Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

Birthweight-for-gestational-age *z*-scores are associated with early childhood cardiometabolic health in the Peri/Postnatal Epigenetic Twin Study

Deborah N. Ashtree[1,2], Mihiri J. Silva[3,4,5], Cong Sun[6], Jeffrey M. Craig[6,7] and Katrina J. Scurrah[1,2]

[1]Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Parkville, Australia; [2]Twins Research Australia, Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Parkville, Australia; [3]Inflammatory Origins, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia; [4]Melbourne Dental School, University of Melbourne, Parkville, Australia; [5]Melbourne Medical School, University of Melbourne, Royal Children's Hospital, Parkville, Australia; [6]Epigenetics, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia and [7]IMPACT – the Institute for Mental and Physical Health and Clinical Translation, School of Medicine, Faculty of Health, Deakin University, Waurn Ponds, Australia

Original Article

Cite this article: Ashtree DN, Silva MJ, Sun C, Craig JM, and Scurrah KJ. (2022) Birthweight-for-gestational-age *z*-scores are associated with early childhood cardiometabolic health in the Peri/Postnatal Epigenetic Twin Study. *Journal of Developmental Origins of Health and Disease* **13**: 514–522. doi: 10.1017/S2040174421000465

Received: 26 April 2021
Revised: 16 June 2021
Accepted: 6 July 2021
First published online: 23 August 2021

Keywords:
Birthweight; adiposity; blood pressure; cardiometabolic health; twins

Address for correspondence:
Dr Katrina J. Scurrah, Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia.
Email: kscurrah@unimelb.edu.au

Abstract

Birthweight has been consistently related to risk of cardiometabolic disorders in later life. Twins are at higher risk of low birthweight than singletons, so understanding the links between birthweight and cardiometabolic health may be particularly important for twins. However, evidence for the association of birthweight with childhood markers of cardiometabolic health in twins is currently lacking. Previous studies have often failed to appropriately adjust for gestational age or fully implement twin regression models. Therefore, we aimed to evaluate the association of birthweight-for-gestational-age *z*-scores with childhood cardiometabolic health in twins, using within-between regression models. The Peri/Postnatal Epigenetic Twins Study is a Melbourne-based prospective cohort study of 250 twin pairs. Birthweight was recorded at delivery, and childhood anthropometric measures were taken at 18-month and 6-year follow-up visits. Associations of birthweight with markers of cardiometabolic health were assessed at the individual, between- and within-pair level using linear regression with generalised estimating equations. Birthweight-for-gestational-age *z*-scores were associated with height, weight and BMI at 18 months and 6 years, but not with blood pressure (twins-as-individual SBP: $\beta = 0.15$, 95% CI: −0.81, 1.11; twins-as-individual DBP: $\beta = 0.22$, 95% CI: −0.34, 0.77). We found little evidence to indicate that the within-between models improved on the twins-as-individuals models. Birthweight was associated with childhood anthropometric measures, but not blood pressure, after appropriately adjusting for gestational age. These associations were consistent across the within-between and twins-as-individuals models. After adjusting for gestational age, results from the twins-as-individuals models are consistent with singleton studies, so these results can be applied to the general population.

Introduction

Cardiometabolic diseases are a major contributor to global morbidity and mortality, accounting for more than 17 million deaths every year.[1] Previous studies have demonstrated a link between birthweight and later-life cardiometabolic health, resulting in the Developmental Origins of Health and Disease (DOHaD) hypothesis.[2] This hypothesis suggests that early-life factors, including fetal programming, and genetic, developmental and environmental factors may permanently affect body structure and function and contribute to diseases later in life.

Birthweight is one of the most investigated of the DOHaD exposures, and many studies have already reported links between birthweight and cardiometabolic health outcomes later in life.[3–5] Previous studies reporting associations of birthweight with anthropometric measures in childhood and adulthood have also demonstrated that height, weight and body mass index (BMI) may track from early life into adulthood.[6,7] If childhood anthropometric measures track into adulthood, they may act as a predictor of obesity and cardiometabolic conditions in later life.[8] Despite the growing popularity of the DOHaD hypothesis, few studies assessing the developmental origins of cardiometabolic health have explored the association of birthweight with cardiometabolic health outcomes in twins.[9]

Twin pregnancies are at higher risk of preterm delivery and intrauterine growth restriction (IUGR) compared to singleton gestations, both of which contribute to smaller size at birth.[10]

CAMBRIDGE UNIVERSITY PRESS

Given the reported association between low birthweight and cardiometabolic outcomes in the general population, the higher risk of low birthweight in twins may predispose them to greater cardiometabolic risk later in life. Many twin studies have explored associations of birthweight with later-life BMI and blood pressure, with some studies reporting that higher birthweight leads to higher BMI but lower blood pressure, and others finding no evidence for an association.[9] Differences in study designs and analytic approaches may have led to differences in conclusions, as many previous studies have failed to appropriately adjust for gestational age or fully implement twin regression models (described below). Furthermore, most twin studies have focussed on a single marker of cardiometabolic health, such as blood pressure.[9] However, many cardiometabolic health markers cluster to provide an individuals' overall cardiometabolic risk profile, and different markers may predict different cardiometabolic health outcomes. Therefore, exploring associations with only a single marker of cardiometabolic health neglects the importance of measuring cardiometabolic health with multiple markers and limits interpretations to only one facet of cardiometabolic health. As such, the evidence for an association of birthweight with later cardiometabolic health in twins is still somewhat unclear.

Though the association of birthweight with later-life health outcomes may be particularly important for twins, results assessing the DOHaD hypothesis in twins can be applied to the general population. Twin studies can be used to reduce familial confounding, and after adjusting for gestational age, results from twin studies are largely applicable to the general population.[11] However, a recent systematic review found that few twin studies assessing associations of birthweight with cardiometabolic health have adjusted for gestational age. Previous studies which have adjusted for gestational age often only included gestational age as a covariate, which introduced an issue of collinearity.[9] Since low birthweight can result from either preterm birth (especially in twins) or from IUGR – which have different risk factors, prevention strategies, postnatal management and even long-term health outcomes[12–15] – studies which do not appropriately account for gestational age cannot disentangle the effect of birthweight from gestational age and may lead to incorrect estimates of associations. In fact, twin studies which do not adjust for gestational age often report an association of birthweight with later-life cardiometabolic health, whereas studies which do adjust for gestational age do not detect an association.[9] Recognising whether low birthweight is a result of preterm birth or IUGR may be important for understanding long-term health outcomes, so it is important for any study reporting associations of birthweight with later-life health to be appropriately adjusted for gestational age.

Finally, few twin studies have fully implemented twin regression models to assess the within- and between-pair specific associations of birthweight with later-life health.[9] Studying the association of birthweight with later-life health outcomes can be difficult due to confounding, particularly by shared factors. However, twin studies provide a unique opportunity to determine the effect of shared versus unshared factors on the association of birthweight with later-life health. Twins share many factors during gestation, so if there are differences in twin birthweight or twin cardiometabolic outcomes, these cannot be due to shared factors.[11] However, not all gestational exposures are shared between twins, such as blood–nutrient supply, which may lead to differences in birthweight, and potentially later-life health outcomes.[11] Therefore, determining whether between-pair (shared) and within-pair (non-shared) associations differ can provide greater insight into the mechanism behind the association of birthweight with later-life cardiometabolic health outcomes.

Based on limitations of previous studies assessing associations of birthweight with later-life cardiometabolic health in twins, especially twin children, we aimed to:

1. Explore the role of birthweight after correcting for gestational age, by using birthweight-for-gestational-age standard deviation scores;
2. Understand the association of birthweight with a range of cardiometabolic markers; and
3. Make full use of the additional information contained in data from twin pairs, by implementing within-pair and between-pair regression models, to establish whether there may be a causal relationship of birthweight with cardiometabolic health.

Method

The Peri/Postnatal Epigenetic Twins Study (PETS) is a prospective twin cohort study established in 2007 in Melbourne, Australia. Women pregnant with twins were recruited during their second trimester from 3 pregnancy clinics in Melbourne (the Royal Women's Hospital, Mercy Hospital for Women and Monash Medical Centre). Women were excluded if they planned to leave the area before delivery or if they had limited English language skills. Of the 287 women recruited during pregnancy, 250 mothers and their twins remained in the study at birth, of which 172 pairs of twins were included in these analyses. Details of loss to follow-up are summarised in Fig. 1.

The primary exposure of interest was twin birthweight. Birthweight was recorded at delivery by trained research assistants or accessed from birth records when birth attendants were not present at delivery. Birthweight-for-gestational-age *z*-scores were calculated using Australian twin birthweight reference charts,[16] and gestational age was estimated from the women's last menstrual period and confirmed with ultrasound.

Participating mothers completed questionnaires on pre- and post-conception lifestyle factors, including smoking habits, alcohol intake and medical history. Follow-up of the twins occurred at age 18 months and at age 6 years, when mothers completed a questionnaire on the health, development and nutritional status of the twins. Anthropometric measurements and blood pressure were used as markers of cardiometabolic health. Anthropometric measurements were taken by a trained research assistant at the 18-month and 6-year follow-up visit. Height was measured using a stadiometer, and weight was measured using a digital weight scale. Skinfold thicknesses were measured using a Holtain calliper – triceps skinfold measurements were taken 1 cm below the mid-point between the shoulder and elbow, and subscapular skinfolds were picked up on a diagonal plane just inferior to the angle of the scapula. Head circumference was measured just above the supra-orbital ridge, arm circumference was measured at the mid-point between the shoulder and elbow and abdominal circumference was measured at the uppermost lateral border of the ilium. Anthropometric *z*-scores, accounting for twin sex and age, were calculated using UK anthropometric charts,[17] and using the Zanthro package in Stata.[18] Growth was calculated as change in anthropometric *z*-score between birth and 18 months and between birth and 6 years. A sphygmomanometer with cuff size at least two-thirds of the upper arm (and able to be wrapped around and secured) was used to measure blood pressure. The cuff was fitted on the upper arm, on bare skin or over light clothing only.

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

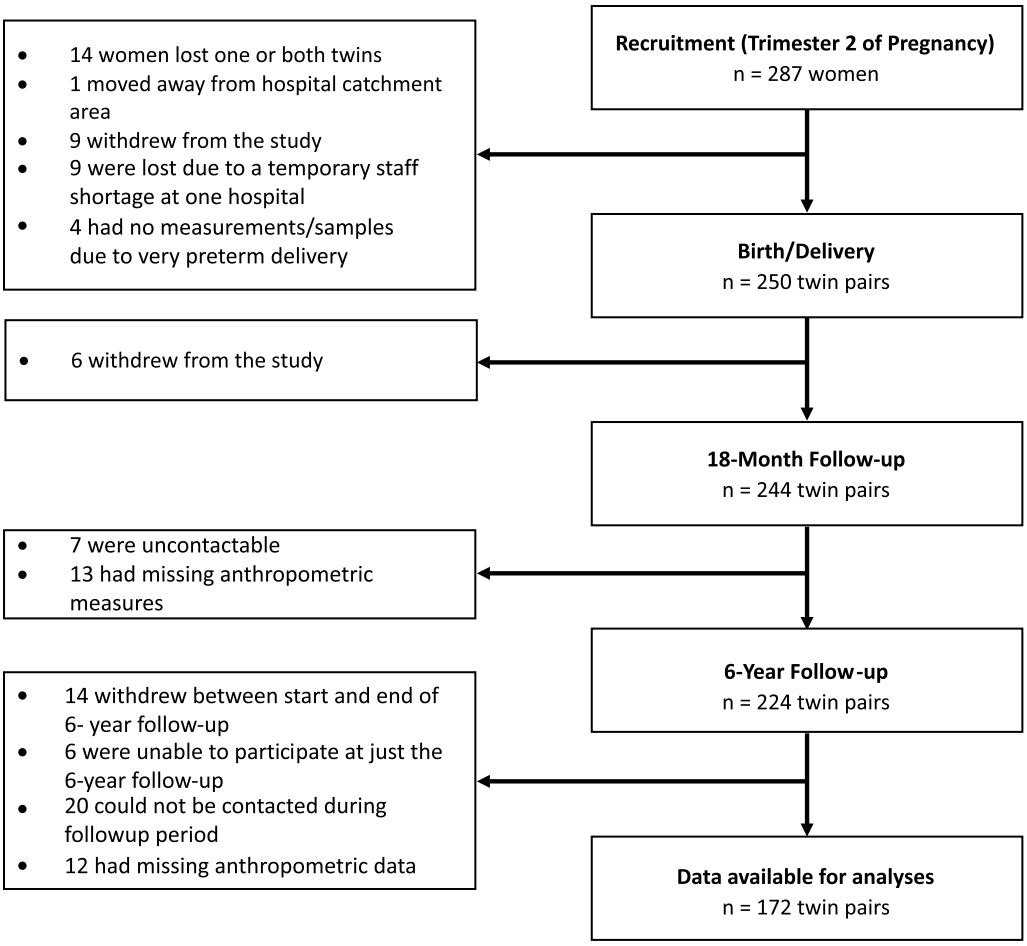

Fig. 1. Flow chart describing participant loss to follow-up from recruitment until the 6-year follow-up visit. A total of 172 pairs of twins were included in these analyses.

Measurements were taken at rest, with elbow resting on a table at approximately heart level. Three measurements were taken, one minute apart. The first of these measurements was excluded, and the average of the 2 subsequent measurements was used in these analyses.

Associations of birthweight with neonatal and childhood cardiometabolic health outcomes were assessed by fitting linear regression models, using generalised estimating equations (GEE) to account for the correlation between twins in each pair. Two regression models were fitted. The first model treated twins as individuals, to determine the overall association of birthweight with later-life cardiometabolic health. The second model allowed the association between birthweight and markers of cardiometabolic health to differ within and between pairs, to determine the within-pair and between-pair specific association of birthweight with later cardiometabolic health. Confounding variables, based on knowledge of the subject area, were assessed for inclusion. Models were adjusted for maternal pre-pregnancy BMI, gestational age at delivery, number of prior pregnancies, total maternal gestational weight gain, smoking during pregnancy, twin sex, chorionicity and zygosity. Simes-adjusted q-values were used to account for multiple testing in all adjusted regression models.[19]

Normality and linearity of each variable were assessed, and variables were transformed where necessary. The distribution and influence of residuals were assessed, and highly influential observations removed in a sensitivity analysis. A further sensitivity analysis, based on inverse probability weighting (IPW), was included to assess the impact of missing data on our results.[20] To determine whether the observed associations were driven by twins discordant for birthweight, we removed twins with a birthweight discordance of 30% or more in an additional sensitivity analysis.

All analyses were performed using Stata 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC.).

Results

Descriptive characteristics of the twins are reported in Table 1. Mean birthweight was 2.47 kg with a mean gestational age of 36 weeks. Most twins were dizygotic (58%) and dichorionic (72%). Smoking during pregnancy was uncommon, with only 12% of women smoking at any point during pregnancy, but 55% of mothers reported drinking alcohol during pregnancy.

18-Month outcomes

When treating twins as individuals, higher birthweight-for-gestational-age z-scores were associated with higher anthropometric measures at 18 months (for example, BMI: $\beta = 0.30$ kg/m^2, 95% CI: 0.14, 0.45, Table 2).

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

Table 1. Descriptive statistics mean (standard deviation) or frequency [percent] for mothers and twin children in the PETS

Mothers at birth	$n = 250$
Twins at birth	$n = 500$
Twins followed-up to 6 years	$n = 344$
Maternal age at delivery (years)	32.69 (5.09)
Mother smoked (at all) during pregnancy	31 [12]
Mother drank alcohol (at all) during pregnancy	137 [54.8]
Gestational age (weeks)	35.87 (2.32)
Caesarean delivery	328 [65.86]
Breastfed (any)	396 [88.20]
Monozygotic twins	208 [41.6]
Monochorionic twins	138 [27.6]
Sex (male)	256 [51.2]
Birthweight (g)	2468.75 (539.63)
Birthweight discordance[a]	
Discordance cut-off: 30%	18 [3.6]
Discordance cut-off: 20%	72 [14.4]
Small for gestational age (based on twin reference charts)	35 [7]
BMI (kg/m^2) at age 6	15.89 (1.77)
SBP (mmHg) at age 6	101.71 (8.11)

[a]Birthweight discordance was calculated as $\frac{(\text{birthweight}_{\text{heaviest twin}} - \text{birthweight}_{\text{lightest twin}})}{\text{birthweight}_{\text{heaviest twin}}} \times 100$; BMI = body mass index; SBP = systolic blood pressure.

Within-pair differences in birthweight were associated with within-pair differences in BMI, weight, head circumference, abdominal circumference and arm circumference, but not height. Pairwise average birthweight was associated with height, weight and head circumference, but not BMI, abdominal circumference or arm circumference (Table 2). For each outcome, the within-pair estimate was approximately equal to the between-pair estimate and there was little evidence to indicate that the within-between model improved on the twins-as-individuals model. As such, the expected difference in each outcome given the difference in birthweight is likely to be the same for twins within a twin pair or between 2 unrelated twin individuals.

Results assessing associations of birthweight with anthropometric measures at 18 months were robust to influential observations. For example, results from the twins-as-individuals model assessing associations of birthweight with 18-month weight z-score changed very slightly from $\beta = 0.27$ (95% CI: 0.15, 0.40) to $\beta = 0.22$ (95% CI: 0.11, 0.32, see Supplementary Table S3) when 11 influential observations were excluded. Interpretations were consistent between the full regression models and IPW models (Supplementary Table S3). Results from the sensitivity analysis removing birthweight discordant pairs led to similar inferences: the twins-as-individuals model changed minimally from $\beta = 0.27$ (95% CI: 0.15, 0.40) to $\beta = 0.23$ (95% CI: 0.10, 0.36, full results not shown).

6-Year outcomes

When treating twins as individuals, higher birthweight-for-gestational-age z-scores were associated with higher anthropometric measures at 6 years (for example, BMI: $\beta = 0.18$ z-scores, 95% CI: 0.04, 0.31, Table 3). Birthweight was not associated with blood pressure at age 6.

Within-pair birthweight was associated with within-pair differences in BMI, but not height, weight or head circumference, and differences in pair-average birthweight were associated with height, weight and head circumference, but not BMI (Table 3). For each outcome, the within-pair estimate was approximately equal to the between-pair estimate, and there was little evidence that the within-between model improved upon the twins-as-individuals model.

Associations of birthweight with twins-as-individuals and within-pair BMI and BMI z-score, between-pair height, and twins-as-individuals and between-pair weight attenuated when removing influential observations (Supplementary Table S4). However, results from all other regression models were robust to influential observations. Inferences from the fully observed models and the IPW models were consistent. Inferences were consistent between the fully observed model and the model with discordant twins removed (for example, BMI: $\beta = 0.20$ z-scores, 95% CI: 0.06, 0.34, full results not shown).

Growth

Higher birthweight-for-gestational-age z-scores were associated with all measures of growth between birth and 18 months and between birth and 6 years (Supplementary Table S5), and the within-pair estimates were approximately equal to the between-pair estimates. Results were robust to exclusion of influential observations, and inferences were consistent between fully observed and IPW models (Supplementary Table S5).

Discussion

Unlike some previous studies,[21,22] we found no evidence for an association of birthweight with blood pressure in twins. Though blood pressure is an indicator of cardiovascular health, no other cardiovascular outcomes were recorded in the PETS. Furthermore, measuring blood pressure at age 6 may not be representative of adult blood pressure. Based solely on results from our study, it is difficult to conclusively determine that birthweight is not related to blood pressure or vascular compromise. Previous studies have often shown an association of birthweight with adult blood pressure or vascular function,[9] but these studies have often failed to appropriately adjust for gestational age. In contrast, previous studies which included gestational age as a covariate in regression models also found no evidence for an association of birthweight with later-life blood pressure in twins.[23–25] Although these studies attempted to adjust for gestational age, including gestational age as a covariate with birthweight is limited, as birthweight and gestational age are highly correlated. Instead, by calculating birthweight-for-gestational-age z-scores, we have not only adjusted for gestational age but have also greatly reduced collinearity between birthweight and gestational age. As such, these results are likely to be more robust than previously reported associations of birthweight with blood pressure.

Similarly, previous studies reporting associations of birthweight with later-life BMI often do not adjust for gestational age,[26–28] and studies which do adjust for gestational age find no evidence for an association of birthweight with later BMI.[9,23,29] These studies rarely use birthweight-for-gestational-age z-scores to account for gestational age, and instead use gestational age as a covariate, again

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

Table 2. Results of the regression models assessing associations of overall, within-pair and between-pair birthweight-for-gestational-age *z*-scores with 18-month anthropometric measures

	Unadjusted β (95% CI), *P*-value	Adjusted[a] β (95% CI), *q*-value[b]	*P*-value[c]
BMI (kg/m^2) $n = 285$			
Twins-as-individuals	0.30 (0.20, 0.40), <0.001	0.29 (0.14, 0.45), <0.001	$P = 0.057$
Between-pair	0.18 (0.01, 0.35), 0.035	0.08 (−0.16, 0.33), 0.593	
Within-pair	0.34 (0.21, 0.48), <0.001	0.37 (0.20, 0.55), <0.001	
BMI (*z*-score) $n = 285$			
Twins-as-individuals	0.24 (0.16, 0.31), <0.001	0.23 (0.11, 0.35), <0.001	$P = 0.060$
Between-pair	0.16 (0.02, 0.29), 0.023	0.07 (−0.11, 0.27), 0.526	
Within-pair	0.27 (0.18, 0.37), <0.001	0.29 (0.16, 0.42), <0.001	
Height (cm) $n = 286$			
Twins-as-individuals	0.64 (0.44, 0.85), <0.001	0.47 (0.10, 0.84), 0.025	$P = 0.335$
Between-pair	0.74 (0.39, 1.09), <0.001	0.70 (0.26, 1.14), 0.005	
Within-pair	0.60 (0.29, 0.92), <0.001	0.38 (−0.05, 0.82), 0.129	
Height (*z*-score) $n = 286$			
Twins-as-individuals	0.22 (0.15, 0.28), <0.001	0.16 (0.02, 0.29), 0.043	$P = 0.537$
Between-pair	0.21 (0.08, 0.34), 0.001	0.21 (0.04, 0.39), 0.032	
Within-pair	0.22 (0.11, 0.33), <0.001	0.13 (−0.02, 0.29), 0.133	
Weight (kg) $n = 285$			
Twins-as-individuals	0.37 (0.27, 0.47), <0.001	0.32 (0.18, 0.46), <0.001	$P = 0.448$
Between-pair	0.32 (0.17, 0.48), <0.001	0.25 (0.05, 0.44), 0.027	
Within-pair	0.38 (0.26, 0.51), <0.001	0.34 (0.18, 0.51), <0.001	
Weight (*z*-score) $n = 285$			
Twins-as-individuals	0.32 (0.26, 0.39), <0.001	0.27 (0.15, 0.40), <0.001	$P = 0.328$
Between-pair	0.25 (0.11, 0.38), <0.001	0.19 (0.02, 0.36), 0.050	
Within-pair	0.35 (0.24, 0.45), <0.001	0.30 (0.15, 0.45), <0.001	
Head circumference (cm) $n = 286$			
Twins-as-individuals	0.29 (0.17, 0.42), <0.001	0.28 (0.11, 0.46), 0.005	$P = 0.539$
Between-pair	0.27 (0.06, 0.47), 0.011	0.35 (0.12, 0.58), 0.007	
Within-pair	0.31 (0.12, 0.50), 0.001	0.25 (0.01, 0.48), 0.060	
Head circumference (*z*-score) $n = 286$			
Twins-as-individuals	0.24 (0.15, 0.32), <0.001	0.23 (0.08, 0.37), 0.005	$P = 0.656$
Between-pair	0.18 (0.04, 0.33), 0.015	0.27 (0.08, 0.46), 0.011	
Within-pair	0.27 (0.13, 0.41), <0.001	0.21 (0.02, 0.40), 0.053	
Abdominal circumference (log cm) $n = 278$			
Twins-as-individuals	0.02 (0.01, 0.03), <0.001	0.016 (0.007, 0.024), <0.001	$P = 0.211$
Between-pair	0.010 (0.000, 0.019), 0.050	0.012 (0.002, 0.022), 0.021	
Within-pair	0.023 (0.016, 0.031), <0.001	0.022 (0.009, 0.036), 0.002	
Upper arm circumference (cm) $n = 280$			
Twins-as-individuals	0.20 (0.11, 0.29), <0.001	0.16 (0.03, 0.29), 0.033	$P = 0.282$
Between-pair	0.18 (0.03, 0.33), 0.022	0.06 (−0.13, 0.25), 0.593	
Within-pair	0.21 (0.09, 0.34), 0.001	0.21 (0.05, 0.37), 0.022	

(*Continued*)

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

Table 2. (*Continued*)

	Unadjusted β (95% CI), *P*-value	Adjusted[a] β (95% CI), *q*-value[b]	*P*-value[c]
Triceps skinfold thickness (cm) $n = 274$			
Twins-as-individuals	0.03 (−0.14, 0.20), 0.704	−0.11 (−0.36, 0.16), 0.520	$P = 0.971$
Between-pair	0.11 (−0.20, 0.41), 0.497	−0.11 (−0.52, 0.30), 0.630	
Within-pair	−0.02 (−0.26, 0.21), 0.848	−0.11 (−0.43, 0.22), 0.593	
Subscapular skinfold thickness (cm) $n = 272$			
Twins-as-individuals	0.10 (−0.01, 0.21), 0.076	0.09 (−0.09, 0.27), 0.440	$P = 0.262$
Between-pair	0.01 (−0.20, 0.23), 0.892	−0.07 (−0.36, 0.22), 0.681	
Within-pair	0.13 (−0.01, 0.27), 0.077	0.15 (−0.06, 0.37), 0.223	

[a]Adjusted for maternal pre-pregnancy BMI, gestational age at delivery, number of prior pregnancies, total maternal gestational weight gain, smoking during pregnancy, twin sex, chorionicity and zygosity.
[b]Simes-adjusted *q*-value.
[c]*P*-value when comparing within-between model to twins-as-individual model.
95% CI = 95% confidence interval; BMI = body mass index.

leading to issues of collinearity. Indeed, when using birthweight *z*-scores to adjust for gestational age, we found evidence for an association of birthweight with BMI at 18 months and 6 years. Including gestational age in addition to using birthweight *z*-scores did not alter our results. For example, the association of birthweight *z*-score with BMI *z*-score at 18 months was consistent with ($\beta = 0.236$, 95% CI: 0.154, 0.318) and without ($\beta = 0.236$, 95% CI: 0.153, 0.319) including gestational age as a covariate (full results not shown).

Although we adjusted for gestational age in our analyses, questions around the mechanism for the observed associations persist. Low birthweight can be a consequence of being born preterm, born small-for-gestational-age (SGA, often used as a proxy for IUGR), or both. Previous studies have shown that infants born SGA are at increased risk of neonatal and long-term mortality and morbidity, including neonatal infections, hypoglycaemia and abnormal postnatal growth.[14] Similarly, preterm infants are at higher risk of adverse health outcomes. However, previous studies have suggested this risk is greatest for those born both preterm *and* SGA.[15] This indicates that the mechanism leading to low birthweight or impaired growth may be important for long-term health. However, it is still unclear whether there is an interaction between preterm birth and SGA, particularly in twins. So, although somewhat outside the scope of this paper, we also wanted to investigate whether the association of low birthweight with later anthropometric measures differed according to whether low birthweight was due to SGA, preterm birth or both (Supplementary Table S6). Our results, although limited by sample size showed that the strongest association was for preterm *and* SGA twins, who were smaller on average at 18 months compared to term appropriate-for-gestational-age (AGA) twins. There was a small association for term-SGA twins compared to term-AGA twins, but no difference between preterm-AGA and term-AGA twins. This indicates that the mechanism behind low birthweight may be important for later-life health. Further investigation into whether associations differ for preterm and SGA infants is warranted.

We also found evidence for an association of birthweight with infant and child anthropometric measures. Previous twin studies have reported associations of birthweight with later BMI, height and weight, but few have also explored associations with skinfold thicknesses and body circumferences.[9] Previous studies have found that abdominal skinfold thickness, subscapular skinfold thickness and upper arm circumference are associated with cardiovascular health,[30,31] and size at birth has been associated with body circumference and skinfold thickness.[32] Therefore, associations of size at birth and later-life cardiovascular health may also be acting through body circumference and skinfold thickness. We found evidence for an association of birthweight with head, abdominal and upper arm circumference at 18 months, but not at 6 years of age. We also found no evidence for an association of birthweight with triceps or subscapular skinfold thicknesses at either 18 months or 6 years. This may indicate that size at birth is only associated with body circumferences in the short term, and that by early childhood, the association attenuates. A widely understood phenomenon, known as postnatal growth catch-up,[33] may explain the association of birthweight with infant anthropometrics but not with childhood anthropometric measures.

Children exposed to poor intrauterine growth undergo a period of rapid compensatory postnatal growth in the first few years following birth.[33] Previous studies have found that most children exposed to IUGR show complete growth catch-up by 2 years of age.[33] However, we found evidence that low birthweight after adjusting for gestational age was associated with growth even at 6 years: twins with a higher birthweight had lower growth between birth and 18 months and birth and 6 years. Postnatal growth catch-up has previously been associated with later-life cardiometabolic health,[34,35] so our finding that birthweight may be associated with long-term growth of twins may have further implications for cardiometabolic health outcomes. Further investigation into the association of birthweight with later-life growth is warranted and an additional follow-up of twins in the PETS at 11-years old has commenced, which will enable this.

These results are from a representative sample of Melbourne twins; however, we recognise the limitations of these findings. First, the PETS may be insufficiently powered to detect some associations. Zygosity and chorionicity may play a role in size at birth and in the development of cardiovascular markers. We included an adjustment for zygosity and chorionicity in our regression models, but additional analyses comparing associations for monozygotic and dizygotic twins, or for monochorionic and dichorionic twins, were outside the aims of this study. Further exploration is warranted into whether the association of birthweight with later-life cardiometabolic health differs according to zygosity or chorionicity. Information regarding clinical IUGR was not available from the PETS, so we were limited to using SGA as a proxy measure

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

Table 3. Results from regression models assessing the associations of overall, within-pair and between-pair birthweight-for-gestational-age *z*-scores with anthropometric measures and blood pressure at 6 years

	Unadjusted β (95% CI), *P*-value	Adjusted[a] β (95% CI), *q*-value[b]	*P*-value[c]
BMI (inverse square (1/(kg/m^2)^2)) *n* = 216			
Twins-as-individuals	-1.63×10^{-4} (-2.38×10^{-4}, -8.91×10^{-5}), <0.001	-1.37×10^{-4} (-2.47×10^{-4}, -2.70×10^{-5}), 0.015	*P* = 0.447
Between-pair	-1.57×10^{-4} (-3.01×10^{-4}, -1.28×10^{-5}), 0.033	-5.69×10^{-5} (-2.40×10^{-4}, 1.26×10^{-4}), 0.543	
Within-pair	-1.66×10^{-4} (-2.54×10^{-4}, -7.79×10^{-5}), <0.001	-1.71×10^{-4} (-2.40×10^{-4}, -3.99×10^{-5}), 0.011	
BMI (*z*-score) *n* = 216			
Twins-as-individuals	0.21 (0.12, 0.30), <0.001	0.18 (0.04, 0.31), 0.025	*P* = 0.113
Between-pair	0.17 (−0.02, 0.35), 0.075	0.01 (−0.22, 0.24), 0.947	
Within-pair	0.23 (0.12, 0.33), <0.001	0.25 (0.09, 0.40), 0.005	
Height (inverse cube (1/cm^3)) *n* = 216			
Twins-as-individuals	-1.17×10^{-8} (-2.10×10^{-8}, -2.40×10^{-9}), 0.014	-8.54×10^{-9} (-2.25×10^{-8}, 5.42×10^{-9}), 0.231	*P* = 0.019
Between-pair	-2.77×10^{-8} (-4.22×10^{-8}, -1.31×10^{-8}), <0.001	-3.08×10^{-8} (-4.84×10^{-8}, -1.32×10^{-8}), 0.001	
Within-pair	-7.07×10^{-9} (-1.77×10^{-8}, 3.60×10^{-9}), 0.194	-4.06×10^{-9} (-1.64×10^{-8}, 1.56×10^{-8}), 0.960	
Height (*z*-score) *n* = 206			
Twins-as-individuals	0.17 (0.05, 0.29), 0.004	0.15 (0.03, 0.28), 0.029	*P* = 0.077
Between-pair	0.34 (0.18, 0.51), <0.001	0.28 (0.07, 0.49), 0.018	
Within-pair	0.09 (−0.05, 0.24), 0.218	0.004 (−0.23, 0.24), 0.978	
Weight (inverse (1/kg)) *n* = 216			
Twins-as-individuals	-1.49×10^{-3} (-2.14×10^{-3}, -8.32×10^{-4}), <0.001	-1.18×10^{-3} (-2.10×10^{-3}, -2.49×10^{-4}), 0.013	*P* = 0.202
Between-pair	-2.21×10^{-3} (-3.41×10^{-3}, -1.01×10^{-3}), <0.001	-1.79×10^{-3} (-3.22×10^{-3}, -3.66×10^{-4}), 0.014	
Within-pair	-1.27×10^{-3} (-2.02×10^{-3}, -5.21×10^{-4}), 0.001	-9.47×10^{-4} (-2.02×10^{-3}, 1.23×10^{-4}), 0.083	
Weight (*z*-score) *n* = 200			
Twins-as-individuals	0.25 (0.14, 0.35), <0.001	0.17 (0.02, 0.32), 0.043	*P* = 0.907
Between-pair	0.50 (0.20, 0.70), 0.001	0.62 (0.24, 0.99), 0.003	
Within-pair	0.28 (0.03, 0.52), 0.026	0.28 (−0.02, 0.59), 0.110	
Head circumference (cm) *n* = 216			
Twins-as-individuals	0.36 (0.15, 0.56), 0.001	0.40 (0.15, 0.66), 0.005	*P* = 0.115
Between-pair	0.38 (0.17, 0.60), 0.001	0.41 (0.14, 0.68), 0.007	
Within-pair	0.24 (0.07, 0.41), 0.005	0.22 (−0.01, 0.46), 0.100	
Head circumference (*z*-score) *n* = 204			
Twins-as-individuals	0.29 (0.15, 0.44), <0.001	0.30 (0.11, 0.49), 0.005	*P* = 0.325
Between-pair	0.32 (0.14, 0.49), 0.001	0.19 (0.02, 0.36), 0.053	
Within-pair	0.22 (0.10, 0.34), 0.001	0.21 (0.09, 0.34), 0.003	
Abdominal circumference (inverse cube (1/cm^3)) *n* = 216			
Twins-as-individuals	-2.26×10^{-7} (-3.57×10^{-7}, -9.49×10^{-8}), 0.001	-1.34×10^{-7} (-3.14×10^{-7}, 4.55×10^{-8}), 0.143	*P* = 0.999
Between-pair	-2.58×10^{-7} (-4.75×10^{-7}, -4.14×10^{-8}), 0.020	-1.35×10^{-7} (-4.03×10^{-7}, 1.32×10^{-7}), 0.320	
Within-pair	-2.14×10^{-7} (-3.68×10^{-7}, -5.98×10^{-8}), 0.007	-1.34×10^{-7} (-3.46×10^{-7}, 7.89×10^{-8}), 0.218	
Upper arm circumference (inverse cube (1/cm^3)) *n* = 216			
Twins-as-individuals	-6.72×10^{-6} (-1.07×10^{-5}, -2.77×10^{-6}), 0.001	-4.69×10^{-6} (-9.81×10^{-6}, 4.39×10^{-7}), 0.073	*P* = 0.817
Between-pair	-9.11×10^{-6} (-1.62×10^{-5}, -1.99×10^{-6}), 0.012	-4.47×10^{-6} (-1.36×10^{-5}, 4.68×10^{-6}), 0.339	
Within-pair	-5.74×10^{-6} (-1.05×10^{-5}, -1.03×10^{-6}), 0.017	-4.79×10^{-6} (-1.11×10^{-5}, 1.38×10^{-6}), 0.128	

(*Continued*)

https://doi.org/10.1017/S2040174421000465 Published online by Cambridge University Press

Table 3. (*Continued*)

	Unadjusted β (95% CI), *P*-value	Adjusted[a] β (95% CI), *q*-value[b]	*P*-value[c]
Triceps skinfold thickness (log cm) $n = 214$			
Twins-as-individuals	0.03 (0.01, 0.05), 0.006	0.02 (−0.01, 0.05), 0.250	$P = 0.956$
Between-pair	0.05 (0.01, 0.09), 0.014	0.01 (−0.04, 0.06), 0.749	
Within-pair	0.02 (−0.00, 0.05), 0.107	0.02 (−0.02, 0.06), 0.269	
Subscapular skinfold thickness (inverse square (1/cm^2)) $n = 211$			
Twins-as-individuals	−0.002 (−0.007, 0.001), 0.186	0.001 (−0.005, 0.007), 0.693	$P = 0.657$
Between-pair	−0.006 (−0.013, −0.0001), 0.048	−0.0001 (−0.0087, 0.0084), 0.978	
Within-pair	−0.001 (−0.006, 0.005), 0.803	0.002 (−0.005, 0.010), 0.593	
Systolic blood pressure (mmHg) $n = 213$			
Twins-as-individuals	0.05 (−0.82, 0.92), 0.915	0.15 (−0.81, 1.11), 0.796	$P = 0.293$
Between-pair	0.46 (−0.74, 1.66), 0.456	0.77 (−0.60, 2.15), 0.359	
Within-pair	−0.28 (−1.47, 0.91), 0.647	−0.30 (−1.81, 1.21), 0.741	
Diastolic blood pressure (mmHg) $n = 213$			
Twins-as-individuals	0.34 (−0.16, 0.84), 0.187	0.22 (−0.34, 0.77), 0.529	$P = 0.619$
Between-pair	0.40 (−0.47, 1.27), 0.369	0.42 (−0.61, 1.46), 0.520	
Within-pair	0.29 (−0.30, 0.89), 0.332	0.10 (−0.64, 0.84), 0.820	

[a]Adjusted for maternal pre-pregnancy BMI, gestational age at delivery, number of prior pregnancies, total maternal gestational weight gain, smoking during pregnancy, twin sex, chorionicity and zygosity.
[b]Simes-adjusted *q*-value.
[c]*P*-value when comparing within-between model to twins-as-individual model.
95% CI = 95% confidence interval; BMI = body mass index.

for IUGR. However, we recognise that IUGR is a defined condition and should not necessarily be derived from birthweight. Birthweight discordance can also be an indicator of impaired fetal growth; however, only 3.6% of twins in the PETS had a birthweight discordance of 30% or more. Therefore, we were unable to conduct further analyses to determine whether the associations of birthweight with childhood markers of cardiometabolic health differ for concordant or discordant pairs. However, results from our sensitivity analyses indicate that twins discordant for birthweight were not influencing these associations. As a twin study, these results may not be representative of the general population; however, after adjusting for gestational age and by implementing a twins-as-individuals regression model, these results are applicable to non-twin pregnancies. Although we implemented the Simes method for *P*-value adjustment to address the issue of multiple testing,[19] we recognise the limitations of frequentist testing approaches,[36] so recommend focussing on effect size and comparing our results with similar studies, rather than relying on *P*-values for significance.

Despite these limitations, our study does have several strengths. First, we adjusted for gestational age by using birthweight for gestational age *z*-scores, so our results are unlikely to be influenced by the collinearity between birthweight and gestational age. We also included multiple measures of cardiometabolic health, and at different ages. As a twin study, results from the within-pair model are unlikely to be confounded by shared genetic or environmental factors, which have been shown to influence both birthweight and later-life cardiometabolic health outcomes. We also made full use of additional information available in data from twins by implementing within- and between-pair regression models. These regression models are more appropriate to use than models which dichotomise data according to whether twins were discordant for birthweight.

Conclusions

After adjusting for gestational age using birthweight *z*-scores, birthweight was associated with childhood anthropometric measures in twins. However, we found no evidence for an association of birthweight-for-gestational-age *z*-scores with blood pressure in 6-year-old twins. This may indicate that the previously observed association of birthweight and blood pressure, may be influenced by gestational age. However, blood pressure at age 6 may not be indicative of blood pressure in later-life. An 11-year follow-up of the PETS is currently underway, so further investigation into the association of birthweight-for-gestational-age *z*-scores with blood pressure is warranted.

Acknowledgments. We wish to thank all the twins, mothers and families who participated in the PETS, as well as the entire PETS research team for their expertise and support.

Financial support. This work was supported by the Australian Government Research Training Program (D.A.); Twins Research Australia, which receives support from the National Health and Medical Research Council through a Centre of Research Excellence Grant, administered by the University of Melbourne (K.S.); the Australian National Health and Medical Research Council (J.C., grant numbers 437015 and 607358); the Bonnie Babes Foundation (J.C., grant number BBF20704); the Financial Markets Foundation for Children (J.C., grant number 032-2007) and the Victorian Government's Operational Infrastructure Support Program.

Conflict of interest. None.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174421000465.

References

1. World Health Organization. Cardiovascular diseases (CVDs). https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* 1989; 2, 577–580.
3. Law CM, Shiell AW. Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. *J Hypertens.* 1996; 14, 935–941.
4. Newsome CA, Shiell AW, Fall CHD, *et al.* Is birth weight related to later glucose and insulin metabolism?—a systematic review. *Diabetic Med.* 2003; 20, 339–348.
5. Yu ZB, Han SP, Zhu GZ, *et al.* Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. *Obes Rev.* 2011; 12, 525–542.
6. Joshi SM, Katre PA, Kumaran K, *et al.* Tracking of cardiovascular risk factors from childhood to young adulthood – the Pune Children's study. *Int J Cardiol.* 2014; 175, 176–178.
7. Yan Y, Hou D, Liang Y, *et al.* Tracking body mass index from childhood to adulthood for subclinical cardiovascular diseases at adulthood. *J Am Coll Cardiol.* 2016; 67, 1006–1007.
8. Bloetzer C, Bovet P, Suris J-C, *et al.* Screening for cardiovascular disease risk factors beginning in childhood. *Public Health Rev.* 2015; 36, 9.
9. Ashtree DN, McGuinness AJ, Plummer M, *et al.* Developmental origins of cardiometabolic health outcomes in twins: a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis.* 2020; 30, 1609–1621.
10. Harrison MS, Eckert LO, Cutland C, *et al.* Pathways to preterm birth: case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine.* 2016; 34, 6093–6101.
11. Morley R, Dwyer T, Carlin JB. Studies of twins: can they shed light on the fetal origins of adult disease hypothesis? *Twin Res.* 2003; 6, 520–525.
12. Tudehope D, Vento M, Bhutta Z, Pachi P. Nutritional requirements and feeding recommendations for small for gestational age infants. *J Pediatr.* 2013; 162, S81–S89.
13. Koullali B, Oudijk MA, Nijman TAJ, Mol BWJ, Pajkrt E. Risk assessment and management to prevent preterm birth. *Semin Fetal Neonatal Med.* 2016; 21, 80–88.
14. Lee AC, Kozuki N, Cousens S, *et al.* Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21st standard: analysis of CHERG datasets. *BMJ.* 2017; 358, J3677.
15. Katz J. Lee AC, Kozuki N, *et al.* Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: A pooled country analysis. *Lancet.* 2013; 382, 417–425.
16. Li Z, Umstad MP, Hilder L, Xu F, Sullivan EA. Australian national birthweight percentiles by sex and gestational age for twins, 2001–2010. *BMC Pediatr.* 2015; 15, 148.
17. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med.* 1998; 17, 407–429.
18. Vidmar SI, Cole TJ, Pan H. Standardizing anthropometric measures in children and adolescents with functions for Egen: update. *Stata J.* 2013; 13, 366–378.
19. Simes RJ. An improved Bonferroni procedure for multiple tests of significance. *Biometrika.* 1986; 73, 751–754.
20. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Stat Methods Med Res.* 2013; 22, 278–295.
21. Dwyer T, Blizzard L, Morley R, Ponsonby AL. Within pair association between birth weight and blood pressure at age 8 in twins from a cohort study. *BMJ.* 1999; 319, 1325.
22. Oberg S, Ge D, Cnattingius S, *et al.* Ethnic differences in the association of birth weight and blood pressure: the Georgia cardiovascular twin study. *Am J Hypertens.* 2007; 20, 1235–1241.
23. Baird J, Osmond C, MacGregor A, *et al.* Testing the fetal origins hypothesis in twins: the Birmingham twin study. *Diabetologia.* 2001; 44, 33–39.
24. Loos RJ, Fagard R, Beunen G, Derom C, Vlietinck R. Birth weight and blood pressure in young adults. *Circulation.* 2001; 104, 1633–1638.
25. McNeill G, Tuya C, Campbell DM, *et al.* Blood pressure in relation to birth weight in twins and singleton controls matched for gestational age. *Am J Epidemiol.* 2003; 58, 150–155.
26. Jelenkovic A, Yokoyama Y, Sund R, *et al.* Association between birthweight and later body mass index: an individual-based pooled analysis of 27 twin cohorts participating in the CODATwins project. *Int J Epidemiol.* 2017; 46, 1488–1498.
27. Kwok MK, Yeung SLA, Leung GM, Mary Schooling C. Birth weight and adult cardiovascular risk factors using multiple birth status as an instrumental variable in the 1958 British Birth Cohort. *Prev Med.* 2016; 84, 69–75.
28. Skidmore PM, Cassidy A, Swaminathan R, *et al.* An obesogenic postnatal environment is more important than the fetal environment for the development of adult adiposity: a study of female twins. *Am J Clin Nutr.* 2009; 90, 401–406.
29. Loos RJ, Beunen G, Fagard R, Derom C, Vlietinck R. Birth weight and body composition in young adult men—a prospective twin study. *Int J Obes Relat Metab Disord.* 2001; 25, 1537–1545.
30. Freedman DS, Katzmarzyk PT, Dietz WH, Srinivasan SR, Berenson GS. Relation of body mass index and skinfold thicknesses to cardiovascular disease risk factors in children: the Bogalusa Heart Study. *Am J Clin Nutr.* 2009; 90, 210–216.
31. de Hollander EL, Bemelmans WJE, de Groot LCPGM. Associations between changes in anthropometric measures and mortality in old age: a role for mid-upper arm circumference? *J Am Med Dir Assoc.* 2013; 14, 187–193.
32. Pomeroy E, Stock JT, Cole TJ, O'Callaghan M, Wells JCK. Relationships between neonatal weight, limb lengths, skinfold thicknesses, body breadths and circumferences in an Australian cohort. *PloS One.* 2014; 9, e105108.
33. van Wyk L, Boers KE, van Wassenaer-Leemhuis AG, *et al.* Postnatal catch-up growth after suspected fetal growth restriction at term. *Front Endocrinol.* 2019; 10, 274.
34. Perng W, Hajj H, Belfort MB, *et al.* Birth size, early life weight gain, and Midchildhood cardiometabolic health. *J Pediatr.* 2016; 173, 122–130.
35. Wu D, Zhu J, Wang X, *et al.* Rapid BMI increases and persistent obesity in small-for-gestational-age infants. *Front Pediatr.* 2021; 9, 625853.
36. Ranganathan P, Pramesh CS, Buyse M. Common pitfalls in statistical analysis: the perils of multiple testing. *Perspect Clin Res.* 2016; 7, 106–107.