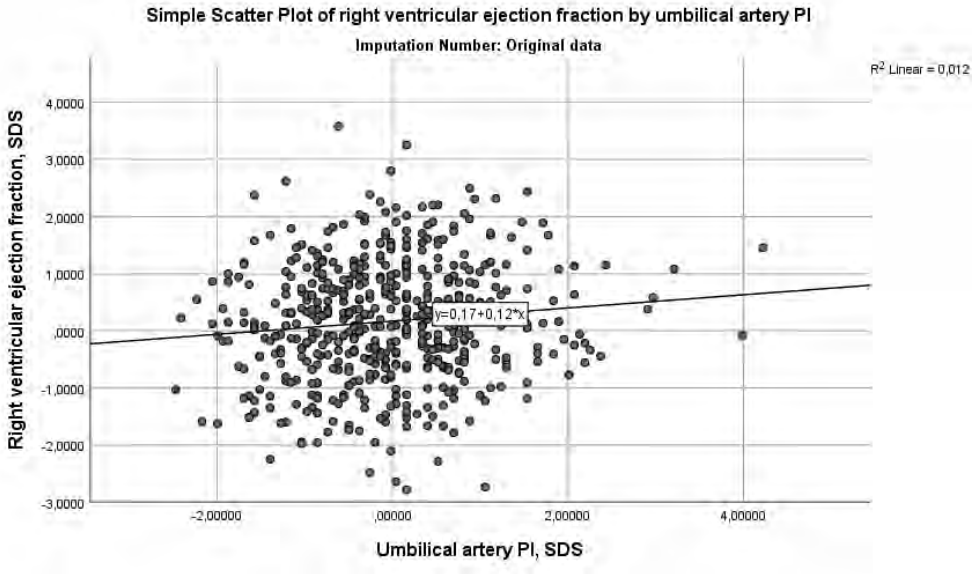


Figure S2.5.2 Correlation scatter plot of right ventricular ejection fraction by umbilical artery PI



Abbreviations: SDS Standard Deviation Score; PI pulsatility index.

Table S2.5.1 Childhood cardiac outcomes standardized on body surface area (N=547)

	Boys	Girls	P-value
Right cardiac outcomes			
Right ventricular end diastolic volume, SDS	0.37 (0.91)	-0.26 (0.89)	<0.01
Right ventricular ejection fraction, SDS	-0.05 (0.99)	0.36 (1.01)	<0.01
Left cardiac outcomes			
Left ventricular end diastolic volume, SDS	0.35 (0.89)	-0.26 (0.86)	<0.01
Left ventricular ejection fraction, SDS	-0.02 (1.01)	0.19 (0.98)	0.14
Left ventricular mass, SDS	0.24 (0.91)	-0.33 (1.04)	<0.01
Left ventricular mass-to-volume ratio, SDS	0.02 (0.96)	-0.02 (1.04)	0.60

Abbreviations: SDS Standard Deviation Score; SD standard deviation; Values represent mean SDS (SD). Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests. Since left ventricular mass-to-volume ratio is not usually standardized on body surface area, we created standard deviation scores as (observed value-mean)/SD.

Table S2.5.2 Correlation coefficients between placental vascular resistance, fetal cardiac hemodynamics and childhood cardiac outcomes

	Childhood cardiac outcomes (SDS)					
	RVEDV	**RVEF**	**LVEDV**	**LVEF**	**LVM**	**LMVR**
Placental vascular resistance (SDS)						
Umbilical artery PI	-0.10*	0.11*	-0.08	0.06	-0.09*	-0.03
Umbilical/middle cerebral artery ratio	-0.08	0.07	-0.08	0.08	-0.04	0.02
Fetal cardiac hemodynamics (SDS)						
Right cardiac output	0.02	0.07	0.10*	0.00	0.25**	0.20**
Pulmonary artery PSV	-0.02	-0.06	0.02	-0.01	0.09	0.08
Tricuspid valve E/A ratio	-0.02	0.10*	-0.03	0.08	0.03	0.06
Left cardiac output	0.01	0.01	0.09	-0.12**	0.19**	0.14**
Aorta ascendens PSV	-0.07	0.04	0.03	-0.05	0.03	0.04
Mitral valve E/A ratio	-0.04	0.06	-0.02	0.04	0.04	0.05
Childhood cardiac outcomes (SDS)						
RVEDV	1	-0.34**	0.86**	0.04	0.44**	-0.22**
RVEF		1	-0.15**	0.62**	-0.07	0.05
LVEDV			1	-0.13**	0.50**	-0.28**
LVEF				1	-0.01	0.09**
LVM					1	0.68**
LMVR						1

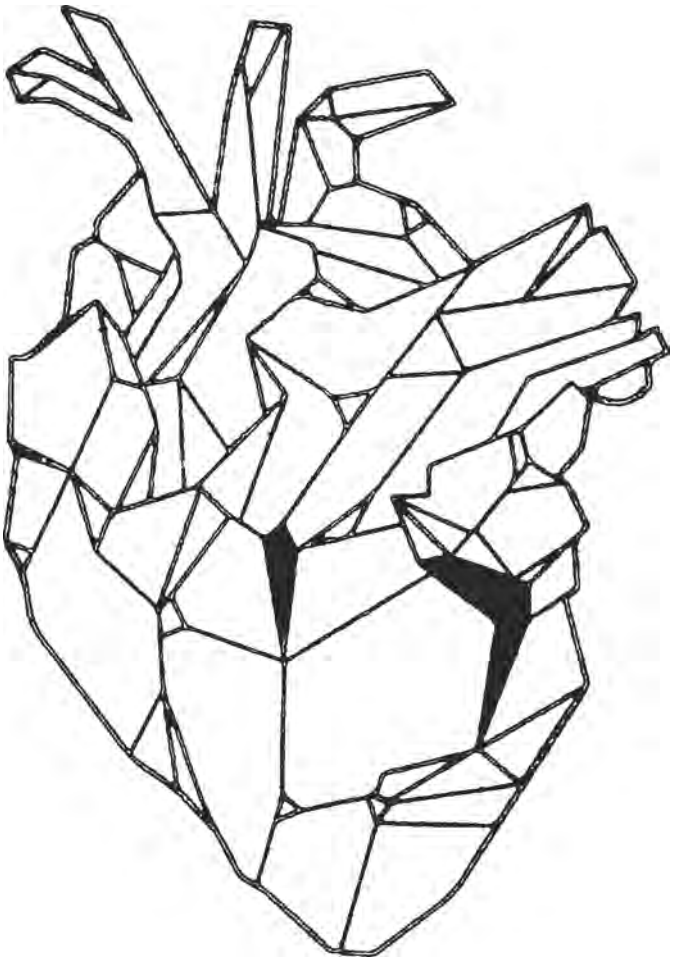
Abbreviations: SDS standard deviation scores; PI pulsatility index; PSV peak systolic velocity; RVEDV right ventricular end diastolic volume; RVEF right ventricular ejection fraction ; LVEDV left ventricular end diastolic volume; LVEF left ventricular ejection fraction; LVM left ventricular mass; LMVR left ventricular mass-to-volume ratio. Values represent Pearson correlation coefficients. Cardiac measures at 10 years are standardized on body surface area (except LMVR). * P-value<0.05; ** P-value<0.01

Table S2.5.3 Non-response analysis (N=1,179)

	Children without cardiac MRI (n=632)	**Children with cardiac MRI (n=547)**	**P value**
Maternal characteristics			
Age, years	31.1 (4.4)	32.1 (3.7)	<0.01
Prepregnancy BMI, kg/m^2	22.3 (18.4, 34.4)	22.6 (18.6, 34.6)	0.52
Folic acid intake during pregnancy, start periconceptional, n (%)	298 (57)	300 (67)	<0.01
Never smoked during pregnancy, n (%)	417 (72)	392 (79)	0.02
High education level, n (%)	390 (66)	388 (76)	<0.01
Third trimester fetal measurements			
Gestational age at measurement, weeks	30.3 (28.5, 32.6)	30.4 (28.4, 32.7)	0.12
Estimated fetal weight, grams	1601 (1155, 2235)	1618 (1181, 2229)	0.42
Vascular resistance parameters			
Umbilical artery PI	0.98 (0.17)	0.96 (0.16)	0.10
Umbilical/middle cerebral artery ratio	0.51 (0.12)	0.50 (0.11)	0.24
Fetal cardiac hemodynamics			
Right cardiac output, ml/min	850 (259)	825 (234)	0.11
Pulmonary artery PSV, cm/s	73.9 (9.4)	73.4 (9.5)	0.39
Tricuspid valve E/A ratio	0.77 (0.08)	0.78 (0.09)	0.74
Left cardiac output, ml/min	608 (179)	606 (170)	0.84
Aorta ascendens PSV, cm/s	91.4 (12.4)	91.1 (12.3)	0.64
Mitral valve E/A ratio	0.78 (0.10)	0.78 (0.10)	0.46
Birth characteristics			
Gestational age at birth, weeks	40.1 (34.9, 42.4)	40.3 (37.0, 42.4)	0.07
Birth weight, grams	3485 (573)	3544 (491)	0.06
Breastfeeding, never, n (%)	69 (12)	45 (8)	0.04
Childhood characteristics			
Age at follow up, years	10.0 (9.2, 11.7)	10.0 (9.4, 11.7)	0.41
Height, cm	142.7 (6.5)	142.2 (6.3)	0.23
Weight, kg	33.6 (26.2, 50.1)	34.0 (25.3, 48.7)	0.77
Body mass index, kg/m^2	16.6 (14.0, 22.5)	16.8 (13.8, 22.6)	0.20
Body surface area, m^2	1.15 (0.98, 1.45)	1.15 (0.96, 1.44)	0.99
Systolic blood pressure, mmHg	103.2 (7.9)	102.9 (7.5)	0.60
Diastolic blood pressure, mmHg	58.3 (6.3)	57.9 (6.3)	0.33

Abbreviations: n; number; SD standard deviation; BPM beats per minute; PI pulsatility index; PSV peak systolic velocity. Values represent means (SD), medians (95% range) or numbers of subjects (valid %) and are based on original data. Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests or Kruskal-Wallis tests for continuous variables and Chi-square tests for proportions.

Chapter 3 | Adiposity



Chapter 3.1

Maternal obesity, gestational weight gain and childhood cardiac outcomes: role of childhood body mass index

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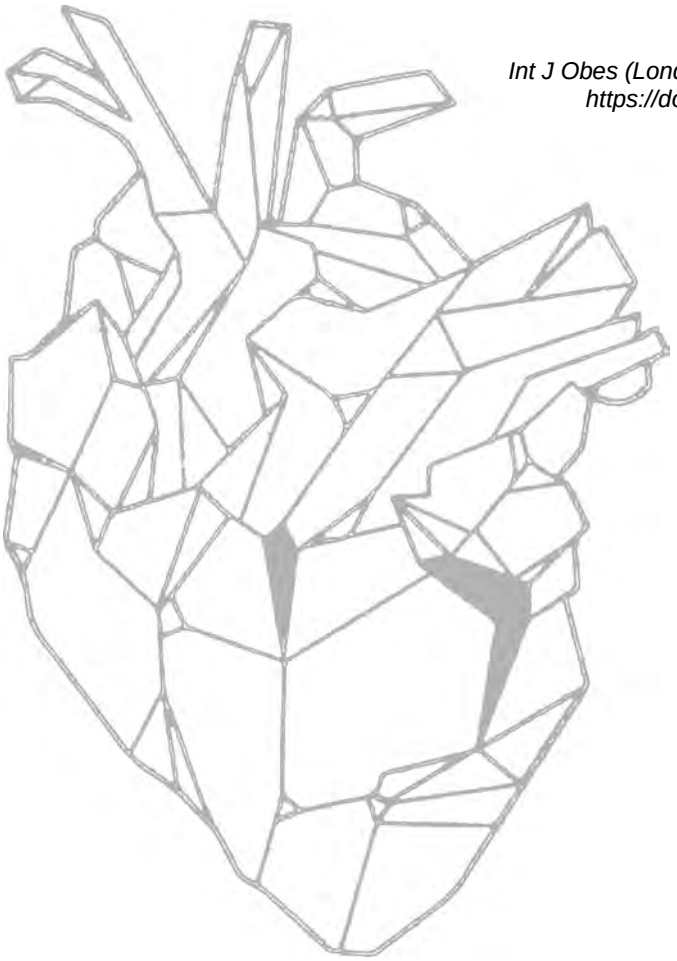


ABSTRACT

Background Maternal obesity may affect cardiovascular outcomes in the offspring. We examined the associations of maternal prepregnancy body mass index and gestational weight gain with childhood cardiac outcomes and explored whether these associations were explained by parental characteristics, infant characteristics, or childhood body mass index.
Methods In a population-based prospective cohort study among 4,852 parents and their children, we obtained maternal weight before pregnancy and in early-, mid- and late-pregnancy. At age 6 years, we measured aortic root diameter (cm) and left ventricular dimensions. We calculated left ventricular mass (g), left ventricular mass index ($g/m^{2.7}$), relative wall thickness ((2*left ventricular posterior wall thickness)/left ventricular diameter), fractional shorting (%), eccentric left ventricular hypertrophy and concentric remodeling.
Results A one standard deviation score (SDS) higher maternal prepregnancy body mass index was associated with higher left ventricular mass (0.10 SDS (95% Confidence Interval (CI) 0.08, 0.13)), left ventricular mass index (0.06 SDS (95% CI 0.03, 0.09)) and aortic root diameter (0.09 SDS (95% CI 0.06, 0.12)), but not with relative wall thickness or fractional shortening. A one SDS higher maternal prepregnancy body mass index was associated with an increased risk of eccentric left ventricular hypertrophy (odds ratio 1.21 (95% CI 1.03, 1.41)), but not of concentric remodeling. When analyzing the effects of maternal weight in different periods simultaneously, only maternal prepregnancy weight and early pregnancy weight were associated with left ventricular mass, left ventricular mass index and aortic root diameter (p-values<0.05), independent of weight in other pregnancy periods. All observed associations were independent of parental and infant characteristics, but attenuated to non-significance after adjustment for childhood body mass index.
Conclusion Maternal prepregnancy body mass index and weight gain in early pregnancy are both associated with offspring cardiac structure in childhood, but these associations seem to be fully explained by childhood body mass index.

INTRODUCTION

Left ventricular hypertrophy and concentricity are associated with cardiovascular events.[28] Left ventricular mass tracks from childhood to adulthood, suggesting that the risk of cardiac diseases is at least partly established in early life.[18] During fetal life, the heart grows due to hyperplasia of cardiomyocytes, whereas after birth heart growth is mainly due to hypertrophic growth.[7] Factors in early life, such as maternal smoking, preterm birth and low birth weight are associated with left ventricular structure and function in childhood.[43, 104, 152]. Therefore, fetal life may be a critical period for heart development. This programming may put an individual at risk for adverse health outcomes.[153] Maternal obesity and excessive gestational weight gain are the most prevalent adverse fetal life exposures in Western countries and are associated with a number of cardiovascular risk factors in the offspring.[154] [48] Maternal obesity is associated with an increased risk of congenital heart disease in the offspring.[155, 156] Animals studies in mice have shown that male offspring of obese dams develop cardiac hypertrophy and cardiac dysfunction, independent of change in offspring body weight or diet.[157] The underlying mechanism is suggested to be the mitogenic action of increased insulin on cardiomyocytes.[158] Human studies show that maternal obesity during pregnancy is associated with increased inter-ventricular septum thickness in the fetus.[159] Maternal weight gain in pregnancy is also positively associated with left ventricular mass in infant offspring.[160] Not much is known about the cardiac outcomes at later age in children from mothers with obesity or excessive weight gain.[48]

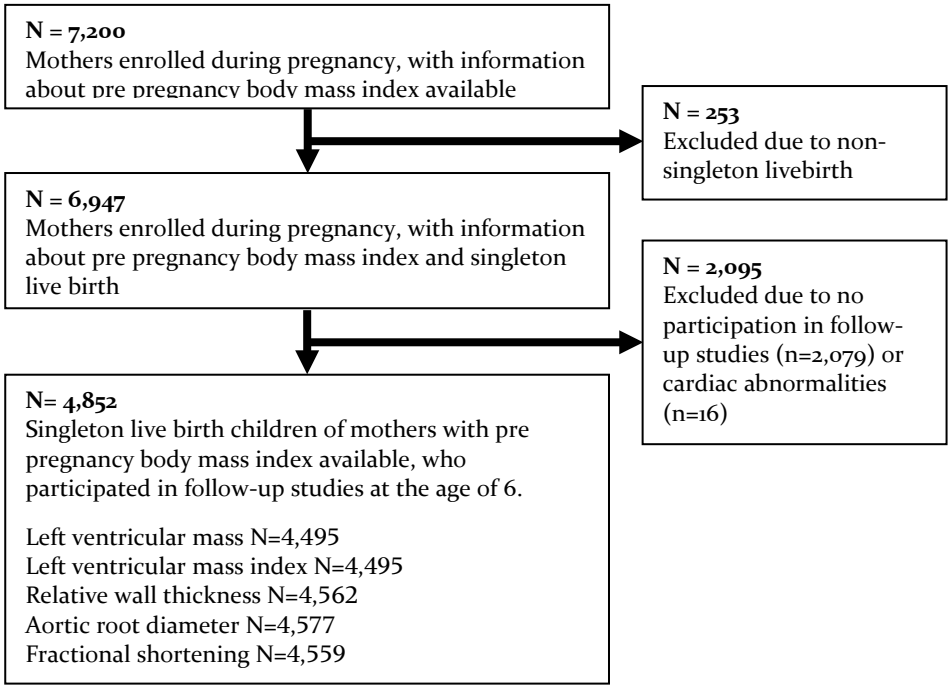
Therefore, in a population-based prospective cohort study among 4,868 children and their parents, we examined the associations of maternal and paternal prepregnancy body mass index (BMI) and maternal weight gain in different periods of pregnancy with childhood cardiac structure and function. We also explored whether these associations are explained by parental characteristics, infant characteristics, or childhood BMI. We compared the effects of maternal BMI and paternal BMI, to help distinguish between the effect of in utero-programming of maternal obesity on cardiac development and the effect of other factors such as shared family-based, lifestyle-related or genetic factors.

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[64] Details of this study have been described previously.[64] In total 7,200 mothers had information about prepregnancy BMI available. We only included the 6,947 of these who had singleton live births. Missing information on maternal prepregnancy BMI was mainly due to later enrollment in the study or non-participation in the first questionnaire. A total of 2,079 children did not participate in cardiac follow-up studies at the age of 6 years. We further excluded 16 children with cardiac abnormalities. The population for analysis included 4,852 children and their parents (flow chart **Figure 3.1.1**). **Table S3.1.1** shows that mothers without offspring follow-up data were more likely to be lower educated, whereas the children without follow-up data were more often from non-European descent, and had lower birth weight. Missing follow-up data is mainly due to no consent for follow up studies at the age of 6 years. Written informed consent was obtained from all parents of participants. The study has been approved by the local Medical Ethics Committee.

Figure 3.1.1 Flow chart of participants included in the analysis



As described previously, maternal and paternal height (cm) and weight (kg) were measured at enrollment, BMI was calculated (kg/m^2).[161] Information about maternal weight before pregnancy was obtained by questionnaire. Prepregnancy BMI was categorized in 4 categories: underweight (<18.5 kg/m^2); normal weight (18.5-24.9 kg/m^2); overweight (25-29.9 kg/m^2); and obese (≥30 kg/m^2). Maternal weight was assessed in early (median 13.4 weeks (95% range 9.9-18.9), mid (median 29.9 weeks (95% range 20.5-31.4) and late (median 39.0 (95% range 32.8-42.0) pregnancy. These periods were defined based on data collection in our cohort and time of self-reported maximum weight.[162] Gestational weight gain until a gestational age of 30 weeks (median 30.2 (95% range 28.5-32.9)) was measured and available for 4,710 women. Information about maximum weight during pregnancy was assessed by questionnaire two months after delivery in a subgroup of 2,638 mothers.[162] Maximum weight from questionnaire two months after delivery and weight measured at 30 weeks were strongly correlated (r = 0.96 (p <0.011)).

Childhood cardiac outcomes

All children were invited to participate in detailed follow up measurements at the age of 6 years.[163] We performed M-mode echocardiographic measurements using methods recommended by the American Society of Echocardiography. We measured aortic root diameter (AOD), left ventricular end-diastolic diameter (LVDD), left ventricular posterior wall thickness (LVPWT) and interventricular septum thickness (IVS). Intraobserver and interobserver intraclass correlation coefficients were calculated previously in 28 children with a median age of 7.5 years, (interquartile range 3.0 - 11.0) and varied between 0.91 to 0.99 and 0.78 to 0.96, respectively.[66] We calculated fractional shortening (FS) and left ventricular mass (LVM), using the formula derived by Devereux et al.[65, 67] To account for differing body sizes, we additionally calculated left ventricular mass index (LVMI) as left ventricular mass /height$^{2.7}$. (ref. [164]) To assess left ventricular concentricity, we calculated relative wall thickness (RWT) as (2*LVPWT)/LVDD, which was subsequently indexed to age (RWT-0.0062*(age in years-10)).[165, 166] The presence of left ventricular hypertrophy (LVH) was defined as increased LVMi, based on the 95% cut off by Khoury et al. (corresponding values: 44.6 g/m$^{2.7}$ for boys and 43.5g/m$^{2.7}$ for girls aged 6-8).[164] Concentricity was defined as increased RWT values (cut off >0.390 in both boys and girls).[166] Since the predictive value of LVH and concentricity for cardiovascular outcomes in adults are higher when these are combined, we defined three different geometric patterns: 1) normal geometry (no LVH and no concentricity); 2) eccentric

LVH (LVH present, but no concentricity), and concentric remodeling (no LVH, but concentricity present).[28]

Covariates

We obtained information about maternal age, education level, folic acid use, alcohol consumption and paternal age and education level from questionnaires.[64] First-trimester total calorie intake was obtained by food frequency questionnaire.[167] Child's ethnicity was classified by the countries of birth of the parents.[58] At birth, information on infant sex, date of birth and weight was obtained from community midwife and hospital registries. We created gestational age- and sex- adjusted birth weight standard deviation score (SDS) by using Growth Analyzer 3.5 (Dutch Growth Research Foundation, Rotterdam, the Netherlands) based on North-European reference standards.[84] Breast-feeding was assessed using questionnaires. Infant weight was measured at community health centers according to standardized procedures at 24 months. Infant growth was defined as the difference in weight SDS between birth and two years of age. At the age of 6 years, we measured height and weight without shoes and heavy clothing and calculated BMI in our dedicated research center. We obtained sex- and age-specific SDS based on Dutch reference growth curves.[86] Maternal total weight gain was defined as the difference in weight between weight measured at 30 weeks gestational age and self-reported weight before pregnancy.

Statistical analyses

First, differences in subject characteristics between maternal prepregnancy BMI categories were examined with 1-way ANOVA tests and χ^2 tests. We examined the associations of maternal and paternal prepregnancy BMI singularly and simultaneously with childhood cardiac outcomes (LVM, LVMi, RWT, AOD, FS) in 3 linear regression models: (1) a basic model including child's age and sex; (2) a confounder model additionally including covariates selected on the basis of their associations with the outcomes of interest based on previous study results or a change in effect estimate >10%; and (3) three mediator models, which additionally included either maternal weight gain, infant characteristics (birth weight, gestational age at birth and infant growth), or childhood BMI. Third, we examined the associations of maternal and paternal prepregnancy BMI with eccentric LVH or concentric remodeling using logistic regression models with the same adjustments. Finally, we performed conditional regression analyses to identify independent associations of maternal weight gain in different periods with cardiac outcomes (LVM, LVMi, RWT, AOD,

FS).[71] Conditional analyses take into account the correlations between maternal prepregnancy weight and weight gain in different periods.[162] We constructed maternal weight gain variables for each period, which are statistically independent from each other, by using standardized residuals obtained from regression of maternal weight at a specific time point on prior maternal weight measurements.[71, 162] For all analyses, we constructed standard deviation scores (SDS) values ((observed value – mean)/SD) of determinants and outcomes to enable comparison of effect estimates. We did not construct SDS curves based on body surface area (BSA), because we aimed to explore the effect of current body size in the mediator models. We examined potential statistical interactions between maternal and paternal BMI and child's sex and with birth weight for these associations, but no interaction was present. Missing data of covariates and maternal gestational weight (for conditional analyses only) were imputed using multiple imputations, five datasets were created and analyzed together.[99] All analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Subject characteristics

Table 3.1.1 shows the subject characteristics according to maternal BMI category. Children of mothers with overweight and obesity had a higher birth weight, were less often breastfed, and had a higher BMI, LVM and AOD, compared to children from mothers with normal weight.

Parental body mass index and childhood cardiac outcomes

Table 3.1.2 shows that in the confounder models a one SDS higher maternal BMI was associated with a 0.10 SDS (95% Confidence Interval (CI) 0.08, 0.13) increase in LVM. Maternal BMI was also positively associated with LVMi (0.06 SDS increase (95% CI 0.03, 0.09)) and AOD (0.09 SDS increase (95% CI 0.06, 0.12)). The different mediator models showed that the associations of prepregnancy BMI with cardiac outcomes were not explained by gestational weight gain, but attenuated slightly after adjustment for infant characteristics. When we adjusted the associations of maternal BMI with LVM, LVMi and AOD for childhood BMI SDS, all effect estimates attenuated and lost significance. Maternal BMI was not associated with RWT or FS. Paternal BMI was associated with increase in LVM, LVMi and AOD, but not with RWT or FS. Effect estimates were comparable with those of maternal BMI (**Table S3.1.2**). Similarly as for

maternal BMI, the associations of paternal BMI with LVM, LVMi and AOD attenuated and lost significance after adjustment for childhood BMI SDS.

Table 3.1.3 shows that a one SDS higher maternal BMI was associated with increased odds for eccentric LVH (Odds Ratio (OR) 1.21 (95% CI 1.03, 1.41)), but not concentric remodeling (OR 0.95 (95% CI 0.85, 1.06). The associations were not explained by gestational weight gain, attenuated only slightly after adjustment for infant characteristics, but attenuated and lost significance after adjustment for childhood BMI SDS. The effect estimates of paternal BMI with these cardiac outcomes were comparable to those for maternal BMI (**Table S3.1.3**).

Gestational weight gain in different periods of pregnancy

Figure 3.1.2 shows the independent associations of maternal prepregnancy weight and gestational weight gain in early-, mid- and late-pregnancy with cardiac outcomes from conditional analyses. Maternal prepregnancy weight was associated with LVM, LVMi and AOD but not with RWT or FS (all p-values <0.05). Also, gestational weight gain in early pregnancy, independent from prepregnancy weight or weight gain in mid- or late-pregnancy was associated with LVM, LVMi, AOD and FS (all p-values <0.05). Weight gain in late pregnancy was also associated with LVM, but not with LVMi. We did not observe associations of gestational weight gain in mid- or late-pregnancy with other cardiac outcomes. Associations of prepregnancy weight and gestational weight gain in early pregnancy were explained by childhood BMI SDS, except the association of weight gain in early pregnancy with FS, which remained the same. Results from the normal linear regression models focused on the associations of weight gain in different periods of pregnancy and childhood cardiac outcomes are shown in **Table S3.1.4.** Weight gain in early pregnancy was associated with LVM, LVMi, AOD and FS, but not with RWT. The associations were largely explained by childhood BMI SDS.

Table 3.1.1 Characteristics of mothers, fathers, and their children (N=4,852)

Characteristics	N	Total group N=4,852	Maternal underweight N=189	Maternal normal weight N=3,319	Maternal overweight N=942	Maternal obesity N=402	P-value
Maternal characteristics							
Age, median (95% range), yrs	4852	30.9 (19.9, 39.4)	28.3 (18.0, 38.4)	31.1 (19.8, 39.5)	30.8 (20.7, 39.5)	30.3 (20.5, 39.4)	<0.01
Education, N. higher education (%)	4731	2193 (46.4)	72 (38.7)	1700 (52.2)	337 (37.0)	84 (22.2)	<0.01
Parity, N. nulliparous (%)	4852	2832 (58.2)	121 (64.0)	2052 (61.8)	470 (49.9)	180 (44.8)	<0.01
Total calorie intake, mean (SD), kcal	3859	2052 (551)	2046 (565)	2085 (546)	1996 (540)	1908 (583)	<0.01
Folic acid supplements use, N. Yes (%)	4852	3847 (79.3)	153 (81.0)	2710 (81.7)	702 (74.5)	282 (70.1)	<0.01
Alcohol consumption during pregnancy, N. Yes (%)	4852	2129 (43.9)	89 (47.1)	1315 (39.6)	475 (50.4)	250 (62.2)	<0.01
BMI, mean (SD), kg/m^2	4852	23.6 (4.2)	17.7 (0.7)	21.7 (1.7)	26.9 (1.4)	33.9 (3.7)	<0.01
Height, mean (SD),cm	4852	168 (7.4)	169 (7.0)	168 (7.2)	167 (7.5)	166 (7.8)	<0.01
Weight, mean (SD), kg							
Prepregnancy	4852	66.5 (12.6)	50.6 (4.8)	61.7 (6.8)	74.7 (7.6)	93.6 (13.4)	<0.01
Early pregnancy	4043	68.9 (12.8)	53.4 (5.7)	64.2 (7.4)	77.4 (8.7)	95.4 (13.4)	<0.01
Mid-pregnancy	4689	75.9 (12.7)	60.8 (6.8)	71.7 (8.4)	83.5 (9.5)	99.7 (14.1)	<0.01
Late-pregnancy	2525	81.2 (12.7)	67.1 (7.1)	77.6 (9.0)	90.4 (10.4)	106.3 (14.7)	<0.01
Paternal characteristics							
Age, median (95% range), yrs	4322	33.2 (22.2, 46.8)	31.7 (20.1, 47.0)	33.3 (22.1, 46.7)	33.1 (22.8, 46.9)	32.9 (23.8, 48.8)	<0.01
Paternal education, N, higher education (%)	3307	1731 (52.3)	64 (56.1)	1326 (55.8)	271 (46.0)	67 (30.0)	<0.01
BMI, mean (SD), kg/m^2	3753	25.3 (3.4)	24.0 (3.1)	25.0 (3.2)	26.1 (3.6)	27.0 (4.5)	<0.01

Table 3.1.1 (continued)

Infant characteristics							
Ethnicity, N. Non-European (%)	4852	1728 (35.6)	79 (41.8)	1013 (30.5)	420 (44.6)	216 (53.7)	<0.01
Males, N. (%)	4852	2434 (50.2)	109 (57.7)	1668 (50.3)	462 (49.0)	195 (48.5)	0.16
Gestational age at birth, median (95% range), wks	4852	40.1 (35.9, 42.3)	39.9 (35.9, 42.0)	40.1 (36.0, 42.3)	40.3 (36.0, 42.4)	39.9 (34.4, 42.4)	<0.01
Birth weight, mean (SD), g	4846	3435 (545)	3180 (483)	3422 (538)	3501 (543)	3500 (588)	<0.01
Ever breastfeeding, N. Yes (%)	3831	3553 (92.7)	130 (92.9)	2499 (93.5)	655 (92.4)	269 (87.3)	<0.01
Childhood characteristics							
Age at follow up, median (95% range), yrs	4852	6.0 (5.6, 8.0)	6.1 (5.7, 8.0)	6.0 (5.6, 7.9)	6.0 (5.6, 7.9)	6.1 (5.6, 8.1)	0.01
BMI, mean (SD), kg/m^2	4850	16.2 (1.9)	15.5 (1.4)	16.0 (1.6)	16.7 (2.0)	17.7 (2.8)	<0.01
Age and sex adjusted BMI SDS, (SD)	4850	0.29 (0.93)	-0.14 (0.84)	0.17 (0.86)	0.50 (0.95)	0.95 (1.10)	<0.01
Left ventricular mass, mean (SD), g	4495	53.4 (11.7)	52.0 (11.9)	52.9 (11.3)	53.9 (12.0)	56.2 (13.2)	<0.01
Left ventricular mass index, mean (SD), g/m$^{2.7}$	4495	32.9 (6.2)	32.6 (6.4)	32.8 (6.1)	33.1 (6.4)	33.7 (6.6)	0.06
Relative wall thickness, mean (SD)	4562	0.3 (0.05)	0.3 (0.05)	0.3 (0.05)	0.3 (0.05)	0.3 (0.05)	0.08
Aortic root diameter, mean (SD), cm	4477	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	2.0 (0.2)	<0.01
Fractional shortening, mean (SD), %	4559	35.3 (4.6)	35.7 (4.8)	35.2 (4.6)	35.3 (4.6)	35.3 (4.7)	0.53
Eccentric LVH, N. (%)	4495	138 (3.1)	5 (3.0)	82 (2.7)	32 (3.7)	19 (5.2)	0.05
Concentric remodelling,N. (%)	4495	428 (9.5)	15 (9.0)	312 (10.1)	66 (7.6)	35 (9.5)	0.18

Abbreviations: Yrs, years; n, number; sd, standard deviation; bmi, body mass index; sds, standard deviation score; lvh, left ventricular hypertrophy. Values represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests or Kruskal-Wallis tests for continuous variables and Chi-square tests for proportions.

Table 3.1.2 Associations of maternal body mass index with childhood cardiac outcomes (N=4,852)

Maternal model	Left ventricular mass (SDS)	Left ventricular mass index (SDS)	Relative wall thickness (SDS)	Aortic root diameter (SDS)	Fractional shortening (SDS)
Basic model[a]	0.08 (0.05, 0.11)**	0.05 (0.02, 0.08)**	0.02 (-0.01, 0.05)	0.08 (0.05, 0.10)**	0.01 (-0.02, 0.04)
Confounder model[b]	0.10 (0.08, 0.13)**	0.06 (0.03, 0.09)**	0.02 (-0.01, 0.05)	0.09 (0.06, 0.12)**	0.00 (-0.03, 0.03)
Mediator models[c]					
Maternal weight gain	0.12 (0.09, 0.15)**	0.07 (0.04, 0.10)**	0.02 (-0.02, 0.05)	0.10 (0.07, 0.13)**	0.01 (-0.02, 0.04)
Infant characteristics	0.04 (0.01, 0.07)**	0.05 (0.02, 0.08)**	0.01 (-0.02, 0.04)	0.04 (0.01, 0.07)**	0.00 (-0.03, 0.03)
Childhood BMI[e]	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.02)	0.00 (-0.03, 0.03)	0.03 (-0.00, 0.06)	-0.01 (-0.04, 0.02)

Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood outcomes in SDS per SDS change in maternal prepregnancy BMI. The effect of paternal BMI is shown in **Table S3.1.2**.

Table 3.1.3 Associations of maternal body mass index with left ventricular hypertrophy (N=4,426)

Maternal model	Eccentric left ventricular hypertrophy (odds ratio) N=138	Concentric remodelling (odds ratio) N=428
Basic model[a]	1.21 (1.04, 1.40)*	0.94 (0.84, 1.04)
Confounder model[b]	1.21 (1.03, 1.41)*	0.95 (0.85, 1.06)
Mediator models[c]		
Maternal weight gain	1.18 (1.00, 1.39)*	0.95 (0.85, 1.06)
Infant characteristics[d]	1.16 (0.99, 1.37)	0.94 (0.84, 1.05)
Childhood BMI[e]	1.04 (0.88, 1.23)	0.92 (0.82, 1.03)

Values are odds ratios (95% confidence interval) from logistic regression models that reflect the risk for a different geometric pattern per SDS change in maternal prepregnancy BMI. Normal geometric pattern was observed in 3,860 children.
Abbreviations: SDS standard deviation score; BMI body mass index;
[a]Basic model is adjusted for child sex and age. [b]Confounder model is additionally adjusted for: maternal age, educational level, alcohol use during pregnancy, folic acid use during pregnancy, total calorie intake during pregnancy, breastfeeding and ethnicity child. [c]Intermediate models are confounder models adjusted for each potential intermediate. [d]Infant characteristics are: birth weight, gestational age at birth and infant growth (change in weight SDS between birth and 24 months). [e]Childhood BMI: adjusted for sex- and age-specific BMI SDS
*P<0.05; **P<0.01.

Figure 3.1.2 Associations of maternal prepregnancy weight and weight gain in each period of pregnancy with childhood cardiac outcomes (N=4,852)

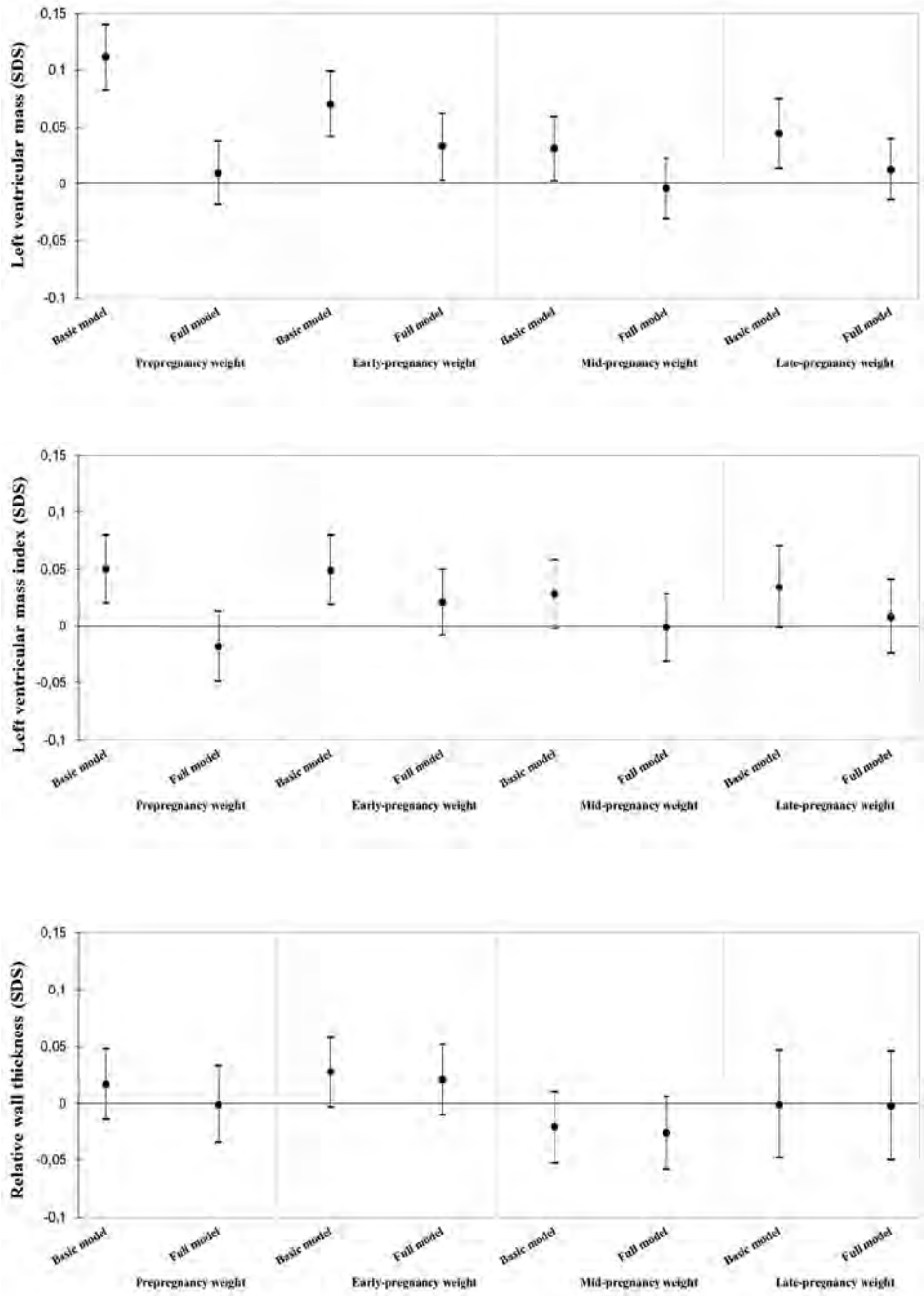
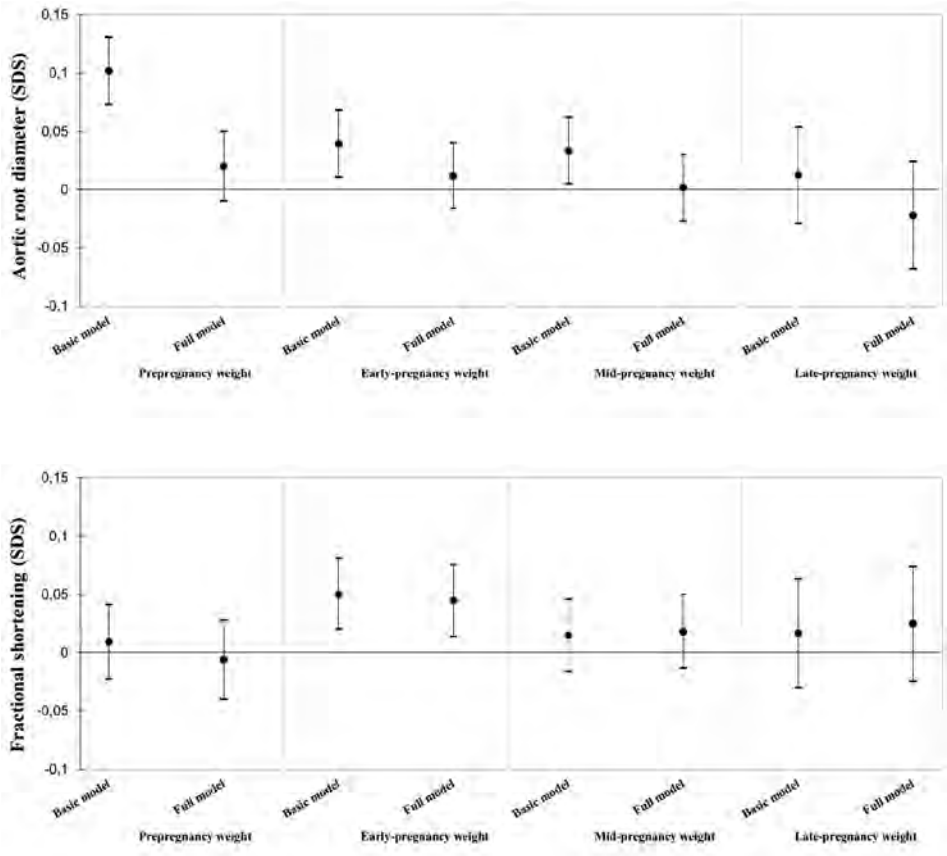


Figure 3.1.2 (continued)



Values are standardized regression coefficients (95% CI) obtained from conditional analyses. The estimates represent differences in cardiac outcomes per standardized residual change of maternal weight gain. Maternal prepregnancy weight is considered as start point. Basic models are adjusted for child age and gender, and for maternal height. Full models are additionally adjusted for age mother, educational level, alcohol use during pregnancy, folic acid use during pregnancy, total calorie intake during pregnancy, breastfeeding, ethnicity child, gestational age at birth, birth weight, infant growth and childhood sex- and age-specific BMI SDS.
Abbreviations: SDS standard deviation score; CI confidence interval; BMI body mass index.

DISCUSSION

We observed that higher maternal prepregnancy BMI and weight gain in early pregnancy are associated with increased offspring LVM, LVMi and AOD in childhood, but not with RWT or FS. Higher maternal BMI also increased the odds for eccentric LVH. Similarly, paternal BMI was

associated with LVM, LVMi, AOD and eccentric LVH. All observed associations were fully explained by childhood BMI SDS.

Methodological considerations

Main strengths of this study are its population-based prospective design starting from pregnancy onwards, the multiple maternal weight measurements throughout pregnancy and the large number of detailed cardiac measurements. Follow up data were available in 68% of our study population. We did not observe differences in BMI of the parents who did and did not participate. However, children who did not participate in follow-up had lower birth weight. The non-response could lead to biased effect estimates if associations of parental BMI with cardiac measurements would be different between children included and not included in our analyses. This cannot be excluded, but seems unlikely. Not all gestational weight measurements were available in all women, due to later enrolment in the study, non-participation in follow up or questionnaires. In order to avoid bias related to complete-case analysis and to maintain statistical power, we used multiple imputations for missing information of maternal weight measurements in the conditional analyses.[99] Information on maternal prepregnancy weight and maximum weight in pregnancy was self-reported. Self-reported weight tends to be underestimated, especially in case of higher maternal weight. This might lead to under- or overestimation of effect estimates of prepregnancy BMI and gestational weight gain in early pregnancy with cardiac outcomes, respectively. Finally, we adjusted for a large number of confounders, but residual confounding might still be an issue by factors such as childhood diet or exercise.

Interpretation of main findings

Maternal obesity is strongly associated with offspring obesity, and an adverse cardiovascular and metabolic profile.[161, 168, 169] These associations may reflect a shared genetic predisposition, shared environmental and lifestyle related factors or direct intra-uterine mechanisms. The fetal or developmental overnutrition hypothesis proposes that maternal obesity leads to high maternal plasma concentrations of glucose, free fatty acids and amino acids crossing the placenta. These might cause permanent changes in appetite control, neuroendocrine functioning and energy metabolism of the developing fetus and thus lead to offspring obesity and an adverse cardiovascular profile.[47, 170] In a previous study, we have found that parental BMI was not only associated with offspring BMI in childhood, but also with adiposity, blood pressure, lipid levels and insulin.[161] Associations for maternal BMI were stronger than with paternal BMI,

suggesting direct intra-uterine effects, besides the shared environmental, lifestyle and genetic effects. Not much is known about the cardiac effects of maternal overnutrition in humans. Studies in mice suggest that there is an association of maternal obesity with offspring cardiac hypertrophy.[157, 158] These associations could be caused by hyperinsulinemia in the fetus, leading to increased mitogenic action of insulin on myocytes.[158] Offspring of obese mice showed increased myocyte width, increased expression of molecular markers of myocyte hypertrophy and reactivation of fetal genes associated with cardiac hypertrophy and function.[157, 158] These associations were found independent from offspring body size.

In the current study, we found that maternal obesity is associated with offspring LVM, LVMi and AOD, but these associations were fully explained by current childhood BMI. A previous study in 76 obese and normal weight pregnant women in Norway showed that maternal obesity during pregnancy was associated with offspring thicker inter-ventricular septum in late pregnancy, and with smaller inner width of both ventricles.[171] Similar effects have been observed in women with gestational diabetes. However, the observed cardiac hypertrophy of offspring normalized in the first months of life.[172] From previous studies we have learned that maternal prepregnancy BMI is associated with childhood BMI and adiposity, whereas childhood BMI is associated with childhood cardiac outcomes. [47, 161, 173-176] Thus maternal obesity might lead to childhood adiposity, which in turn leads to cardiac hypertrophy. It is possible that more detailed cardiac measurements are necessary to show subclinical altered cardiac function and geometry independent of body size, such as strain imaging.[177]

Next to maternal prepregnancy BMI, also gestational weight gain in early- and mid-pregnancy to be associated with LVM, LVMi and AOD. These associations were independent from prepregnancy weight or weight gain in the other pregnancy periods. The associations were fully mediated by offspring BMI. Maternal weight gain in early pregnancy mainly consists of increased fat deposition, whereas in mid- and late-pregnancy the weight gain reflects extracellular fluid expansion and growth of uterus, placenta and fetus.[178] A previous study among 5,154 mother offspring pairs in the UK showed that gestational weight gain in early pregnancy was associated with offspring adiposity. In this latter study gestational weight gain between 14-36 weeks was associated with offspring adiposity only among women who gained 500g per week or more. Gestational weight gain in second and third trimester was also associated with offspring concentrations of triglycerides, HDL, apoA1 and IL-6, which was fully explained by offspring adiposity.[179] Previously we have also found that maternal weight gain in early pregnancy was associated with increased adiposity levels in offspring, independent of

birthweight or infant growth.[162] We also observed that gestational weight gain was associated with a higher increase in growth of LVM in infancy.[160] Thus it seems that increasing maternal adiposity levels in early pregnancy are associated with increased adiposity levels in offspring, which is subsequently associated with increased cardiac size.

Obese individuals not only have an increase in fat mass, but also in lean mass. [180] In children, this increased lean mass, rather than increased adipose tissue, explains most of the variability in heart mass.[22] Previously, we observed that specific adiposity measures, conditional on BMI, were inversely associated with LVM, suggesting that lean mass is a better predictor of heart mass than fat mass.[74] The increase in lean mass increases the total body oxygen consumption, which may in turn lead to increased stroke volume and cardiac output.[181] A study in 281 children observed that obesity was associated with eccentric and concentric LVH. Children with concentric LVH showed the worst cardio-metabolic profile.[175] Obesity in childhood was the most important risk factor for eccentric LVH in adulthood in 824 young adults.[182] Similar results were observed in a study that tracked childhood BMI to adulthood.[183] Our results suggest that maternal obesity is associated with eccentric LVH, but not concentric remodeling in offspring. The associations were fully explained by childhood BMI.

Altogether, we did not find evidence to support the hypothesis that maternal obesity leads to cardiac hypertrophy through direct intra-uterine programming effects. In our study, the association between maternal obesity and gestational weight gain with LVM, LVMi and AOD seems to be explained by shared genetic or shared lifestyle factors that lead to increased childhood BMI or programming of child BMI, which in turn leads to increased cardiac size. However, it is still possible that there is programming of the myocytes, but that the effects of the programming show at a different stage of life, as is seen in the transient cardiac hypertrophy in children born of diabetic mothers.[172] It could also be possible that we need more specific cardiac imaging methods such as strain imaging or more specific geometry measures to identify subtle effects on the cardiac structure and function. Thus far, previous studies and our results suggest that interventions focused on reduction of maternal prepregnancy BMI and gestational weight gain may lead to reduced risks of childhood adiposity and subsequent cardiac adaptations.[161]

CONCLUSION

We observed that both maternal and paternal prepregnancy BMI are associated with cardiac structures in offspring. Gestational weight gain in early pregnancy was also associated with cardiac structures, independent from prepregnancy BMI. All associations were fully explained by childhood BMI. Our results suggest that maternal prepregnancy BMI influences cardiac structures through shared family-based and lifestyle related characteristics and genetic factors leading to higher childhood BMI.

SUPPLEMENTAL MATERIAL

Table S3.1.1 Non-response analysis for childhood follow-up data at 6 years (N=7,200)

Characteristics	Follow-up at 6 years N=4,868*	Lost to follow-up at 6 years N=2,332	P-value
Maternal characteristics			
Age, median (95% range), yrs	30.9 (19.9, 39.4)	29.0 (18.5, 38.3)	<0.01
Education, N. higher education (%)	2198 (46.3)	767 (34.5)	<0.01
Parity, N. nulliparous (%)	2830 (58.1)	1249 (53.6)	<0.01
Total calorie intake, mean (SD), kcal	2052 (551)	2035 (593)	0.31
Folic acid supplements use, N. Yes (%)	3861 (79.3)	1620 (69.5)	<0.01
Alcohol consumption during pregnancy, N. Yes (%)	2140 (43.9)	1274 (54.6)	<0.01
BMI, mean (SD), kg/m^2	23.6 (4.2)	23.7 (4.6)	0.32
Height, mean (SD),cm	168 (7.4)	167 (7.4)	<0.01
Weight, mean (SD), kg			
Prepregnancy	66.5 (12.6)	66.0 (13.3)	0.17
Early pregnancy	68.9 (12.8)	68.5 (13.3)	0.24
Mid-pregnancy	75.9 (12.7)	75.2 (13.7	<0.05
Late-pregnancy	81.2 (12.7)	77.1 (13.4)	<0.01
Paternal characteristics			
Age, median (95% range), yrs	33.2 (22.2, 46.8)	31.7 (20.2, 45.5)	<0.01
Paternal education, N, higher education (%)	1737 (52.4)	547 (47.2)	<0.01
BMI, mean (SD), kg/m^2	25.3 (3.4)	25.4 (3.7)	0.52
Infant characteristics			
Ethnicity, N. Non-European (%)	1734 (35.6)	1051 (45.1)	<0.01
Males, N. (%)	2442 (50.2)	1069 (51.4)	0.17
Gestational age at birth, median (95% range), wks	40.1 (35.9, 42.3)	39.9 (33.4, 42.4)	<0.01
Birth weight, mean (SD), g	3434 (545)	3384 (581)	<0.01
Ever breastfeeding, N. Yes (%)	3565 (92.7)	1109 (90.2)	<0.01

Abbreviations: yrs, years; N, number; SD, standard deviation; wks, weeks.
Values represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests or Kruskal-Wallis tests for continuous variables and Chi-square tests for proportions. * Children with cardiac abnormalities were not excluded from non-response analysis.

Table S3.1.2 Associations of paternal body mass index with childhood cardiac outcomes (N=4,852)

Model	Left ventricular mass (SDS)	Left ventricular mass index (SDS)	Relative wall thickness (SDS)	Aortic root diameter (SDS)	Fractional shortening (SDS)
Paternal model					
Basic model[a]	0.08 (0.05, 0.11)**	0.05 (0.02, 0.09)**	0.02 (-0.02, 0.05)	0.07 (0.04, 0.10)**	-0.02 (-0.06, 0.01)
Confounder model[b, f]	0.09 (0.06, 0.12)**	0.06 (0.03, 0.09)**	0.01 (-0.02, 0.05)	0.07 (0.04, 0.10)**	-0.03 (-0.06, 0.01)
Mediator models[c]					
Infant[d]	0.03 (0.00, 0.06)*	0.05 (0.02, 0.08)**	0.01 (-0.03, 0.04)	0.03 (-0.00, 0.06)	-0.03 (-0.07, 0.00)
Childhood BMI[e]	0.01 (-0.02, 0.04)	0.00 (-0.03, 0.03)	-0.01 (-0.04, 0.03)	0.02 (-0.01, 0.05)	-0.04 (-0.08, -0.01)*
Combined paternal and maternal model					
Basic model[a]					
Maternal BMI	0.07 (0.03, 0.10)**	0.04 (0.00, 0.07)*	0.02 (-0.02, 0.05)	0.07 (0.04, 0.11)**	0.03 (-0.01, 0.06)
Paternal BMI	0.07 (0.04, 0.10)**	0.05 (0.01, 0.08)**	0.01 (-0.02, 0.05)	0.05 (0.02, 0.08)**	-0.03 (-0.06, 0.01)
Confounder model[b,f, g]					
Maternal BMI	0.09 (0.05, 0.12)**	0.05 (0.02, 0.09)**	0.02 (-0.02, 0.05)	0.08 (0.05, 0.12)**	0.02 (-0.02, 0.05)
Paternal BMI	0.08 (0.04, 0.11)**	0.05 (0.02, 0.08)**	0.01 (-0.02, 0.05)	0.06 (0.02, 0.09)**	-0.03 (-0.07, 0.00)
Mediator models[c]					
Maternal weight gain					
Maternal BMI	0.10 (0.07, 0.14)**	0.06 (0.03, 0.10)**	0.02 (-0.02, 0.05)	0.10 (0.06, 0.13)**	0.03 (-0.01, 0.07)
Paternal BMI	0.07 (0.04, 0.10)**	0.05 (0.02, 0.08)**	0.01 (-0.02, 0.05)	0.05 (0.02, 0.08)**	-0.03 (-0.07, 0.00)
Infant characteristics[d]					
Maternal BMI	0.04 (0.00, 0.07)*	0.04 (0.00, 0.07)*	0.01 (-0.03, 0.05)	0.04 (0.01, 0.08)*	0.02 (-0.02, 0.05)
Paternal BMI	0.03 (-0.00, 0.06)	0.04 (0.01, 0.08)**	0.00 (-0.03, 0.04)	0.02 (-0.01, 0.05)	-0.04 (-0.07, -0.00)*
Childhood BMI[e]					
Maternal BMI	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.03)	0.00 (-0.04, 0.04)	0.03 (-0.00, 0.07)	0.01 (-0.03, 0.04)
Paternal BMI	0.01 (-0.02, 0.04)	0.00 (-0.03, 0.04)	0.00 (-0.04, 0.03)	0.01 (-0.02, 0.04)	-0.04 (-0.08, -0.01)*

Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood outcomes in SDS per SDS change in maternal and paternal prepregnancy BMI. Abbreviations: SDS standard deviation score; BMI body mass index.

[a,b,c,d,e:] see Supplemental Table S3.1.3

Table S3.1.3 Associations of paternal body mass index with left ventricular hypertrophy (N=4,426)

	Eccentric left ventricular hypertrophy (odds ratio) N=138	**Concentric remodelling (odds ratio) N=428**
Paternal model		
Basic model[a]	1.29 (1.07. 1.55)**	1.04 (0.93, 1.17)
Confounder model[b, f]	1.31 (1.08, 1.58)**	1.04 (0.93, 1.17)
Mediator models[c]		
Infant characteristics[d]	1.26 (1.04, 1.53)*	1.02 (0.91, 1.15)
Childhood BMI[e]	1.15 (0.94, 1.40)	1.02 (0.90, 1.14)
Combined paternal and maternal model		
Basic model[a]		
Maternal BMI	1.18 (0.98, 1.42)	0.92 (0.81, 1.04)
Paternal BMI	1.24 (1.03, 1.50)*	1.06 (0.95, 1.19)
Confounder model[b, f, g]		
Maternal BMI	1.21 (1.00, 1.48)	0.93 (0.81, 1.06)
Paternal BMI	1.25 (1.03, 1.52)*	1.06 (0.94, 1.19)
Mediator models[c]		
Maternal weight gain		
Maternal BMI	1.20 (0.98, 1.47)	0.93 (0.81, 1.06)
Paternal BMI	1.25 (1.03, 1.52)*	1.06 (0.94, 1.19)
Infant characteristics[d]		
Maternal BMI	1.17 (0.95, 1.42)	0.92 (0.81, 1.05)
Paternal BMI	1.22 (1.00, 1.48)	1.04 (0.92, 1.17)
Childhood BMI[e]		
Maternal BMI	1.21 (1.00, 1.48)	0.93 (0.81, 1.06)
Paternal BMI	1.25 (1.03, 1.52)*	1.06 (0.94, 1.19)

Values are odds ratios (95% confidence interval) from logistic regression models that reflect the risk for hypertrophy per SDS change in maternal and paternal prepregnancy BMI, compared to children with normal left ventricular structure (N=3,860). Abbreviations: LVM left ventricular mass; LVMi left ventricular mass index (left ventricular mass/ height$^{2.7}$); RWT relative wall thickness (2 left ventricular posterior wall thickness/left ventricular diameter, age adjusted); SDS standard deviation score; BMI body mass index; [a]Basic model is adjusted for child sex and age. [b]Confounder model is additionally adjusted for: maternal age, educational level, alcohol use during pregnancy, folic acid use during pregnancy, total calorie intake during pregnancy, breastfeeding and ethnicity child. [c]Intermediate models are confounder models adjusted for each potential intermediate. [d]Infant characteristics are: birth weight, gestational age at birth and infant growth (change in weight SDS between birth and 24 months). [e]Childhood BMI: adjusted for sex- and age- specific BMI SDS. [f]Paternal confounder model is adjusted for paternal age and paternal education instead of maternal age and maternal education. [g]Combined paternal and maternal confounder model is adjusted for all previously mentioned confounders. *P<0.05; **P<0.01.

Table S3.1.4 Associations of weight gain in different periods of pregnancy with childhood cardiac outcomes

Model	Left ventricular mass (SDS)	Left ventricular mass index (SDS)	Relative wall thickness (SDS)	Aortic root diameter (SDS)	Fractional shortening (SDS)
Early pregnancy	**N=3,747**	**N=3,747**	**N=3,802**	**N=3,808**	**N=3,803**
Basic model[a]	0.06 (0.03, 0.09)**	0.04 (0.01, 0.08)**	0.02 (-0.01, 0.06)	0.03 (0.01, 0.06)*	0.05 (0.01, 0.08)**
Confounder model[b]	0.06 (0.03, 0.09)**	0.04 (0.01, 0.08)**	0.02 (-0.01, 0.06)	0.04 (0.01, 0.07)*	0.05 (0.01, 0.08)**
Infant model[c]	0.04 (0.02, 0.07)**	0.04 (0.01, 0.07)*	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.04 (0.01, 0.08)**
BMI model[d]	0.03 (0.01, 0.06)*	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.04)	0.04 (0.01, 0.07)*
Mid-pregnancy	**N=3,630**	**N=3,630**	**N=3,683**	**N=3,689**	**N=3,684**
Basic model[a]	0.01 (-0.02, 0.04)	0.02 (-0.02, 0.05)	-0.03 (-0.06, 0.00)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)
Confounder model[b]	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.05)	-0.03 (-0.06, 0.00)	0.01 (-0.02, 0.04)	0.02 (-0.02, 0.05)
Infant model[c]	0.00 (-0.03, 0.03)	0.01 (-0.03, 0.04)	-0.03 (-0.07, 0.00)	0.00 (-0.03, 0.03)	0.02 (-0.02, 0.05)
BMI model[d]	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	-0.03 (-0.06, 0.00)	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.05)
Late-pregnancy	**N=2,258**	**N=2,258**	**N=2,286**	**N=2,292**	**N=2,286**
Basic model[a]	0.04 (0.00, 0.08)*	0.04 (-0.00, 0.07)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.05)	0.01 (-0.03, 0.05)
Confounder model[b]	0.04 (0.01, 0.08)*	0.03 (-0.01, 0.07)	0.02 (-0.03, 0.06)	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.05)
Infant model[c]	0.03 (-0.01, 0.06)	0.03 (0.01, 0.05)*	0.02 (-0.03, 0.06)	0.00 (-0.04, 0.04)	0.02 (-0.02, 0.06)
BMI model[d]	0.03 (-0.00, 0.07)	0.03 (-0.01, 0.06)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.05)

Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences of childhood outcomes in SDS per SDS change in gestational weight gain.

Abbreviations: SDS standard deviation score; BMI body mass index.

[a]Basic model is adjusted for maternal height, child sex and age. [b]Confounder model is additionally adjusted for: maternal age, educational level, alcohol use during pregnancy, folic acid use during pregnancy, total calorie intake during pregnancy, child ethnicity and breastfeeding. [c]Infant model is adjusted for confounders and is adjusted for gestational age at birth, birth weight and infant growth (change in weight SDS between birth and 24 months).[d]BMI model is adjusted for confounders and is additionally adjusted for sex- and age-specific childhood BMI SDS. *P<0.05; **P<0.01.

Chapter 3.2

Body fat distribution, overweight and cardiac structures in school-age children

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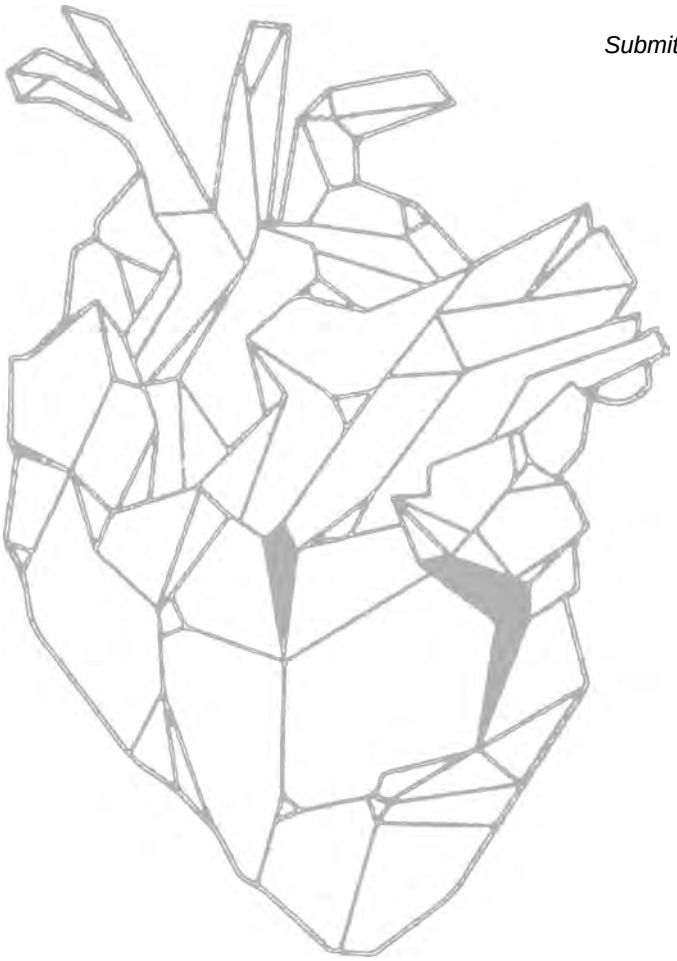

ABSTRACT

Background: Adiposity is associated with larger left ventricular mass in children and hypertrophy in adults. The role of body fat distribution in these associations is not clear.
Objective: We examined the associations of body fat distribution and overweight with cardiac measures obtained by cardiac Magnetic Resonance Imaging (cMRI) in school-age children.
Methods: In a population-based cohort study among 2,836 children aged 10 years, we used anthropometric measures, Dual-energy X-ray absorptiometry (DXA) and MRI scans to collect information on body mass index (BMI), lean mass index (LMI), fat mass index (FMI), and abdominal visceral adipose tissue index (VATI). Indexes were standardized on height. Cardiac measures included right ventricular end-diastolic volume (RVEDV), left ventricular end-diastolic volume (LVEDV), left ventricular mass (LVM), and left ventricular mass-to-volume ratio (LMVR) as marker for concentricity.
Results: All body fat measures were positively associated with RVEDV, LVEDV and LVM, with the strongest associations for lean mass index (all p-values <0.05). These associations were only partly explained by blood pressure. Obese children had 1.12 SDS (95% CI 0.94, 1.30) larger LVM and 0.35 SDS (95% CI 0.14, 0.57) higher LMVR than normal weight children. Conditional on body mass index, higher lean mass index was associated with higher RVEDV, LVEDV and LVM, while higher fat mass measures were inversely associated with these cardiac measures (all p-values <0.05).
Conclusion: Higher childhood body mass index is associated with a larger right and left ventricular size. These associations are explained by higher lean mass. In childhood, lean mass may be a stronger determinant of heart growth than fat mass. Fat mass may influence cardiac structures at older ages.

Chapter 3.3

Pericardial adipose tissue, cardiac structures and cardiovascular risk factors in school-age children

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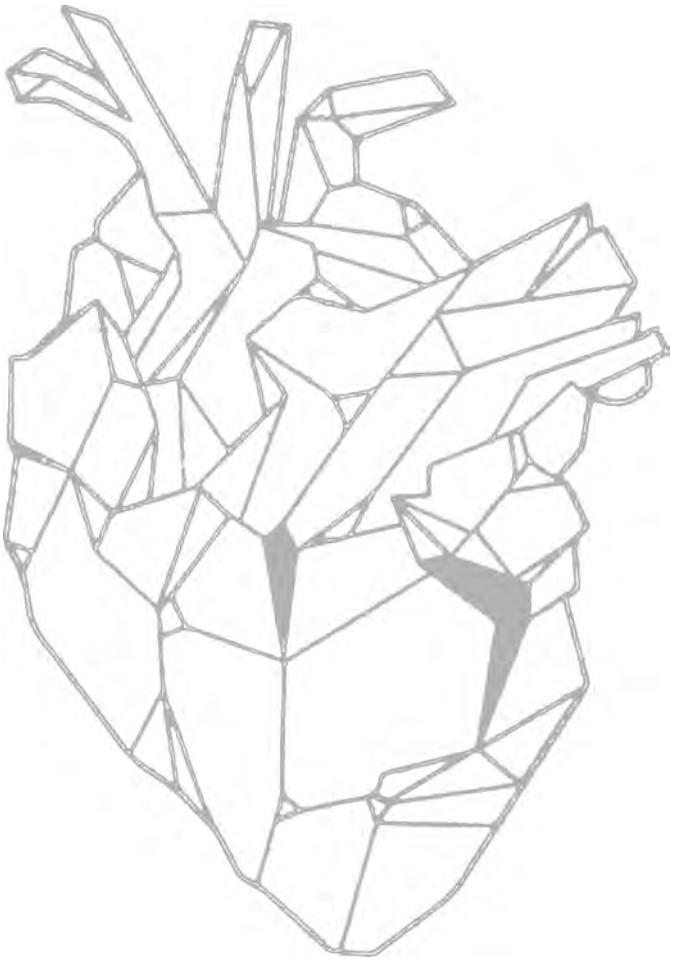
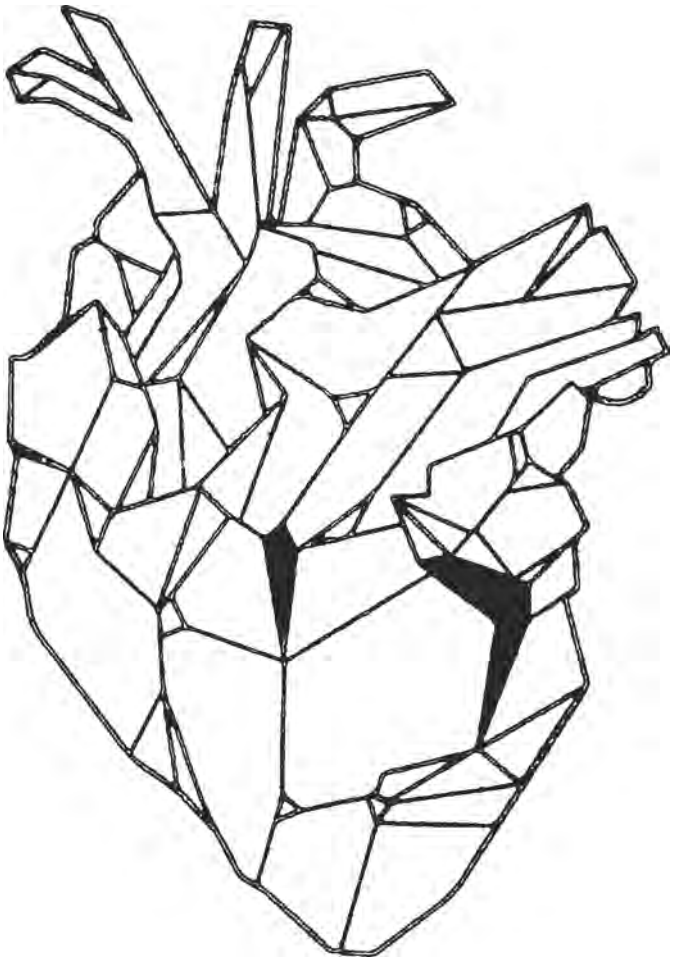
Submitted to Eur Heart J Cardiovasc Imaging



ABSTRACT

Background: Pericardial adipose tissue in adults is associated with cardiovascular disease. We examined the associations of pericardial adipose tissue with cardiac structures and cardiovascular risk factors among both normal weight and overweight children.
Methods: We performed a cross-sectional analysis in a population-based cohort study among 2,892 children aged 10 years (2,404 normal weight and 488 overweight/obese). Pericardial adipose tissue mass was estimated by magnetic resonance imaging (MRI) and indexed on height3. Left ventricular mass (LVM) and left ventricular mass-to-volume ratio (LMVR) were estimated by cardiac MRI. Cardiovascular risk factors included android adipose tissue percentage obtained by Dual-energy X-ray absorptiometry, blood pressure, and glucose, insulin, cholesterol and triglycerides concentrations. Adverse outcomes were defined as values higher than the 75 percentile.
Results: The median pericardial adipose tissue index was 3.6 (95% range 1.6- 7.1) among normal weight children and 4.7 (95% range 2.0- 8.9) among overweight children. In the full group, a one-standard deviation (1-SD) higher pericardial adipose tissue index was associated with higher LMVR (0.06 SDS (95% Confidence Interval (CI) 0.02, 0.09)) but not with LVM. Also, a 1-SD higher pericardial adipose tissue index was associated with increased odds of high android adipose tissue (Odd Ratio (OR) 2.08 (95% Confidence Interval (CI) 1.89, 2.29)), high insulin concentrations (OR 1.17 (95% CI 1.06, 1.30)), an atherogenic lipid profile (OR 1.22 (95% CI 1.11, 1.33)) and clustering of cardiovascular risk factors (OR 1.56 (95% CI 1.36, 1.79)). Pericardial adipose tissue index in was not associated with blood pressure and glucose concentrations. As compared to the full group, the associations showed largely the same directions but tended to be weaker among normal weight than among overweight children.
Conclusions: Pericardial adipose tissue is associated with cardiac adaptations and cardiovascular risk factors already in childhood in both normal weight and overweight children.

Chapter 4 | General discussion



General discussion

INTRODUCTION

Cardiovascular disease is a major public health burden worldwide. In 2015, an estimated 17.7 million people died from cardiovascular disease. This is over 30% of all global deaths.[227] In the Netherlands, cardiovascular disease accounts for 27% of all deaths.[228] The burden of cardiovascular disease usually affects people later in the life course. Therefore, most studies focus on cardiovascular risk factors in adults. However, the origins of cardiovascular disease could lie in the earliest phase of life.

Circumstances in pregnancy, around birth and in infancy could trigger developmental pathways that increase the chance of short-term survival. But, if these past circumstances don't match with the situation later in life, this could have adverse effects on health.[153] Adults who were born with a low birth weight, a sign of suboptimal in utero nutrition, followed by an affluent childhood, indicated by increased childhood weight gain, were at increased cardiovascular risk in adulthood.[3] The effects of suboptimal fetal nutrition can already be observed in childhood. Offspring of mothers with gestational hypertensive disorders have higher childhood blood pressure.[229] Not just undernutrition in utero, but also overnutrition could condition the structure, physiology and functioning of various organ systems.[5]

Not only factors during pregnancy and infancy, but also later in childhood could affect adult cardiovascular disease risk. Many traditional cardiovascular risk factors, such as obesity, lipid levels and blood pressure track from childhood to adulthood.[16] But increased childhood risk factors also influence adult cardiovascular health independently of adult obesity and blood pressure.[35, 36] The prevalence of obesity in both adults and children is increasing worldwide.[230] Obesity in adults is associated with higher all-cause mortality, with cardiovascular disease as the most important cause of death, followed by diabetes, kidney disease and cancer.[231] Since childhood obesity is associated with adult obesity and cardiovascular health, the increasing rates of childhood obesity are worrying. It is therefore important to understand the mechanisms by which early life factors influence later obesity and cardiovascular health, in order to start prevention of cardiovascular disease as early as possible.

The main aim of the studies presented in this thesis was to identify early-life growth and adiposity measures related to cardiac structure and adverse cardiovascular outcomes in childhood. We also aim to identify critical periods in early life for these associations. This chapter provides a

general discussion of the main findings of the studies in this thesis, discusses general methodological issues and provides suggestions for future research.

INTERPRETATION OF MAIN FINDINGS

Growth

Tracking

As stated previously, cardiovascular disease could have its origins in early life. Studying cardiovascular risk factors in childhood, such as BMI, lipid levels, blood pressure and cardiac structure within a normal population can be useful, if these risk factors tend to be stable over time. If a child has a blood pressure in the higher range than its peers, and this stays during adolescence and into adulthood, then the blood pressure level in childhood can possibly predict the cardiovascular health in adulthood. Therefore, it is important to study the stability of rank order of different cardiovascular risk factors.

Longitudinal studies have shown moderate to high tracking coefficients for BMI and cholesterol levels, medium tracking of triglycerides levels and blood pressure, and low tracking of fasting insulin and insulin resistance from young childhood to adulthood.[16, 232] Tracking of clustering of cardiovascular risk factors is high, children who have multiple risk factors in the higher ranges in childhood, are more likely to remain in the higher range of those cardiovascular risk factors in adulthood.[233, 234] Left ventricular mass tracks from childhood into young adulthood.[17, 18, 25] Results from studies in this thesis (**Chapter 2.1**) suggest that not only left ventricular mass, but also aortic root diameter and left atrial diameter track between the ages of 6 weeks and 10 years. Tracking became stable after the age of 24 months. The tracking coefficients we observed were low to moderate. Although these results are interesting and show that larger cardiac size in childhood might influence later cardiovascular health, these results are not strong enough to warrant routine echocardiography in childhood to identify children at risk for adult cardiovascular disease, who might benefit from early intervention. The predictive value of childhood cardiac size on later cardiovascular health remains unclear. Since other risk factors, mainly BMI, are easier to measure and show stronger tracking, they are more likely candidates for routine measuring in a child health setting focused on prevention of later cardiovascular disease.

Conclusions on tracking

- Cardiac structures track moderately from early infancy into school-age
- Cardiac structure might be determined early in life and influence cardiovascular health later in life

Fetal and infant growth

Fetal growth restriction is associated with cardiovascular disease later in life.[3] Children who are born small, but experience fast growth in infancy and early childhood have a greater risk of cardiovascular disease in later life. [3, 14] However, an observational study on birth weight showed that not only low birth weight, but also high birth weight places individuals at risk for higher blood pressure and overweight in childhood.[42] Therefore, fetal and infant life seem to be critical periods for development of cardiovascular health.

Fetal and infant growth and vascular health

The effects of fetal growth and birth weight on blood pressure in later life have been studied often. Although most studies find an effect of low birth weight on increased blood pressure later in life, the effect estimates reported are small, especially in the larger observational studies. Also, many studies don't adequately adjust for current body size or other confounders.[105] When studying birth weight and later growth patterns, it becomes clear that both birth weight, and infant or childhood growth are independently associated with increased blood pressure later in life. However, the association of infant and childhood growth with blood pressure is stronger than that of birth weight.[106] In line with these results, in the studies presented in this thesis (**Chapter 2.2**) we observed independent associations of both birth weight and increased infant weight gain with higher blood pressure in childhood. Weight gain in infancy showed the strongest effect estimates, and could therefore be more important in predicting blood pressure values across the life course than birth weight. A possible mechanism by which suboptimal fetal growth could lead to increased blood pressure, is through impaired elastin synthesis, and subsequently stiffer arteries and increased blood pressure.[108] In the studies presented in this thesis, we examined the associations of early growth with pulse wave velocity, a measure of arterial stiffness. However, we did not observe any associations with this measure.

Conclusions on fetal and infant growth and vascular health

- Both birthweight and infant weight gain are associated with increased childhood blood pressure
- Infant weight gain could be more important than birth weight in predicting blood pressure across the life course

Fetal and infant growth and cardiac health

The in utero environment not only affects the vessel structure and functioning, but also has an effect on cardiac structure and function. Children with fetal growth restriction show altered cardiac structure, with more global shaped cardiac ventricles.[11] In preterm adults, increased left ventricular mass, altered cardiac shape and lesser function have been observed.[104] In contrast to these studies, we observed (**Chapter 2.2**) that lower gestational age and lower birth weight were associated with smaller aortic root diameter and lower left ventricular mass in childhood at the age of 6 years, relative to childhood body size. Children with higher birth weight had larger relative aortic root diameter and left ventricular mass. Children with higher birth weight also have higher lean mass later in life.[114] Lean mass is a major determinant of cardiac size in childhood, which could explain why we observed that higher birth weight was associated with increased cardiac size in childhood.[22] However, we also observed that fetal growth deceleration followed by infant growth deceleration was associated with larger left ventricular mass. This has also been observed in adults, were a low weight at the age of 1 year was associated with left ventricular hypertrophy in adulthood.[46] In fetal life, cardiac growth is mainly determined by myocardial cell hyperplasia, while after birth this switches to myocardial cell hypertrophy.[7] Possibly, growth in utero affects cardiac size through altered hemodynamics, which can affect the maturation and sarcomere structure of cardiomyocytes.[19-21] This could affect the size and function of the cardiomyocytes and the heart around the time of birth, and program cardiomyocyte development after birth. Therefore, different mechanisms related to fetal growth and infant growth might be at play and cause increased left ventricular mass. It remains unclear how these different mechanisms of increased left ventricular mass affect later cardiovascular health.

Conclusions on fetal and infant growth and cardiac health

- Higher birth weight is associated with relatively larger aortic root diameter and left ventricular mass in childhood, this could be mediated by higher lean mass

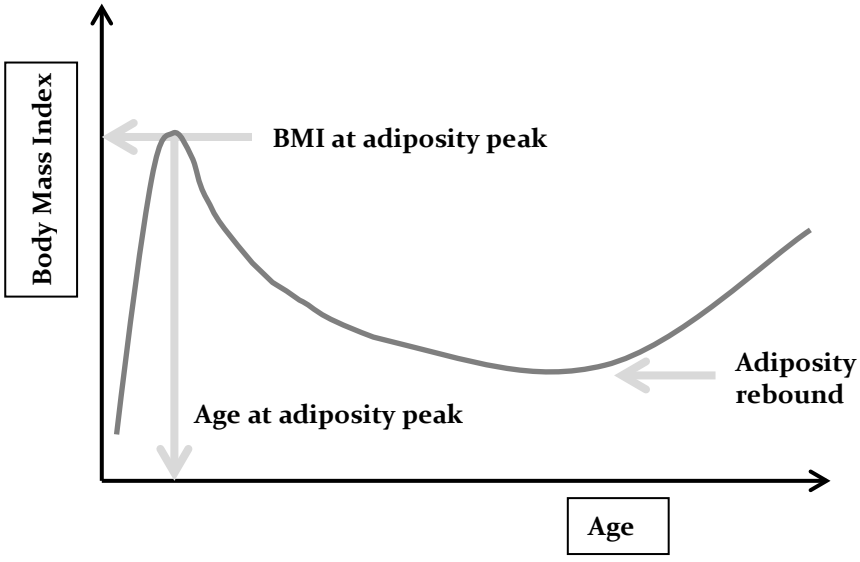
- Fetal growth deceleration followed by infant growth deceleration is also associated with relatively larger left ventricular mass; this might be caused by a different mechanism

Infant growth and childhood growth

Infant growth and cardiometabolic health

As stated previously, rapid infant growth is associated with adult obesity, increased blood pressure and adverse lipid profiles.[44, 123, 235] Most studies use change in length or weight between two time points as indicators of growth. However, more detailed growth patterns can be derived from longitudinally collected anthropometric measures. For most infants, BMI increases after birth till around 9 months, after which it declines, and rebounds again around the age of 6 years.[45] From repeatedly measured anthropometrics in infancy, we can determine at what age this BMI peak was reached, and how high the maximum BMI was (**Figure 4.1**). We can also calculate peak weight velocity, which is the greatest weight growth in infancy. In the days after birth, newborns first tend to lose some bodyweight, after which they reach the peak weight velocity within the first month of life. Fast growth in the first month after birth, measured by higher peak weight velocity, was associated with higher blood pressure, weight circumference and BMI in adulthood.[120]

Figure 4.1 Body Mass Index curve in early life



Previous work in the Generation R study showed that higher peak weight velocity and BMI peak were associated with higher childhood BMI, body fat percentage, and abdominal adiposity.[45] Other childhood studies have shown that peak weight velocity was also associated with higher blood pressure in childhood, independent of current BMI.[131] Timing and magnitude of BMI peak were associated with childhood blood pressure, although timing of BMI peak was mediated through current BMI.[132] In the studies discussed in this thesis (**Chapter 2.3**), we observed that peak weight velocity was associated with higher blood pressure, and that the association of higher BMI peak with higher blood pressure was mediated through current BMI. We did not observe any consistent associations of infant weight velocity patterns with metabolic factors, such as triglycerides, cholesterol or insulin concentration.

Conclusions on infant growth and cardiometabolic health

- Peak weight velocity is associated with higher blood pressure in childhood
- A higher BMI peak in infancy is associated with higher childhood blood pressure, but this can be explained by childhood BMI

Fetal, infant and childhood growth and cardiac structure

In this thesis I previously discussed the effects of fetal and infant growth on childhood left ventricular mass and aortic root diameter at the age of 6 years. In the studies discussed in this thesis (**Chapter 2.4**), we also examined the effects of fetal, infant and childhood growth on cardiac structure in more detail at the age of 10 years. In adults, cardiac remodeling resulting in left ventricular hypertrophy is associated with cardiac disease and mortality.[26, 27] Besides left ventricular mass, left ventricular geometry is important. More concentric remodeling, in which the ratio between mass and volume is increased, is also associated with cardiac disease and mortality.[27, 28] Since right ventricular size is associated with cardiac disease independently of left ventricular mass, in the studies discussed in this thesis, we also examine right ventricular volume.[30] In this thesis, we observed that fetal growth in length and weight were associated with larger left and right ventricular volumes, and left ventricular mass in childhood, relative to childhood size. We did not find strong effects of infant height or weight gain on cardiac structure, but the period after 24 months seemed important. Children who gained more height, had larger ventricular volumes and mass, relative to their current size. Children who gained less height, but more weight, showed smaller volumes and mass. The ratio

between mass and volume was greater in these children, indicating increased concentricity of the left ventricle. This is in line with another study, which showed that both birth weight, and current weight were associated with left ventricular mass in adolescents, and that obesity was associated with concentric remodeling patterns.[136] The differences of childhood growth on cardiac structure might be explained by differences in body composition, which will be discussed more extensively in the next section.

Conclusions on fetal, infant and childhood growth and cardiac structure

- Both fetal and childhood growth are critical for development of cardiac dimensions
- Children who are smaller at birth and shorter and heavier in childhood have increased concentricity of the left ventricle

Fetal hemodynamics as possible mechanism relating fetal growth to cardiac health

As stated previously, one of the possible mechanisms of altered cardiac structure related to fetal growth retardation could be hemodynamical changes in the fetus. Fetal nutrition and hemodynamics depend on placental function. Changes in placental vascular resistance may lead to fetal growth and hemodynamic adaptations.[145] An adverse fetal environment leads to redistribution of blood flow to the upper parts of the body at expense of the trunk, this is called 'brain sparing'.[143] This in turn, leads to altered blood flow through the left and right ventricle, the flow through the left ventricle increases, and more blood is pumped to the brain circulation.[144] The increased pressure on the left ventricle, increasing the wall stress, in combination with chronic hypoxia and undernutrition have been suggested as possible mechanism why fetal growth retardation leads to more globular shaped ventricles in childhood.[149] Within the Generation R study, it was previously observed that increased third-trimester placental vascular resistance was associated with decreased fetal growth.[147] In the studies presented in this thesis (**Chapter 2.5**), we observed that higher uterine artery resistance and a stiffer, less compliant right ventricle in fetal life were associated with childhood right ventricular function. Fetal left cardiac output was associated with lower childhood left ventricular function and altered cardiac structure. The population under observation consisted of relatively healthy pregnancies. Possibly, the effects of fetal environment on cardiac structure are more pronounced in the more

compromised pregnancies, or show in different periods of the life course. Also, other factors might influence the relation between fetal environment and cardiac structure. For example, a study on cardiac structure in preterm born adults showed that breast feeding could attenuate the negative effects of the preterm birth on cardiac structure.[236] Therefore, it remains important to study the effects of fetal environment, and other factors during fetal life, in infancy and childhood and how they affect cardiac development and cardiovascular health throughout the life course.

Conclusions on fetal hemodynamics and cardiac structure

- Fetal growth is associated with fetal hemodynamical changes, which could influence later cardiac structure and functioning

Adiposity

Maternal obesity and child cardiac size

In the previous paragraphs we have mainly discussed fetal growth restriction, but we have not extensively discussed overnutrition in utero. Maternal obesity during pregnancy is associated with adverse metabolic profiles in the offspring with increased insulin resistance, adverse body composition and lipid profile, and subclinical inflammation.[161, 168, 169, 237] These associations might be caused by shared genetic predisposition, shared environment and lifestyle factors, or by direct intra-uterine processes. Maternal obesity leads to high maternal plasma concentrations of nutrients which cross the placenta. These might cause changes in appetite control, neuroendocrine functioning and energy metabolism of the developing fetus and thus program for later obesity and an adverse cardiovascular profile.[47, 170] Excessive weight gain in early pregnancy might have similar effects.[162, 179] The offspring heart might be directly involved as well. Maternal obesity is associated with congenital heart disease in the offspring.[155] Animal studies have shown that offspring of obese mice develop cardiac hypertrophy, independent of offspring weight or diet.[157] Hyperinsulinemia in the fetus caused by the overnutrition could lead to increased mitogenic activity of insulin on cardiomyocytes.[158] In the studies discussed in this thesis (**Chapter 3.1**) we observed that prepregnancy BMI and weight gain in early pregnancy were associated with larger left ventricular mass and aortic root diameter. However, the association of paternal BMI with these outcomes was of similar strength, suggesting not a direct intra-uterine effect, but a shared genetic or lifestyle effect.[238] Also, after adjusting for child BMI, the effect on cardiac size disappeared,

suggesting that maternal and paternal BMI are associated with child BMI, which in turn is associated with cardiac size.

Conclusions on maternal obesity and child cardiac size

- We did not observe a direct intra-uterine effect of maternal BMI or gestational weight gain on childhood cardiac size
- Shared genetics with both parents or family lifestyle might affect childhood cardiac size through childhood BMI

Childhood body composition and cardiovascular health

Obesity is a very important risk factor for cardiovascular disease in adulthood.[184] Obesity in childhood is associated not just with obesity in adulthood, but also with cardiovascular disease, irrespective of adult size.[35, 239, 240] Obesity and overweight are determined by BMI. This measure is very useful to identify people at risk in the general population, but it is not a very precise measure. BMI cannot distinguish between lean mass and fat mass. Adipose tissue is more strongly associated with cardiovascular health than lean mass. When examining different adipose tissue depots, visceral adipose tissue is more pathological than subcutaneous adipose tissue.[51, 186, 241] In obesity, usually lean mass, fat mass and abdominal fat mass are all increased.[192, 193] To understand the mechanisms by which obesity leads to cardiovascular disease, it is important to study body composition.

Body composition and cardiac health

In this thesis, we have studied the associations of body composition with cardiac structure (**Chapter 3.2**). Previous literature in adults has shown that adiposity in the hip region was associated with an increase in both left ventricular mass and volume, which is called eccentric remodeling.[52] Central obesity was also associated with concentric remodeling, in which the mass-to-volume ratio was increased.[52] Eccentric remodeling is associated with heart failure, while concentric remodeling is also associated with stroke and coronary heart disease.[27] Studies in children show that obesity is associated with increased left ventricular mass and volume.[174] In line with these results, we observed that higher BMI was associated with larger left ventricular mass and volume. In children, one of the main determinants of cardiac size is lean body mass.[22] Indeed, when we examined body composition independent of BMI, we observed that lean body mass was associated with larger cardiac mass and volume, while higher fat mass was associated with relatively smaller cardiac mass and volume. Lean body mass is associated with an increase in blood volume,

leading to a higher preload and thus an increase in volume and mass. Adipose tissue is less metabolically active, and will not lead to these hemodynamical changes.[23] Concentric remodeling was often thought to be caused by hypertension.[242] However, in obese adults concentric remodeling has been observed independently of blood pressure or of aortic stiffness.[197, 243] Possibly another mechanism, on top of the hemodynamic effects is involved, using adipokine-mediated mechanisms.[243] Higher concentricity was associated not only with more abdominal adiposity, but also with insulin resistance and inflammation.[198]
Concentric remodeling is already present in obese children.[56] We also observed more concentricity of the left ventricle in the obese children, but we did not observe an association with visceral adipose tissue.

Conclusions on body composition and cardiac health

- Childhood obesity is associated with larger cardiac structures
- Lean mass may be a stronger determinant of cardiac size than fat mass

Pericardial adipose tissue and cardiometabolic health

Higher visceral adiposity causes endocrine and immune responses that affect cardiovascular structure and function directly and through worsening of other cardiovascular and metabolic risk factors.[191] Pericardial adipose tissue is a visceral adipose depot, directly attached to the heart. It consists of epicardial adipose tissue, and paracardial adipose tissue.[206] Besides the endocrine and immune responses of visceral adipose tissue that affect cardiovascular health, pericardial adipose tissue might also affect cardiac health directly, because of its closer proximity to the heart. In coronary artery disease, pericardial adipose tissue released inflammatory factors that could influence insulin resistance and cardiovascular functioning.[220] In adults, higher pericardial adipose tissue is associated with cardiovascular disease, but studies are not consistent on the role of general and visceral adipose tissue in these observed associations.[53, 54] Pericardial adipose tissue was associated with cardiac structure, and with numerous cardiovascular and metabolic risk factors, such as insulin resistance, atherogenic lipid profile, hypertension and metabolic syndrome.[53, 55, 216, 217] Again, it was unclear if these associations were independent of general and visceral obesity. In obese children studies have shown that pericardial adipose tissue was associated with cardiac structure, blood pressure, triglycerides and HDL cholesterol.[207, 222] In line with the current literature, we observed that both pericardial adipose tissue and

measures of cardiac structure were larger in overweight and obese children (**Chapter 3.3**). The absence of a similar relationship in strata of normal weight and overweight children suggests that general adiposity may explain this association. Pericardial adipose tissue was associated with an adverse body fat distribution and unfavorable lipid concentrations, independently of general obesity. It remains unclear if pericardial adipose tissue is mainly a marker for increased visceral adipose tissue, or if it exerts direct pathological effects to cardiovascular health as well in childhood. Since pericardial adipose tissue can be easily visualized with echocardiography, it can possibly have a role as a measure of visceral adipose tissue in routine screening for risk prediction. Independently of the possible mechanisms and causality, it remains clear that adiposity in childhood is associated with an adverse cardiovascular phenotype, and prevention or early treatment of adiposity in childhood could possibly influence later cardiovascular health.

Conclusions on pericardial adipose tissue and cardiometabolic health

- Obesity is associated with pericardial fat mass
- Pericardial fat mass is not associated with cardiac mass and concentricity, independently of general obesity
- Pericardial fat mass is associated with adverse body fat distribution and unfavorable lipid concentrations, independently of general obesity

METHODOLOGICAL CONSIDERATIONS

Specific strengths and limitations for the studies presented in this thesis have been described in **Chapter 2** and **Chapter 3** of this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias and confounding are discussed.

Selection bias

Selection bias is the bias in the estimated associations observed, when the association between determinant and outcome of interest is different in the participants than in the non-participants. Selection bias may occur because of selective non-response at baseline, or selective loss to follow-up. Of all children eligible at birth, response to participate in the Generation R Study was 61%. This non-response is not likely to be random. Participating women more often belonged to the Dutch ethnicity than would be

expected from population numbers of the study area. Highest finished education and household income suggested a higher socioeconomic status in participants than in non-participants.[58] Percentages of preterm birth or low birth weight were lower than expected from the population figures, suggesting a more healthy study population.[244] This selection towards a more affluent and healthy population could lead to bias in etiological association studies if the selection mechanisms are related to both determinant and outcome measures. However, several studies have shown that in cohort studies, associations are not strongly influenced by selection at base-line.[101, 245] The more homogenous study population could affect generalizability of our findings and reduce statistical power. Selection bias can also occur due to loss to follow up if associations of those remaining in the analyses are different in those who were lost to follow up. At the age of 10 years, children were invited to our dedicated research center for anthropometrics measures, echocardiography and cardiovascular measurements. This also entailed a separate visit for more detailed body composition and cardiac structure imaging by MRI. In total, 76% of the original cohort participated in this follow-up, of whom almost 80% visited the research center.[59] A lower percentage of children participated in blood measurements, due to non-consent for venous puncture. Participation in the MRI studies was lower because of later start of the MRI study. Unsuccessful abdominal or cardiac MRI scans were due to logistical or time constraints, or to low quality of imaging. Mothers of children who did not visit the research center more frequently had unhealthy lifestyle habits and were less well educated than the total study population. Similar differences were observed in mothers of children who did and did not participate in the MRI studies. Overall, this selective loss to follow up could lead to bias in the associations presented in this thesis, but this bias is difficult to quantify.

Information bias

Information bias is a bias that can arise from misclassification in a study of determinant or outcome measurements.[246] Non-differential misclassification occurs when the determinant status is not related to the outcome status, and vice versa, this can lead to an underestimation of observed effect estimates. In differential misclassification the determinant status is related to the outcome status and this leads to biased results, which can be under- or overestimations. Exposure data in our study were collected longitudinally and before assessment of the outcomes. Parents and data-collectors were unaware of the research questions under study. This makes differential misclassification unlikely. However, non-

differential misclassification might have occurred in the studies discussed in this thesis. In some of the studies we examined fetal growth as determinant of cardiovascular health. Pregnancy dating was performed using ultrasound measurements at the first visit. This method neglects variation of growth in the first trimester and as a consequence growth variation in second and third trimester might be underestimated and random measurement error might have occurred in pregnancy duration estimation. In some of the studies we examine the associations between anthropometrics, body composition and cardiac structure. Although the time difference between the two visits was less than 2 months for the majority of the children, and we adjusted all our analyses for this time difference, some non-differential misclassification might exist and have diluted the observed effect estimates in our cross sectional studies or in studies where MRI imaging was later standardized on anthropometrics. In most of the studies the determinants and outcome were assessed using medical records, or standardized hands-on assessments of body composition and cardiovascular measures, which makes differential misclassification less likely.

Confounding

A confounding factor is an extraneous variable associated with both the determinant and the outcome, and this variable is not an intermediate variable in the causal pathway between the exposure and the outcome.[246] If a confounding factor is not taken into account, this might bias the effect estimate of the association observed. To take into account confounding, all our analyses were adjusted for multiple potential confounders. Covariates were usually selected based on previous studies, or a change in effect estimate of more than 10%. As in any observational study, residual confounding might still be an issue. Some of the unmeasured confounding can relate to parental or child diet, activity, fitness or sedentary behavior. Also, information on some of the confounding factors was self-reported and measurement error could have occurred. This residual confounding could have led to under- or overestimation of observed associations.

FUTURE RESEARCH

As stated previously, cardiovascular disease is a major public health burden worldwide and an important cause of premature death, which can partly be influenced by modifiable lifestyle factors.[227] Childhood cardiovascular risk factors not only track into adulthood, but also influence later cardiovascular health directly.[233, 234] In this thesis we discussed some maternal, fetal, infant and childhood factors that impact childhood cardiovascular health. These results provide more insight into the development of cardiovascular disease and cardiovascular risk factors. However, the effect estimates we observed were often small and of limited clinical importance for the individual. They remain important in a developmental perspective, and possibly also in population health. Although the observed associations were often small, the combination of all these small differences could transfer to larger cardiovascular risk in later life. It is therefore important to perform long-term follow up studies from early life into adulthood in order to examine how growth patterns in early life and body composition in childhood, and the differences in cardiovascular measures we observed in childhood relate to later cardiovascular risk factors and cardiovascular disease.

Fetal and childhood exposures

Because of the observational design of the Generation R Study, we can only observe associations, and cannot conclude on causality of these observations. To establish causality, experimental study designs are necessary. Since randomized controlled trials are not possible in relation to fetal growth retardation in humans, animal studies in mice or sheep are often used to study this topic. In animal models an adverse fetal environment can be created for example by feeding a protein limited diet to the pregnant animal, or by surgically restricting placenta function.[6, 19] Although useful insights into mechanisms by which fetal growth retardation leads to cardiovascular adaptation have been obtained by animal studies, we can never be sure if these experimental designs relate directly to the in utero situation in humans and if these changes in cardiac structure and function relate to cardiovascular disease. Randomized controlled trials in which the placental function in humans is experimentally reduced is not possible, but there might be some medications that can improve placental function, and thus improve later cardiovascular health in childhood. Some therapeutic options currently under study in animal or human studies aiming to increase placental function are sildenafil, adenovirus-mediated delivery of vascular

endothelial growth factor, metformin, statins, insulin like growth factor, and artificial oxygen carriers.[247, 248] Since most experiments are preclinical, hopefully in the upcoming years therapeutic options to treat the dysfunctional placenta will become available. Lifestyle intervention studies focusing on improving cardiovascular health of pregnant women could be useful to determine if lifestyle changes in or before pregnancy can help in increasing cardiovascular health of offspring in the general or the at risk population. Since randomization or intervention of many of the studied exposures in this thesis will not be possible, more detailed assessment of these exposures might provide further insight into possible mechanisms or associations by which cardiovascular health and structure are affected. Further studies will need more information on placenta function, first trimester fetal growth, hemodynamic adaptations and fetal and newborn cardiac function. One possible method is by using 3D ultrasound images in a virtual reality system to develop new imaging markers for placental vascular development.[249]

Infant and childhood exposures, such as breastfeeding, diet and exercise can also affect cardiovascular health. In preterm born adult who received donor breast milk, cardiac structure was less modified than in preterms who received formula feeding.[236] Diet and exercise not only have beneficial effects on weight, but also on body composition.[225] They also benefit cardiac structure and cardiovascular health.[250, 251] Interventions aiming to change lifestyle can be difficult to implement or have small effects. However, starting with healthier habits early in life might have great effects. Interventions don't need to be life changing, small changes can also be beneficial and can also be implemented in the school environment. For example, children walking or running for one mile day in school seem to benefit and have improved cardiovascular health and fitness.[252]

Cardiac measures

Main childhood outcomes studies in this thesis were blood pressure, left ventricular mass and left ventricular mass-to-volume ratio. Measures of cardiac structure might provide further insight into the underlying mechanisms linking early life exposures with cardiac alterations and later cardiovascular disease. In adults, left ventricular hypertrophy and concentricity are associated with cardiovascular disease.[28] Cardiac hypertrophy can be physiological and pathophysiological. Hypertrophic remodeling takes place in order to decrease ventricular wall stress in response to various stimuli. Physiological stimuli can be postnatal growth, pregnancy, or exercise. This physiological hypertrophy is mainly mild, the

volume of the ventricle increases with a coordinated increase in wall thickness. Cardiomyocytes grow in both length and width.[253] After removal of the stimulus, the ventricular volume and wall thickness decrease again. In sedentary people following an exercise training program the ventricular mass increased by 45% in two weeks.[251] Pathophysiological remodeling can be observed in patients with myocardial infarction, valvular disease and metabolic syndrome. The ventricular volume is reduced, while the wall thickness is increased. Cardiomyocytes grow more in thickness than in length. Later ventricular dilation takes place with lengthening of cardiomyocytes, resulting in contractile dysfunction and cardiac failure.[253] This type of remodeling is accompanied not only by the changes in cardiomyocytes, but also with increased fibrosis, type 1 collagen levels, myofibroblast activation, cardiomyocyte death, insufficient increase of the capillary network and activation of fetal genes.[253]

In the studies discussed in this thesis we observed that taller, but lean children had highest cardiac mass. This is probably physiological in response to a larger body, more lean mass or higher cardiac fitness. In contrast, the relatively smallest hearts were observed in shorter children with weight gain. Since they also showed increased concentricity, this could possibly be an early sign of pathophysiological remodeling. Since it is difficult to distinguish physiological and pathophysiological remodeling in the study design we used, more research is necessary to understand the pathways of cardiac remodeling in childhood, whether remodeling is physiological, when will it become pathophysiological and how this relates to later cardiac disease. Possibly there is a role for more detailed measures of cardiac geometry or function. We studied ejection fraction, but this is a crude measure of cardiac function. Even in cardiac failure, this measure can still be within normal limits.[254] Strain analyses derived from echocardiography or MRI could possibly provide more information on cardiac function and hold prognostic information.[177] This method differentiates between active and passive movement of myocardial segments and evaluates myocardial function, such as longitudinal myocardial shortening.[177] A study in children with fetal growth retardation has showed that longitudinal strain was decreased, while circumferential strain was increased. This was also accompanied by changes in cardiac geometry.[21] The children had smaller ventricles that were more spherical. This is also present in adults who were born preterm.[104] In obesity more conically shaped ventricles have been observed.[199] Both are associated with cardiac failure.[203] A more detailed analyses of cardiac geometry, for example by creating 3-dimensional images from cardiac MRI in populations at risk for cardiac disease could possibly give insight into adverse

remodeling patterns.[255] It would then become possible to study these remodeling patterns in large population based studies in both children and adults and learn if there is any predictive value of cardiac geometry early in life.

CLINICAL IMPLICATIONS

Worldwide, the burden of cardiovascular disease remains massive.[227] In the Netherlands, cardiovascular disease is accountable for 27% of deaths.[228] The associations of cardiovascular risk factors, such as hypertension, hyperinsulinemia, adverse lipid profile, metabolic syndrome and obesity on cardiovascular morbidity and mortality are well-known.[227] Also, adverse cardiac geometry and greater left ventricular mass in late adulthood are associated with cardiovascular events.[28] We already know that cardiovascular risk factors track from childhood to adulthood, and that cardiovascular disease might originate in early life. From this thesis, we have identified several maternal, fetal and childhood factors that might influence cardiac structure and cardiovascular health in childhood. Growth and weight gain patterns in fetal life, infancy and childhood influence later cardiovascular health. Obesity, body composition and pericardial fat in childhood are already associated with adverse cardiovascular risk profiles. Fetal life and early childhood are critical periods for cardiac structure and cardiovascular health of children.

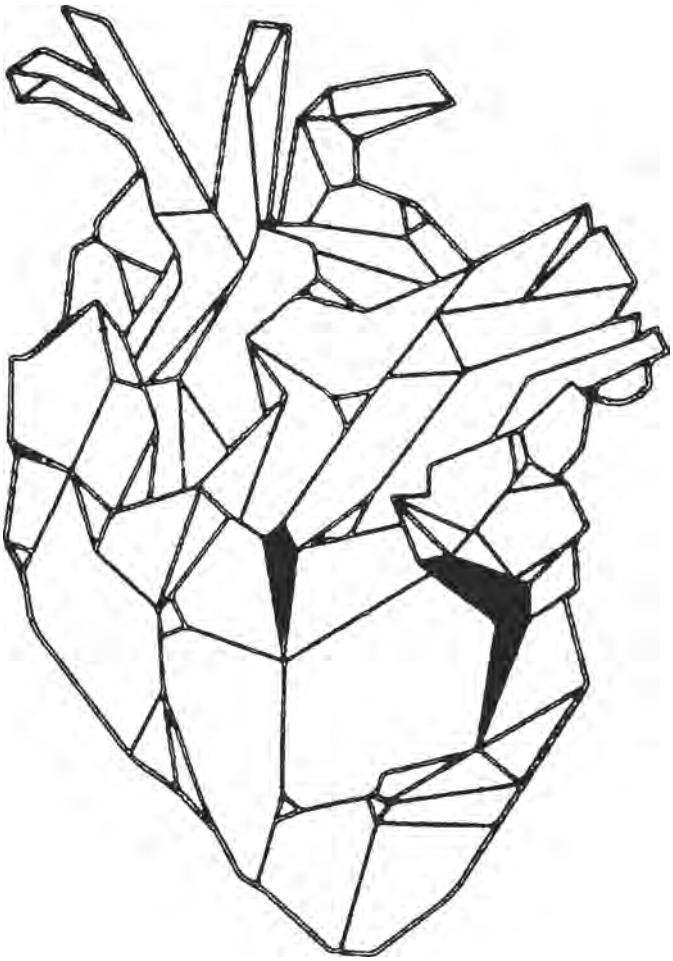
The associations we observed were often small, but might be important on a population level. Possibly, they can contribute in identifying at risk individuals or populations. They are also important for the development of intervention strategies that focus on promoting a healthy lifestyle early in life. Weight loss in adolescents and adults improves cardiovascular health.[195, 256] However, weight loss or life style changes are very difficult to achieve for most people. Prevention should start earlier in life. A good time to start would be in the preconception period. Future parents might be more motivated and when future parents start to live healthier, the lifestyle changes might promote better health in pregnancy. Hopefully, the lifestyle changes will last, and thus influence infant and childhood growth and health. Some interventions should focus on healthy prepregnancy weight and habits, but concluding from this thesis, infant and childhood growth are also very important. In the Netherlands we have a unique system of child well centers, routinely measuring child anthropometrics. They are ideally situated for screening of children at risk for adverse growth patterns, obesity and cardiovascular disease. However, funding and evidence-based, long-term, family-oriented

interventions are often lacking. More interventions should be researched and funding should be made available to implement the already existing interventions. Interventions could focus on healthy diet, lifestyle, sedentary time and physical activity for the whole family.

CONCLUSION OF THIS THESIS

Findings from this thesis suggest that early-life growth and adiposity related factors are associated with cardiac structure and adverse cardiovascular outcomes in children. Although the associations were small, they might be important on a population level. Both fetal life and early childhood are critical periods for later cardiovascular health.

Chapter 5 | Summary



Summary

Chapter 1 describes the background and hypothesis for the studies presented in this thesis. Cardiovascular disease is a major public health problem worldwide and might originate in early life. The developmental origins hypothesis suggests that adverse exposures in fetal and postnatal life might lead to cardiovascular adaptations. This enables the individual to survive in the short term, but these adaptations can also predispose individuals to cardiovascular disease later in life. This is supported by experimental studies in animals and by large observational studies in humans. The effects on the cardiovascular system are already present in childhood. Not only fetal life, but also early postnatal life is important for cardiovascular health. Common risk factors for cardiovascular disease, such as blood pressure, lipid levels and obesity track from childhood to adulthood. Thus, cardiovascular disease might originate in early life. Both an adverse fetal environment and an affluent postnatal environment seem to adversely affect cardiovascular health from childhood to adulthood. Identifying the risk factors and the sensitive periods in early life and the mechanisms through which early life affects cardiovascular health are important for future preventive strategies to ensure cardiovascular health later in the life course. Therefore, studies presented in this thesis were designed to identify fetal, infant and childhood factors associated with cardiovascular health outcomes in childhood. The studies were particularly focused on the role of growth and adiposity in specific early-life periods.

In **Chapter 2** studies on fetal, infant and childhood growth on cardiovascular outcomes are described. In **Chapter 2.1** we observed that cardiac structures track moderately from early infancy into school-age. Tracking was stronger from 24 months of age into school-age. Moderate tracking of cardiac structures suggests that cardiac structures are at least partly determined in early life, but if this related to cardiac disease should be further studied. The influence of growth patterns in fetal life and infancy on cardiovascular health was discussed in **Chapter 2.2**. We observed that both birthweight and infant weight gain were associated with increased childhood blood pressure. Infant weight gain could be more important than birth weight in predicting blood pressure across the life course. Higher birth weight was also associated with larger cardiac structures in childhood. A possible mediation mechanism might be in place, where these children have a higher lean mass and thus larger cardiac structures. Fetal growth deceleration followed by infant growth deceleration was also associated with larger left ventricular mass, but this might be caused by a different mechanism. In **Chapter 2.3** we have studied the associations of infant

growth velocity patterns with cardiometabolic health in childhood. Peak weight velocity was associated with higher blood pressure in childhood. A higher BMI peak in infancy was also associated with higher childhood blood pressure, but this was explained through higher childhood BMI. Thus, faster infant growth places an individual not only at risk for increased blood pressure, but also for later adiposity. We examined the effects of fetal, infant and childhood growth on cardiac structure in more detail at the age of 10 years (**Chapter 2.4**). Both fetal and childhood growth, but not infant growth were critical for the development of cardiac dimensions. Children larger at birth, but taller and leaner in childhood had larger cardiac mass, but no adverse geometry. Children who were smaller at birth, and shorter and heavier in childhood had increased concentricity of the left ventricle. How these differences in cardiac structure relate to cardiac disease remains to be studied. In **Chapter 2.5** we studied the effects of fetal hemodynamics on childhood cardiac structure and function. An adverse fetal environment not only influences fetal growth, but also fetal hemodynamics. We observed that increased placental resistance and fetal cardiac blood flow redistribution may have long term effects on cardiac function and structure.

In **Chapter 3** we presented studies on the associations between maternal and child obesity and body composition on cardiovascular health. **Chapter 3.1** discussed the associations between maternal obesity, gestational weight gain and childhood cardiovascular health. Maternal obesity and weight gain in early pregnancy, and paternal obesity were associated with larger childhood cardiac structures. However, we did not observe a direct intra-uterine effect of maternal BMI or gestational weight gain on childhood cardiac size, the associations were explained by childhood BMI. Shared genetics with both parents, or family life style might affect cardiac size through childhood BMI. In **Chapter 3.2** we observed that childhood obesity was associated with larger cardiac structures. However, differences in body composition might be important. Lean mass was a stronger determinant of cardiac size than fat mass. Obese children also showed more concentricity of the left ventricle, which might place them at increased risk for cardiac disease in later life. Pericardial adipose tissue was examined in **Chapter 3.3**. We observed that obesity was associated with pericardial fat mass. Independently of general obesity, pericardial fat mass was not associated with larger cardiac mass or concentricity. However, higher pericardial fat mass in children was associated with an adverse body fat distribution and unfavorable lipid
concentrations independently of general obesity, possibly influencing cardiovascular health across the life course.

In **Chapter 4** we provide a general discussion, in which the studies described in this thesis were described in a broader context. Also, implications and suggestions for future research were discussed. In conclusion, findings from this thesis suggest that early-life growth and adiposity related factors are associated with cardiac structure and adverse cardiovascular outcomes in children. Future studies should focus on how changes in cardiac structure and cardiovascular outcomes in childhood relate to cardiovascular disease risk in adulthood. Preventive strategies should start early in life and focus on (pre)pregnancy weight and habits. Since not only fetal life, but also early childhood is an important critical period for later cardiovascular health, preventive strategies and early interventions in early childhood might be effective in improving cardiovascular health.

Samenvatting

Hoofdstuk 1 beschrijft de achtergrond en hypothese voor de studies die gepresenteerd worden in deze thesis. Hart- en vaatziekten zijn een enorm probleem voor de volksgezondheid wereldwijd en ontstaan waarschijnlijk in het vroege leven. De `developmental origins´ hypothese stelt dat ongunstige blootstellingen in de foetale en postnatale periode kunnen leiden tot aanpassingen aan hart en vaten. Op de korte termijn zorgt dit ervoor dat het individu in leven blijft, maar op de lange termijn kunnen deze aanpassingen juist zorgen voor verhoogde vatbaarheid voor hart- en vaatziekten. Deze hypothese wordt ondersteund door experimentele studies in dieren en door grote observationele studies in mensen. De effecten van ongunstige blootstellingen vroeg in het leven zijn al zichtbaar in de kindertijd. Niet alleen de foetale periode, maar ook het de vroege postnatale periode zijn belangrijk voor cardiovasculaire gezondheid. Bekende risicofactoren voor hart- en vaatziekten, zoals hoge bloeddruk, lipidenniveaus, en obesitas tracken van de kindertijd tot in volwassenheid. Hart- en vaatziekten ontstaan dus mogelijk al in het vroege leven. Een nadelige foetale omgeving en een overvloedige postnatale omgeving lijken een negatief effect te hebben op gezondheid van hart en vaten vanaf de kindertijd tot aan volwassenheid. Het identificeren van risico factoren en de gevoelige periodes vroeg in het leven, en de mechanismen over hoe factoren in het vroege leven de latere gezondheid beïnvloeden zijn belangrijk voor het ontwikkelen van preventie strategieën en bevorderen van cardiovasculaire gezondheid later in het leven. Daarom zijn de studies die gepresenteerd worden in deze thesis gericht op het identificeren van foetale, zuigeling en kind factoren die samenhangen met cardiovasculaire gezondheid op de schoolleeftijd. De studies richten zich in het bijzonder op de kritische periodes in het vroege leven.

In **Hoofdstuk 2** werden studies over de groei in de foetale periode, zuigelingentijd en kindertijd en het effect op cardiovasculaire uitkomsten beschreven. In **Hoofdstuk 2.1** zien we dat hartstructuren redelijk tracken van de vroege zuigelingenperiode tot aan de schoolleeftijd. Het tracken was het sterkste tussen 24 maanden en schoolleeftijd. Redelijk tracken van hartstructuren suggereert dat afmetingen van hartstructuren ten minste deels al vroeg in het leven worden bepaald, maar hoe dit samenhang met hart- en vaatziekten behoeft meer onderzoek. De invloed van groeipatronen in de foetale en zuigelingenperiode op cardiovasculaire gezondheid is besproken in **Hoofdstuk 2.2**. We observeerden dat geboortegewicht en zuigelingen gewichtstoename beiden samenhangen met verhoogde bloeddruk op de kinderleeftijd. Gewichtstoename in de

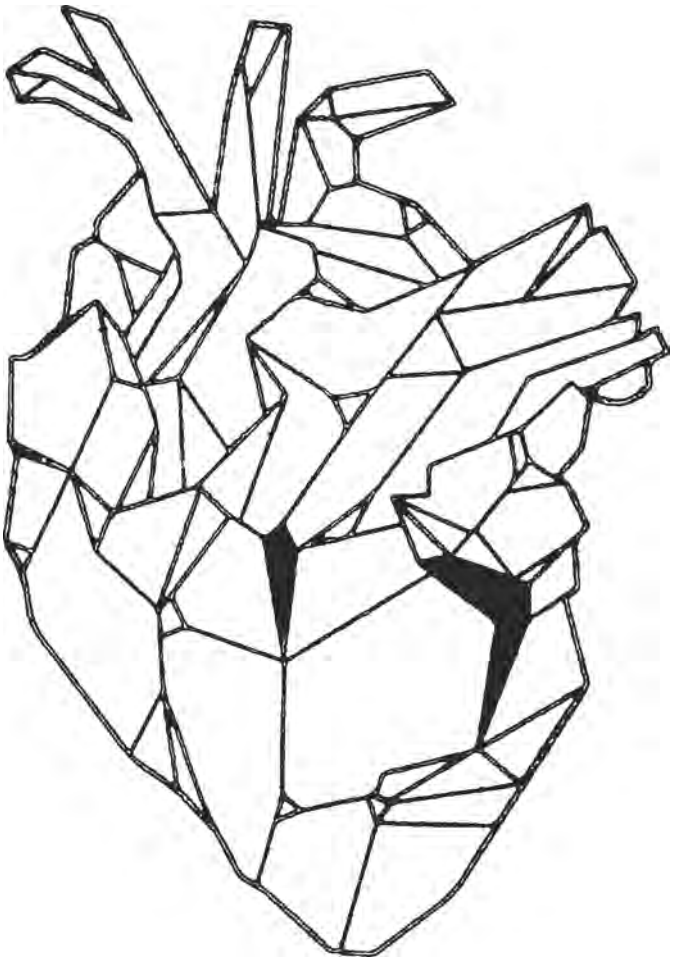
zuigelingenperiode is mogelijk belangrijker in het voorspellen van bloeddruk over de levensduur dan geboortegewicht. Hoger geboortegewicht hing ook samen met grotere hartstructuren op kinderleeftijd. Mogelijk speelt hier een mediërend mechanisme, waarbij deze kinderen een hogere vetvrije massa hebben en dus een groter hart. Foetale groei deceleratie gevolgd door deceleratie van gewicht in de zuigelingenperiode hing ook samen met een grote massa van het linker ventrikel, maar dit kan ook veroorzaakt worden door weer een ander mechanisme. In **Hoofdstuk 2.3** hebben we de associaties van zuigelingengroei en zuigelingengroei snelheid met cardiometabole gezondheid bestudeerd. Maximale snelheid van gewichtstoename hing samen met hogere bloeddruk op de kinderleeftijd. Een hogere BMI-piek in de zuigelingenperiode ging ook samen met hogere bloeddruk op de kinderleeftijd, maar dit werd verklaart door hogere kinderleeftijd BMI. Snellere groei als zuigeling verhoogt dus niet alleen het risico op hoge bloeddruk, maar ook voor overgewicht. De effecten van foetale, zuigelingen kindergroei op hartstructuren hebben we in meer detail bestudeerd op de leeftijd van 10 jaar (**Hoofdstuk 2.4**). Foetale en kindergroei, maar niet zuigelingengroei, waren belangrijk voor de ontwikkeling van de hartafmetingen. Kinderen die groter waren bij de geboorte, en langer en slanker als kind hadden relatief grotere hartstructuren. Kinderen die juist kleiner waren bij de geboorte, en in de kindertijd minder snel groeiden, maar juist wel in gewicht meer aankwamen, hadden een grotere concentriciteit van het hart. Hoe deze veranderingen in hartstructuur samenhangen met hart- en vaatziekten moet verder onderzocht worden. In **Hoofdstuk 2.5** hebben we gekeken naar de effecten van foetale hemodynamica of hartfunctie en structuur op kinderleeftijd. Een ongunstige foetale omgeving heeft niet alleen invloed op de groei van de foetus, maar ook op de foetale hemodynamica. We observeerden dat verhoogde weerstand in de placenta, en redistributie van bloedstroming door het foetale hart lange termijn effecten kunnen hebben op functie en structuur van het hart.

In **Hoofdstuk 3** presenteerden we de studies over de associaties tussen moeder en kind overgewicht en lichaamssamenstelling op cardiovasculaire gezondheid. **Hoofdstuk 3.1** bediscussieerde de associaties tussen maternaal overgewicht, gewichtstoename vroeg in de zwangerschap, en gezondheid van hart en vaten op kinderleeftijd. Maternaal overgewicht, gewichtstoename vroeg in de zwangerschap en paternaal overgewicht waren geassocieerd met grotere hartstructuren in het kind. We zagen echter geen direct intra-uterien effect van maternaal BMI of gewichtstoename op hartafmetingen van het kind. De associaties

werden verklaard door BMI van het kind. Gedeelde genetica met de ouders, of familie levenswijze hebben mogelijk effect op hartafmetingen via BMI op kinderleeftijd. In **Hoofdstuk 3.2** hebben we gezien dat obesitas op de kinderleeftijd samen hing met grotere hartstructuren. Waarschijnlijk zijn verschillen in lichaamssamenstelling van belang. Vetvrije massa was een sterkere voorspeller van hartafmetingen dan vetmassa. Kinderen met obesitas hadden een meer concentrisch hart, wat een hoger risico geeft op latere hartziekten. Pericardiale vetmassa was onderzocht in **Hoofdstuk 3.3**. Obesitas ging samen met hogere pericardiale vetmassa. Onafhankelijk van algehele obesitas hing hogere pericardiale vetmassa niet samen met grotere hartstructuren. Daarentegen hing hogere pericardiale vetmassa in kinderen wel samen met een nadelige lichaamsvetverdeling en lipidenconcentraties in het bloed, wat een verhoogd risico op latere hart- en vaatziekten kan geven.

In **Hoofdstuk 4** bieden we de algehele discussie, waarin de studies die beschreven worden in deze thesis in een bredere context worden gezet. Ook bespreken we de implicaties en suggesties voor verder onderzoek. In conclusie suggereert deze thesis dat groei in het vroege leven en factoren samenhangend met adipositas zijn geassocieerd met hartstructuur en nadelige cardiovasculaire uitkomsten in kinderen. Toekomstige studies zouden zich kunnen richten op hoe deze veranderingen op kinderleeftijd samen hangen met later risico op hart- en vaatziekten. Preventie strategieën zouden vroeg in het leven moeten starten, en zich richten op gezond gewicht en gewoonten net voor en tijdens de zwangerschap. Omdat niet alleen de foetale periode, maar ook de vroege kindertijd een belangrijke periode is voor de ontwikkeling van een gezond hart en vaten, zouden preventie en interventie strategieën in de vroege kindertijd ook effectief kunnen zijn in het verbeteren van latere cardiovasculaire gezondheid.

Chapter 6 | Appendices



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About the author

Liza Toemen was born March 8, 1989 in Oirschot, the Netherlands. She graduated from secondary school (gymnasium) at the Jacob-Roelandslyceum in Boxtel in 2007. In the same year she started her medical education at Maastricht University, Maastricht, the Netherlands. In the first year of her studies she started her scientific career (2008-2010) as data coordinator in the TIF vs PPI Study in the department of surgery of the Maastricht University Medical Center under supervision of prof. dr. N. Bouvy and dr. B. Witteman. After obtaining the bachelor's degree in medicine, her medical internships were mostly in the Eindhoven area (Catharina Ziekenhuis). However, some internships were a bit farther away. Starting with an elective in pediatrics in Kampala, Uganda, later she also went to Sint-Truiden, Belgium for ophthalmology and to Cape Town, South Africa for gynecology and obstetrics. The last clinical internship was in pediatrics in the Maxima Medisch Centrum, Veldhoven. The last stage of her medical training was a scientific internship under supervision of dr. M. Kroupina in the International Adoption Clinic, University of Minnesota, Minneapolis, USA. The scientific career progressed from this into the current PhD-project entitled 'Early life growth, adiposity and cardiovascular health in childhood' under supervision of prof. dr. V.W.V. Jaddoe (Departments of Epidemiology and Pediatrics, Erasmus MC, Rotterdam, the Netherlands) and prof. dr. W.A. Helbing (Department of Pediatrics, Erasmus MC, Rotterdam, the Netherlands). The results of this work are presented in this dissertation. During her PhD-project, she obtained her Master of Science in Clinical Epidemiology, NIHES, Rotterdam (2014-2016). From August 2018 onwards she has been slowly working to finish this dissertation, while also starting work as a jeugdarts (youth healthcare physician) at the Centrum voor Jeugd en Gezin (CJG) Den Haag. She hopes to be admitted to the residency program in the near future.

PhD Portfolio

SUMMARY PHD TRAINING AND TEACHING ACTIVITIES

Name of PhD student:	Liza Toemen
Erasmus MC Department:	Epidemiology
Research School:	Netherlands Institute for Health Sciences
PhD period:	December 2013 – December 2019
Promotors:	Prof. dr. V.W.V. Jaddoe
	Prof. dr. W.A. Helbing

	Year	Workload (ECTS)
1. PhD Training		
General courses		
Master´s degree Health sciences, Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2014-2016	70
Common Core		
Study Design		4.3
Biostatistical Methods I: Basic Principles		5.7
Development Research Proposal		2.5
Biostatistical Methods II: Classical Regression Models		4.3
Research period PIN Health Sciences		29.6
Oral Research Presentation		1.4
Advanced courses		
Clinical Epidemiology		5.7
Methodologic Topics in Epidemiologic Research		1.4
Principles of Research in Medicine and Epidemiology		0.7
Methods of Public Health Research		0.7
Clinical Trials		0.7
Health Economics		0.7
The Practice of Epidemiologic Analysis		0.7
Fundamentals of Medical Decision Making		0.7
Elective Courses		
Missing Values in Clinical Research		0.7
Topics in Meta-analysis		0.7
Causal Inference		0.7
History of Epidemiologic Ideas		0.7
Advanced Analysis of Prognosis Studies		0.9
Principles of Epidemiologic Data-analysis		0.7
Health Services: Research and Practice		0.9
From Problem to Solution in Public Health		1.1
Courses for the Quantitative Researcher		1.4
Introduction to Public Health		0.7
Repeated Measurements in Clinical Studies		1.4

	Year	Workload (ECTS)
General academic skills		
Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC, the Netherlands	2014	0.7
MRI Safety Course, Erasmus MC, the Netherlands	2014	0.7
Biomedical English Writing and communication, Erasmus MC, the Netherlands	2016	3.0
Research Integrity Course, Erasmus MC, the Netherlands	2016	0.7
Seminars		
Generation R Research Meetings	2013-2018	1.0
Generation R Maternal and Child Health meetings	2013-2018	1.0
ACE Pregnancy & Childhood meetings	2017-2018	1.0
(Inter)national congresses and presentations		
Power of Programming, Munich, Germany. *Poster Oral Presentation*	2014	0.7
Developmental Origins of Health and Disease (DOHaD), Cape Town, South Africa. *Poster Oral Presentation*	2015	0.7
Power of Programming, Munich, Germany. *Poster Presentation*	2016	0.7
Sophia Research Day, Erasmus MC, Rotterdam. *SLAM Oral Presentation*	2016	*1.4*
Pregnancy complications and offspring cardiovascular health, Wolfson College, Oxford, UK. *Invited Speaker*	2016	*1.4*
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands. *SLAM Oral Presentation*	2017	1.4
Grants		
Vereniging Trustfonds Erasmus Universiteit Rotterdam, several travel grants	2013-2017	
Other		
Reviewed article for the Journal of Pediatrics	2018	
Participating in abstract reviewing and volunteer for DOHaD, Rotterdam	2017	

	Year	Workload (ECTS)
2. Teaching		
Supervision Master's and Bachelor's theses		
Gavro Jelic, Master of Science, Clinical Epidemiology, NIHES, the Netherlands. Project title: *Obesity and cardiac structure in school-age children, evaluated by magnetic resonance imaging.*	2015	2.0
Tamara Marinkovic, Master of Science, Clinical Epidemiology, NIHES, the Netherlands. Project title: *Maternal blood pressure during pregnancy and intima-media thickness in school-aged children.*	2015	2.0
Iris Pieters, Medical Student, Erasmus MC, the Netherlands. Project title: *Ethnic Differences in Childhood Carotid Intima-Media Thickness.*	2016	2.0
Gavro Jelic, Doctor of Science, NIHES, the Netherlands. Project titles: *Third trimester fetal cardiac blood flow and cardiac outcomes in school-age children assessed by magnetic resonance imaging.* *Associations of blood pressure with cardiac outcomes evaluated by magnetic resonance imaging in school-age children.*	2016-2017	4.0
Tamara Marinkovic, Doctor of Science, NIHES, the Netherlands. Project titles: *Early infant growth velocity patterns and bone health in school-aged children. A multi-ethnic population-based prospective cohort study.* *Early infant growth velocity patterns and cardiovascular and metabolic outcomes in childhood.*	2016-2017	4.0
Wouter van Genuchten, Medical Student and Clinical Epidemiology student, NIHES, the Netherlands. Project title: *Ethnic differences in childhood right and left cardiac structure and function assessed by cardiac magnetic resonance imaging.*	2017-2018	2.0
Teaching Assistant		
Practical skills in Clinical Trials for Medical students	2016-2017	0.4

Dankwoord

Eindelijk mag ik het dankwoord schrijven. De laatste loodjes. Of eigenlijk niet, want ik moet nog best wel wat doen aan de lay-out of aan papers die die al dan niet onder revisie zijn, of aan het regelen van een feestje, en een drukker, en een lekenpraatje en ik weet niet eens wat nog meer allemaal. Afijn, het dankwoord is het laatste wat jullie lezen, of misschien wel het enige. Want een dankwoord leest toch net iets makkelijker dan 3 hoofdstukken in academisch Engels. En als je geluk hebt wordt je zelfs nog vernoemd. Tip: op bladzijde 222 begint de Nederlandse samenvatting, dus als je indruk wil maken en wil doen of je mijn hele proefschrift hebt gelezen, zou ik daar verder gaan lezen. Voel je zeker niet verplicht om alle hoofdstukken door te nemen. Hoewel ik met veel plezier 6 jaar aan dit project heb gewerkt, vind ik het zelf ook best saai om alles terug te lezen. Een spannende detective, historische roman of een fantasyboek leest toch lekkerder weg.

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zouden kunnen schrijven. 6 jaar verder kunnen we concluderen dat ik iets meer tijd nodig had. Dat lag zeker niet aan jou, Vincent, het is ongelooflijk hoe snel ik reacties kreeg met commentaar op papers, of met nieuwe ideeën. Helaas duurde het toch allemaal iets langer, en was het heel veel meer werk dan ik van tevoren had ingeschat om alle hart MRI data op orde te krijgen. En daar komt ook nog eens bij dat ik niet op zondagnacht om 3 uur aan het werk ging en je nuttige commentaar dus pas op maandagochtend weer zag. Beste Janine, ik schreef dat ik mijn promotoren ging bedanken in deze paragraaf, maar toch wijd ik hier een regeltje aan jou. Jij hebt me enorm geholpen met de start van mijn traject, en hoewel ons eerste project niet in deze thesis staat, ben ik je wel dankbaar. Beste Wim, niet betrokken bij mijn project vanaf mijn eerste werkdag, maar wel vanaf de start van mijn projecten waarbij het hart betrokken was. Hartelijk dank voor uw hulp bij het verbeteren van de hartscans, het beoordelen en scoren van de scans, en uw nuttige input op cardiologisch gebied voor mijn manuscripten.

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Het loopbaantraject richting wetenschapper begon niet pas 6 jaar geleden. Ik weet niet meer precies wat en wanneer mijn eerste stappen waren, maar mogelijk begon het met het leren lezen aan de ontbijttafel toen ik een jaar of 4 was: melk, hagelslag, relativiteitstheorie. Bedankt paps en mams, jullie hebben me altijd gesteund en gezorgd dat ik kon leren, studeren en reizen. Bedankt voor alle liefde, steun en goede zorgen. En voor het helpen met 9 keer verhuizen. Of juist me onderdak bieden als ik door coschappen in het buitenland tijdelijk zonder woonruimte zat. Iris en Erik, ik kan jullie niet echt bedanken dat jullie mijn grote zus en kleine broer zijn, daar kunnen jullie ook niks aan doen. Maar, ik had geen betere broer en zus kunnen wensen, en ben blij dat jullie me vroeger ook ooit dwongen om sociaal te zijn en te spelen, als ik weer eens een leeso was ([1]leeso: (de; m, v; meervoud: leeso´s) (Helleweikes, Nederland, informeel) bijvoeglijk naamwoord: samenvoeging van lezen+aso). En jullie snappen tenminste dat goed werk tijd nodig heeft en dat sommige studies nou eenmaal 6 jaar kunnen duren.

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