Longitudinal association between caesarean section birth and cardio-vascular risk profiles among adolescents in Australia

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Abstract

Objective: To examine the association of cesarean section (C-section) with cardiovascular disease (CVD) risk biomarkers among Australian children.

Methods: The Longitudinal Study of Australian Children (LSAC) birth cohort was prospectively followed for body mass index (BMI) trajectory, and then linked with CVD risk indicators of children; waist circumference (WC), systolic blood pressure (SBP), blood glucose, high-density lipoprotein (HDL), triglyceride (TG), fat mass index (FMI) and composite metabolic syndrome (CMetS) score. Multivariable linear regression analysis was done to assess the association of C-sections with CVD risk biomarkers.

Results: Of 1,874 study children, 30% had C-sections; the mean age (SD) was 11.50 (0.50) years, and 49% were female. Against the vaginally-born cohort, Caesarean-born children showed a higher Z- score for five of the seven CVD risk indicators in regression analysis; WC (0.15; p=0.003), SBP (0.16; p=0.003), inverse HDL (0.15; p=0.003), FMI (0.12; p=0.004), and CMetS (0.45; p=0.004) score. Children with accelerated BMI trajectory had higher CMetS scores for both the delivery types while the C-section cohort showed statistical association only (1.69; p=0.006)

Conclusion: C-section was independently associated with increased CVD risk profiles of children, further increased with high BMI trajectory.

Implication for public health: The chronic disease risk of C-sections should be discussed with families to reduce clinically unrequired C-sections.

Key words: cardiovascular risk, continuous metabolic syndrome score, caesarean section birth, adolescents, developed country

Cardiovascular (CVDs) diseases are the major cause of death and disabilities for all world regions.[1,2] Globally, in 2019, around 18.6 million people died from CVDs, and the disability-adjusted life years for CVDs have doubled to 34.4 million from 17.7 million in 1990.[2] In Australia, around 26% of deaths per year were reported from CVD in the adult population, making it the second most common cause of disease burden in 2020.[3] Consequently, the chronic disease spectrum of CVDs costs trillions due to health service-related expenditures and loss of economic productivity of the affected people.[4] Although CVDs are an ailment of adulthood, the atherosclerotic and metabolic change of the body leading to overt diseases can also be detected during childhood.[5,6] Additionally, various studies have identified the presence of subclinical states of CVDs such as hypertension, obesity during adolescents and a high degree of continuity of these events towards adulthood.[5-7] Following up, research has also been directed to test the feasibility of using Metabolic syndrome (MetS), a composite index of multiple CVD risk indicators for the CVD risk assessment among the paediatric population.[8] By definition, the MetS is not a single disease – instead it is a combination of multiple cardiovascular risk profiles including hypertension, altered lipid level, abdominal obesity and increased blood glucose.[4] One study from Finland reported that children with high MetS risk scores at 9-18 years of age had a 30%-78% increased chance of developing diabetes and a 12%- 61% higher risk of having atherosclerotic deposition in great vessels in their adulthood at 24-43 years of age.[9] Therefore, the mainstay of the preventive strategy lies on the diagnosis of surrogate CVD markers and early life predictors of CVDs at its earliest course.[10]

In recent days, the association between CVD risk profiles and the mode of birth has come into discussion considering the rising evidence of obesity among children delivered by caesarean section (C-section).[11,12] Parallel to increased overweight and obesity in childhood, the epidemic rise of C-section birth has also been noticed across the world with a disproportionately higher rate in developed countries.[13] In Australia, the C-section birth rate has increased from 18.5% in 1990 to 36% in 2019.[14] However, being

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an early life predictor of adult-onset CVDs, C-section has not received much research attention as do other perinatal factors such as maternal obesity, hypertension, diabetes and birth weight of the fetus.[15-17] To our knowledge, only four studies have examined the association between C-sections and CVD risk factors with inconclusive research findings.[11,12,18,19] A birth cohort study in Brazil found a positive association between C-sections with increased BMI and blood pressure but not with other CVD risk indicators at 20 years of age.[11,12] C-section birth was associated with abnormal lipid profiles but not with hypertension and high blood glucose level in a study population of more than 20 years of age in a Danish study.[19] The shortcoming of current evidence is that none of them considered paediatric age groups as a study population and a composite MetS score has never been applied to assess the association between C- section birth and CVD risk profiles.[20] However, with the evidence of a 50% increased risk of MetS for adolescents with obesity,[21] it is important to ascertain whether C-section itself or a joint association of C-section and obesity in childhood is driving the abnormal CVD risk profiles in adolescents.[22]

We aimed to fill the current gaps in the literature by analysing a birth cohort in Australia with data on CVD risk profiles at 10 to 12 years of age. This study aimed to assess the association of C-section birth with multiple CVD risk factors and a composite MetS (CMetS) score after adjusting for potential perinatal confounders. We also assessed the moderating effect of the increased BMI in childhood on the observed association between C-section birth and CMetS score. Our study findings can contribute to clinical health research to initiate an evidence-informed discussion on the long-term impact of C-section birth.

Methods

Study setting and design

We used data from a nationally representative study, the Longitudinal Study of Australian Children (LSAC).[23] A detailed description of LSAC's study design and participant recruitment is mentioned elsewhere.[23] In brief, LSAC followed a two-stage randomised clustered design to recruit two cohorts of study children: B (aged '0–1' years) and K (aged '4–5' years) cohorts. Starting in 2004, data collection continued biennially, and eight waves of data were collected until 2018.[23] A nested cross-sectional checkpoint survey was done between waves six and seven in 2016, where a major cluster of the birth cohort (2004) from 30 major cities participated. The child health checkpoint survey collected a range of biomarkers and physical assessment data with a predominant focus on cardiovascular health.[24]

Ethical approval to conduct the LSAC study, including the checkpoint survey, was given by the Australian Institute of Family Studies Ethics Committee and the Royal Children's Hospital Melbourne Human Research Ethics Committee.[24] Participation was voluntary and informed consent was taken from study children, parents and guardians.[24] We report the accuracy of our study methodologies in line with the STROBE checklist in Supplementary File 1.[25]

Analytic sample

For this study, we limited the analysis to the 'B cohort' of the LSAC study as CVD risk indicators were available only for this cohort, and compared with the 'K cohort', they had minimum losses of birth history data.[26] Children who attended the checkpoint survey at 'wave 6.5' (n=1,874) were linked with 'wave 1' (n=5,107) and with a longitudinal data set on BMI trajectory from wave two to wave six (n=4,520) to reach the final analytic sample of 1,874 children. Figure 1 describes the flow chart of study participants from baseline to check point survey.

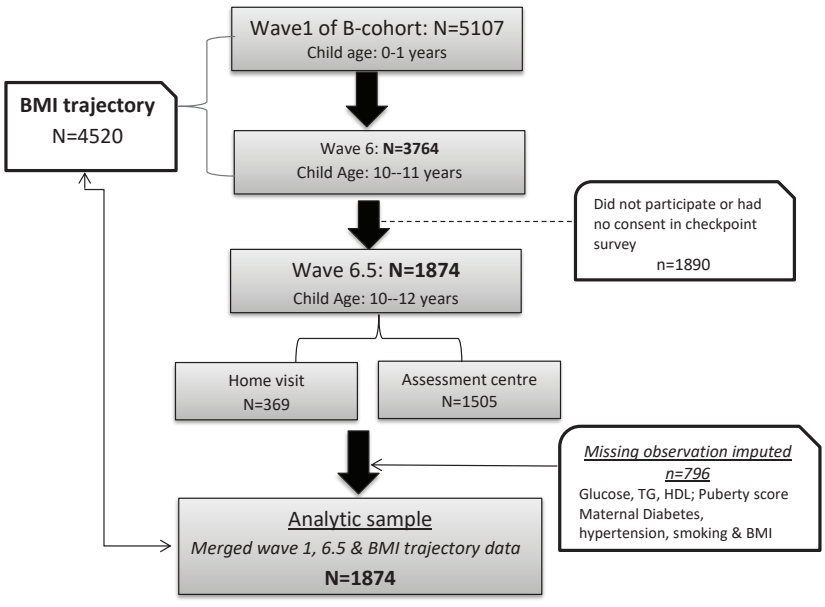
Measurements

Outcome measure

Primary outcomes were six individual CVD risk factors and a composite metabolic syndrome (CMetS) score. Individual CVD risk indicators were waist circumference (WC), systolic blood pressure (SBP), blood glucose, high-density lipoprotein (HDL) cholesterol, triglyceride (TG) and Fat Mass Index (FMI).[20,27] The CMetS score was generated from the first five components.[20] These five components were chosen based on their strong inter-correlation with CMetS score, reported by previous studies.[20,28] We have used SBP instead of mean arterial pressure since it has strong predictive power toward insulin resistance[29] and other CVD risk outcomes.[20,27]

The majority of the participants (80%) attended the fixed data collection centres where project-appointed trained research assistants and phlebotomists did the physical assessment and venous blood collection respectively after maintaining all standard procedures.[24] A study protocol by Clifford SA, et al. (2019) described the detailed data collection and storage procedure for the checkpoint survey.[24] In short, waist circumference was taken with a steel anthropometric measuring tape at the narrowest point between the 10th rib and iliac crest or the midpoint in the absence of visible narrowing. Measurement was taken twice or three times when the first

Figure 1: Flow chart on sample selection criteria to assess CVD risk profiles in LSAC birth cohort.



Note: BMI: Body Mass Index; TG: Triglyceride, HDL: High Density Lipoprotein Cholesterol

two differed by ≥1cm and the average of the closest two was used in the analysis.[24] SBP was measured in the right arm in a supine position with a child BP cuff.[24] Three measurements of SBP were taken at one-minute intervals and the mean value was used in the analysis. Semi-fasting (median 4.2 h after last caloric intake) venous blood was collected to report blood glucose and lipid profiles. Blood was processed on-site at the data collection centre within four hours of collection.[24] Total Fat Mass Index (FMI) was calculated by dividing the total body fat in kilogram by the child's height in meters squared.[30] The height of the study child was measured through a portable rigid stadiometer, and body fat was measured using four limbs segmental or two limb body composition scale.[24] The mean value of all the assessments were used during analysis.[24]

Pubertal status at outcome measurement was used for the standardisation of CVD risk factors. A sex-specific self-administered questionnaire was used to assess the pubertal development that included hair growth in the body and face, voice deepening for boys, and breast development and menarche for girls. All these questions were reported on a scale of 1 to 4 where 1 denotes 'not yet started', 2 'barely started', 3 'definitely started' and 4 'seems complete'. The summary pubertal score was between 3 to 12 points for boys and 2 to 8 points plus an additional point for menarche in girls.[31] The mean pubertal score was used in our analysis.

Exposure variable and confounders

A dichotomous variable of C-section birth (yes or no) was our exposure variable, which was collected from the child health record book during wave one of data collection. In absence of missing child health records, birth mothers of study children were interviewed to collect the mode of birth and other pregnancy-related information.[23]

A range of maternal and childbirth characteristics was chosen as perinatal confounders based on their association with the C-section birth and CVD risk factors.[32,33]

Maternal characteristics included sociodemographic status at birth and maternal risk factors during pregnancy such as; maternal age, ethnicity, number of children at home, family socioeconomic status (SES), diabetes, hypertension and smoking during pregnancy.[23] The family SES was generated from the Socio-Economic Index for Areas (SEIFA) released from national census data of 2006 and 2011.[34] We have created quintiles of SEIFA scores where quintile one denotes the most disadvantage and quintile five as the most advantage family SES.

The child characteristics encompassed weight for gestational age (WGA) z-score and child sex. The WGA z- score was generated from gestational weeks at birth and birth weight of children and re-categorised into small for gestational age (SGA) with mean value < = -1.28, large for gestational age (LGA) with mean +1.28, and rest as normal.[35]

Child BMI data from wave two to wave six were used to develop a group-based trajectory and used as an interaction term with C-section birth to assess its moderation effect on CMetS score.[36] The International Obesity Task Force (IOTF) recommended age-specific cut-off value of BMI for the corresponding age groups was used to define overweight and obesity categories for each trajectory group.[37]

Statistical analysis

A multiple-chained equation method was used to impute the missing observation under the assumption of missing at random.[38] The missing observation was imputed that include Glucose, TG, HDL cholesterol from outcome variables and maternal country of birth, BMI, diabetes, hypertension and smoking during pregnancy from the exposure variables list. Sample weights and cluster variables (strata) from wave one along with variables without missing observation were declared as registered variables in the multiple imputation model. In total, 20 imputations were requested and an augmented regression option was used to handle perfect prediction.[38] Statistical software package STATA 16 was used in analyses.[39]

Computation of CMeTS score

All six metabolic components were regressed with age, sex and pubertal development score, and saved as standardised residual z-score for the corresponding variables. The HDL Z-score was multiplied by -1, considering its negative relationship with the overall CVD risk predictors. Subsequently, the CMetS Z- score was generated by summing up the standardised residual Z-score of five CVD risk components (Z-WC, Z-SBP, Z-glucose, Z-TG and Z-HDL).[20] A four-component CMetS Z-score was also generated by dropping the WC for sensitivity analysis. The higher CMetS score represents a poorer cardiometabolic status for this study sample.[20]

Predictors of CVD risk

Descriptive statistics, i.e. frequency percentage for the categorical variables and mean with standard deviation (SD) for the continuous variable, were used to describe the study sample. The difference in distribution for the confounders and outcome variable across C-section birth was measured through bivariable analysis using the Chi-square test statistic. In addition, multivariable linear regression analysis was done to show the association of C-section birth with CVD risk predictors. Five different models were tested by adding groups of confounders one by one. Model 1 started with C-section and CVD risk components without adjustment for the confounders. Following up, Model 2 was adjusted for maternal sociodemographic characteristics, Model 3 for pregnancy risk factors, Model 4 for childbirth characteristics, and Model 5 for maternal and childbirth characteristics. The regression output was presented as standard mean difference (SMD) and considered significantly different than mean zero with a p-value <0.05.

A separate regression analysis was done to understand the interaction effect of BMI trajectories and C-sections on the CMetS z-score. We used latent class growth modeling (LCGM) to identify the distinct BMI trajectory of the children from age two to 10 years.[40] The details of the LCGM methodology are described in our previous paper, where we report the BMI trajectory of children from age two to 13 years (wave two to wave seven).[36]

Sensitivity analysis

We conducted a series of sensitivity analyses. Firstly, we checked the distribution of covariates between baseline and checkpoint survey data to understand the representativeness of the analytic sample to LSAC's original study population. Additionally, we explored the distribution of covariates across imputed and complete data set of checkpoint survey to ensure observation was missing at random. Categorical variables were reported by frequency percentage and continuous variables by histogram. Secondly, the multivariable regression findings from the imputed data set were compared with the complete case findings to ensure imputation did not alter the true association. Finally, the joint association of BMI trajectory and

C-section births were compared by using both five- and four-components MetS scores. Since both the BMI value and WC are the indicators of anthropometric assessment, the four-component CMeTS score helped us to overcome the issue of residual effect.

Results

Sample characteristics

Of 1,874 study children, 30% (n=560) were delivered by C-section. Table 1 highlights the sample characteristics and bivariable association of perinatal factors with C-section birth. During enrollment, one-third of birth mothers were 35 years of age or over and 52 % of the families were from average socioeconomic positions. Regarding pregnancy risk factors, around 12% of the birth mothers were active smokers and 7% had hypertension during pregnancy. Around 49% of the study children were female and 5% followed an accelerated BMI trajectory with a mean BMI value in the overweight or obesity range. Maternal age at birth, diabetes, hypertension during pregnancy and accelerated BMI trajectory were found significant for the C-section occurrence and suggested to be the potential confounders in CVD risk prediction. We report the comparison between the checkpoint survey and baseline study population in Supplementary Table S1. The comparison between the imputed and completed data set for the analytic sample is presented in Supplementary Table S2 and Figure S1. As the data suggest the proportion for categorical variables was similar across the data set and the mean value of the continuous variable in the imputed data set followed a similar curve as those are in the completed data set.

Table 1: Sample characteristics by mode of births in Check point survey of LSAC.

Co-variates	Total sample	Vaginal delivery	C-section	P-Value
	N (%)			
	N=1,874	1,313 (70.06)	560 (29.88)	
A. Maternal sociodemography				
Maternal age				
<35 years	1,277(68.14)	938(73.51)	338(26.49)	<0.01
>=35 years	597(31.86)	375(63.77)	222(36.23)	
Country of birth				
Australian born	1,481(80.71)	1,047(70.74)	433(29.26)	0.16
Immigrant	354(19.29)	237(66.95)	117(33.05)	
Family socioeconomic position				
Most disadvantage	466(24.87)	336(72.26)	129(27.74)	0.36
2nd Quintile	291(15.53)	201(69.07)	90(30.93)	
3rd Quintile	399(21.29)	282(70.68)	117(29.32)	
4th Quintile	387(20.65)	276(71.32)	111(28.68)	
Most advantage	331(17.66)	218(65.86)	113(34.14)	
Number of children at home				
None	773(41.25)	530(68.56)	243(31.44)	0.46
One	709(37.83)	502(70.9)	206(29.1)	
More than two	392(20.92)	281(71.68)	111(28.32)	
B. Pregnancy risk factors				
Diabetes at pregnancy				
No	1,601(94.4)	1,128(70.46)	473(29.54)	0.01
Yes	95(5.6)	55(57.89)	40(42.11)	
Hypertension at pregnancy				
No	1,582(92.95)	1,118(70.67)	464(29.33)	<0.01
Yes	120(7.05)	70(58.33)	50(41.67)	
Smoking at pregnancy				
No	1,501(88.14)	1,043(69.49)	458(30.51)	0.60
Yes	202(11.86)	144(71.29)	58(28.71)	
Maternal BMI (mean ±SD)	25.15±5.11	24.68±4.83	26.26±5.58	<0.01
C. Childbirth characteristics				
Weight for gestational age				
Small for age	68(3.63)	46(67.65)	22(32.35)	0.71
Large for age	270(14.41)	184(68.4)	85(31.6)	
Normal	1,536(81.96)	1,083(70.51)	453(29.49)	
Multiple birth				
Single	1,806(96.42)	1,290(71.47)	515(28.53)	<0.01
Twin/triplets	67(3.58)	23(34.33)	44(65.67)	
Gender				
Female	919(49.04)	646(70.29)	273(29.71)	0.86
Male	955(50.96)	667(69.92)	287(30.08)	

Notes:
BMI: Body Mass index; SD: Standard deviation.
Variables with a P-value of ≤0.05,differed significantly by the mode of birth

During outcome measurement, the mean age of study children was 11.50 years with a standard deviation of (*SD*) ±0.50 years, and their pubertal development score was in a category of the prepubertal stage with a mean score of 2.18 points with *SD* ±0.60 points. Table 2 shows the standard mean Z-score of outcome variables by the mode of birth. Compared to those delivered vaginally, children delivered by C-section had a statistically significant higher mean Z-score for the WC, SBP, inverse value of HDL cholesterol, FMI and the CMetS score.

Association between C-section and CVD risk indicators

Table 3 shows the multivariable findings from final adjusted model. In the final adjusted model, five of seven CVD risk indicators observed a positive association with C-section birth. Children delivered by C-section had a higher mean Z-score of SBP, obesity parameters and lower HDL value. Against the vaginally delivered children, the standardised Z-score was higher for WC by 0.15 units (95% CI: 0.05-0.25; $p \leq 0.01$) and by 0.12 units for the FMI (95% CI: 0.04-0.21; $p \leq 0.01$) for caesarean born children. Since the reported Z-HDL score was an inverse value of HDL, a 0.15-units increased score of HDL signifies children delivered by C-section had a 0.15-unit lower HDL cholesterol than the vaginally delivered child. However, children delivered by C-section birth did not show a significant association with higher blood glucose or TG- concentration. Regarding the CMetS score, caesarean-born children had 0.45 units higher CMetS -Z score (95% CI: 0.15-0.75; $p \leq 0.01$) than the vaginally born children. Among the perinatal confounders, the number of children at home, maternal BMI, and child sex showed a statistical association with C-sections. Increasing mean BMI of

mothers during pregnancy showed a positive association with an increased CVD risk score for all seven indicators among the C-section cohort While the male gender was a positive risk factor for the majority of the CVD risk indicators, it was negatively associated with higher WC Z-score for the C-section cohort. In contrast, having more siblings at home shows a lower CVD risk score in comparison to having no siblings at home.

Finally, the direction and strength of association observed in the imputed data set for the final adjusted model were similar to the complete case findings in the sensitivity analysis (Supplementary Table S3). The findings from five tested models are presented in Supplementary Table S4. In general, the positive association observed in the unadjusted model did not change after adjustment with potential perinatal confounders. For example, the SBP of children was 0.17 units higher for the C-section cohort in the unadjusted model and showed similar strength of association in model 3 (0.13; 95%CI: 0.03-0.24) after adjustment for maternal hypertension and other adverse clinical conditions during pregnancy.

Interaction effect of C-section and BMI trajectory on the combined CVD risk score

Similar to previous work, three distinct BMI trajectory groups were identified as the best fit model in the group-based trajectory (GBT) model.[36] The reported three BMI trajectory groups were low stable for 65% (1,212/1,874); moderate for 30% (567/1,874) and accelerated for 5% (n=93/1,874) of study children in checkpoint survey The mean BMI value was in normal to overweight range for children with moderate BMI trajectory and in overweight to obesity range for accelerated BMI trajectory from age two to ten years.[37] The mean BMI was always in the normal range for the children with low stable BMI trajectory.

Figure 2 presents the interaction effect of C-section and BMI trajectory over CMeTS score of children during checkpoint survey from the unadjusted model (Panel A) and final adjusted model (Panel B) after adjustment for the potential perinatal confounders. As shown in Figure 2, the composite score CVD risk indicators (CMetS Z-score) increased linearly with increasing BMI trajectory and has been augmented in presence of C-section birth in the model. The direction and strength of association observed in the unadjusted model did not alter in presence of perinatal confounders in the adjusted model. While children with moderate and accelerated BMI trajectories both had a higher value than the mean zero, the mean value of CMeTS Z-score was always higher for children with C-section birth in comparison to Vaginally delivered children. For example, while the CMeTS Z-score was 1.69 units (95%CI: 0.49 to 2.90; $p<0.01$) for the children having C-section birth with accelerated BMI trajectory, it was only 0.33 units (95%CI: -0.42 to 1.08 $p=0.28$) for children who delivered vaginally and followed an accelerated BMI trajectory in their childhood In sensitivity analysis, the direction of association was not different with the four component CMetS score as an outcome variable, as shown in Supplementary Figure S2.

Table 2: Association between C-section birth and cardio-vascular risk profiles during early adolescence: Bivariable findings

CVD risk profiles	Vaginal delivery	C-section	P-value
	Mean Z-score[a] (Standard error)		
Z-WC	-0.05(0.03)	0.12(0.04)	<0.01*
Z-WC	-0.05(0.03)	0.12(0.04)	<0.01*
Z-glucose	0.02(0.03)	-0.05(0.06)	0.70
Z-HDL[b]	-0.05(0.03)	0.11(0.04)	<0.01*
Z-TG	-0.01(0.03)	0.03(0.05)	0.51
Z-FMI	-0.04(0.03)	0.10 (0.04)	0.03*
Z- CMetS score[c]	-0.15(0.10)	0.35(0.16)	0.01*

Notes:

a: CVD risk profiles were standardized for age, sex, and pubertal development score of children during check point survey

b: HDL cholesterol is the inverse value

c: Continuous metabolic syndrome (CMetS) score was generated from the combined Z-score of SBP, WC, glucose, HDL, and TG

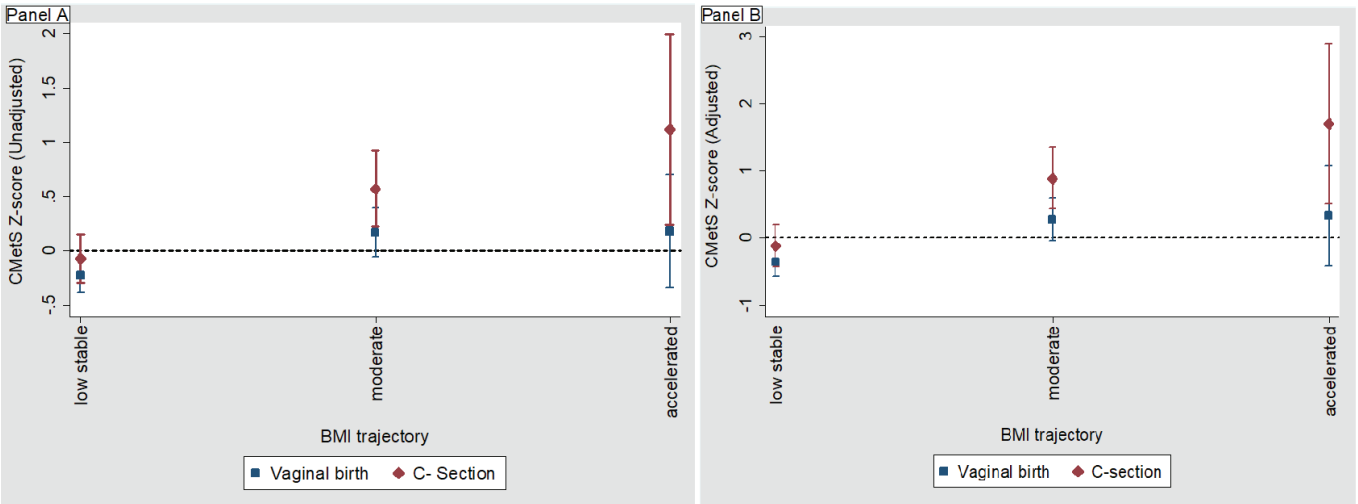
** P-value ≤0.05 denotes the observed mean Z-score of CVD risk indicators differed significantly by the delivery methods*

WC: Waist circumference; SBP: Systolic blood pressure; TG: Triglyceride, HDL: High-density lipoprotein cholesterol. FMI: Fat Mass Index; CMetS: Continuous Metabolic Syndrome score.

Discussion

This is the first study to show a positive relationship between C-section births and CVD risk factors in the paediatric

Figure 2: Joint association of mode of birth and BMI trajectory with the continuous metabolic syndrome score (Panel A: unadjusted model; Panel B: model adjusted for perinatal factors).



population. Four out of six individual CVD risk components (WC, SBP, HDL cholesterol and FMI) and the composite index of the five CVD risk components, CMetS score observed a positive association with C-section birth. Using different parameters of obesity, our study provided a direct relationship between C-section and increased fat deposition with a higher Z-score of WC and FMI among children at 10-12 years of age. Moreover, the association between C-section and CMetS score was found independent in relation to the observed BMI trajectory of children. Caesarean-born children who followed a persistent obesity trajectory in their childhood had significantly higher CMetS scores than their counterparts of the vaginally delivered children with a similar persistent obesity trajectory.

The background for the current research was built on our previous work on the LSAC study population where children delivered by C-section birth have shown twice the increased risk of persistently

Table 3: Association between C-section birth and Cardiovascular risk indicators during early adolescence: Multivariable findings.

Covariates	CVD risk indicators[a]						
	Z-WC	Z-SBP	Z-Glucose	Z-TG	Z-HDL[b]	Z-FMI	Z-CMET[c]
	Mean Z-score (95% CI)						
Mode of birth							
Vaginal birth	Ref	Ref	Ref	Ref	Ref	Ref	Ref
C-section	0.15(0.05-0.25) *	0.16(0.05-0.26)*	-0.05(-0.19-0.09)	0.04(-0.03-0.11)	0.15(0.05-0.25)*	0.12(0.04-0.21)*	0.45(0.15-0.75)*
Maternal age							
<35 years	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>35 years	-0.04(-0.14-0.06)	-0.04(-0.14-0.06)	-0.04(-0.15-0.06)	0.01(-0.04-0.07)	-0.04(-0.14-0.05)	-0.02(-0.1-0.06)	-0.15(-0.45-0.15)
Country of birth							
Australian born	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Immigrant	-0.06(-0.17-0.06)	-0.06(-0.17-0.06)	0.07(-0.06-0.19)	-0.04(-0.1-0.03)	-0.05(-0.16-0.06)	-0.06(-0.15-0.04)	-0.13(-0.48-0.21)
Family socioeconomic status							
Most disadvantage	0.12(-0.01-0.25)	0.12(-0.01-0.25)	0.02(-0.14-0.18)	0(-0.08-0.08)	0.13(0-0.25)	0.08(-0.03-0.19)	0.39(0-0.79)*
2ndquintile	0.06(-0.09-0.2)	0.05(-0.1-0.19)	0.17(0-0.33)*	-0.07(-0.17-0.03)	0.08(-0.06-0.23)	0(-0.12-0.12)	0.29(-0.16-0.73)
3rd Quintile	Ref	Ref	Ref	Ref	Ref	Ref	Ref
4th Quintile	0.05(-0.09-0.19)	0.05(-0.09-0.19)	0(-0.14-0.15)	0(-0.07-0.08)	0.05(-0.08-0.19)	0.04(-0.08-0.15)	0.16(-0.25-0.58)
Most advantage	-0.08(-0.23-0.06)	-0.08(-0.22-0.07)	-0.05(-0.21-0.11)	0.01(-0.07-0.1)	-0.09(-0.23-0.05)	-0.05(-0.17-0.07)	-0.29(-0.72-0.14)
Number of children at home							
None	Ref	Ref	Ref	Ref	Ref	Ref	Ref
One	-0.14(-0.24--0.04)*	-0.14(-0.24--0.04)*	0.04(-0.1-0.17)	-0.03(-0.1-0.04)	-0.14(-0.23--0.04)*	-0.11(-0.19--0.03)*	-0.41(-0.72--0.1)*
More than two	-0.09(-0.21-0.03)	-0.1(-0.22-0.02)	0.15(0.01-0.29)	-0.08(-0.16-0)*	-0.07(-0.19-0.05)	-0.1(-0.21-0) *	-0.19(-0.57-0.19)
Maternal diabetes at pregnancy							
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Yes	0.08(-0.12-0.28)	0.08(-0.12-0.29)	-0.03(-0.25-0.19)	0.03(-0.09-0.14)	0.08(-0.12-0.27)	0.07(-0.1-0.24)	0.23(-0.38-0.85)
Maternal hypertension at pregnancy							
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Yes	-0.04(-0.21-0.14)	-0.04(-0.22-0.14)	0.07(-0.12-0.26)	-0.03(-0.13-0.06)	-0.03(-0.2-0.14)	-0.04(-0.19-0.1)	-0.07(-0.59-0.46)
Maternal smoking at pregnancy							
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Yes	0.12(-0.03-0.27)	0.12(-0.03-0.27)	-0.06(-0.23-0.11)	0.04(-0.04-0.12)	0.11(-0.03-0.26)	0.1(-0.02-0.22)	0.34(-0.11-0.78)
Maternal mean BMI	0.02(0.01-0.03)*	0.02(0.02-0.03)*	-0.02(-0.04-0)	0.01(0-0.02)*	0.02(0.01-0.03)*	0.02(0.01-0.03)*	0.06(0.03-0.1)*
Multiple births							
Single	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Twin/triplets	-0.17(-0.42-0.08)	-0.17(-0.42-0.09)	-0.03(-0.31-0.26)	-0.01(-0.16-0.14)	-0.17(-0.41-0.06)	-0.12(-0.33-0.09)	-0.55(-1.3-0.2)
Child's weight for age							
Normal	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Small for age	0(-0.24-0.25)	0(-0.25-0.24)	0.08(-0.16-0.33)	-0.04(-0.17-0.09)	0.02(-0.22-0.25)	-0.02(-0.22-0.18)	0.07(-0.65-0.78)
Large for age	0.08(-0.04-0.2)	0.08(-0.04-0.21)	-0.06(-0.2-0.08)	0.04(-0.03-0.11)	0.07(-0.05-0.19)	0.07(-0.03-0.18)	0.21(-0.17-0.59)
Child gender							
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Male	-0.65(-0.74--0.56)*	0.57(0.42-0.71)*	-0.32(-0.74-0.1)	1.72(1.4-2.04)*	0.76(0.47-1.04)*	1.23(1.12-1.33)*	2.07(1.45-2.7)*

Notes:

a: CVD risk indicators were standardized for age, sex, and pubertal development score of children during check point survey

b: Z-HDL uses inverse value of HDL cholesterol

c: CMetS score was generated from the combined Z-score of SBP, WC, glucose, HDL, and TG

**P-value ≤0.05 denotes the observed mean Z-score of CVD risk indicators was significantly different than mean zero in comparison to reference category*

WC: Waist circumference; SBP: Systolic blood pressure; TG: Triglyceride, HDL: High-density lipoprotein cholesterol.

FMI: Fat Mass Index; CMetS: Continuous Metabolic Syndrome score.

high BMI and WC trajectory from age two to 13 years than their vaginally delivered counterparts.[24] Consistent with our earlier findings, adolescents in this subsample of LSAC have also shown a 0.15 unit higher WC (95%CI: 0.05 to 0.25; *p*-value ≤0.01), and an additional risk of 0.12 unit higher FMI (95%CI: 0.04 to 0.21; *p*-value ≤.01) for the C-section cohort. Body fat composition is considered more valid to represent obesity status in childhood[10] and was available only at the LSAC checkpoint survey. The direction and strength of association between C-section and obesity parameters did not vary after adjustment for important confounders like child size at birth and maternal BMI and socioeconomic status.[41] At the same time, evidence suggests that increased body fat proportion is directly linked with dyslipidemia and hypertension in children.[42] For example, a study from Columbia among those aged 10-20 years had shown an increased TG and reduced HDL cholesterol level by 0.04 mg/dL for every one unit increase in total fat proportion.[42] Thus, the high SBP Z-score observed in our study could have resulted from excess fat deposition along with the reduced concentration of scavenger HDL cholesterol. Additionally, the observed positive association between C-section and increased SBP Z-score (0.16 unit) of children did not attenuate by the confounding effect of maternal hypertension in our study.[32,33] Therefore, our findings clearly showed that C-section birth has an independent association with obesity and it also increases the concomitant risk of high SBP in early adolescence.

Although our study infers evidence from the early adolescent age group, a positive association observed between C-section and individual components of CVD risk factors was similar to earlier studies done among adult offspring in Brazil[11,12] and Denmark.[19] In contrast to our study findings, a study done in North America among one-year-old offspring did not show a significant difference in individual CVD risk components by the mode of birth.[18] However, the generalisability of that study is limited by low statistical power from a small sample size (n=104) and a comparatively very young study population to observe the desired metabolic changes.[18]

Though C-section did not show a positive association with blood glucose and TG, the composite index of five CVD risk indicators (WC, SBP, TG, HDL and blood glucose) showed a positive association with C-section birth. Compared to vaginally delivered study children, the CMetS Z-score was 0.45 units higher for the Caesarean-born children. Secondly, we observed that the child cohort of C-sections with accelerated BMI trajectory had a higher CMetS score (1.69; 95%CI: 0.49-2.9; *p*-value <0.01) than the child cohort who delivered vaginally and followed a similar accelerated BMI trajectory (0.33, 95%CI: -0.42 to 1.08; *p*-value 0.28). Our observed study finding supports an earlier study from the same LSAC study population where persistently high BMI trajectory has shown a positive association with CMetS score during early adolescence.[43] Additionally, with an observation of a non-significant relationship between the interaction effect of vaginal delivery and accelerated BMI trajectory with CMetS score, we would like to conclude that C-section is an independent predictor of CVD risk during early adolescents.

The biological plausibility of CVD risk factors with C-section and obesity is based on its shared exposure to altered microbial load from C-section birth.[44] Children born through elective C-section, particularly before rupture of the amniotic membrane, do not receive the benefits of commensal microbiota of maternal perineum and continue to harbor the obesogenic microbiota.[44] Subsequently, this altered microbial ecosystem hampers the 'gut-brain axis' and releases some pathogenic toxins to cause metabolic damage to distant organs of the body. One of these toxins, Trimethylamine N-oxide, is further related to atherosclerotic changes and thrombus growth in target organs, increasing the risk of metabolic diseases.[45] On the contrary, the pathway of emergency C-sections towards increased CVD risk factors in later life has been postulated by the altered fetal stress concerning physiological or pharmacological induction of labour.[46] To support this hypothesis, earlier research has also shown an increased risk of MetS in adult offspring with emergency C-section history.[46] Thus, our inability to differentiate the effect of C-section over emergency vs elective nature may not have a biased effect on the observed association with CVD risk profiles.

Strength and limitation

The strength of our study lies in its strong methodology in data collection, preparation and analysis. We imputed missing observation for all the required variables and the resultant analytic sample was not much different than the 'lost to follow-up' sample of the original birth cohort. In addition, we used the continuous metabolic score to report the composite CVD risk profile of MetS, which is considered the best approach for the paediatric population.[47] Most importantly, all the CVD risk predictors were standardized for the age, sex and pubertal development of children to adjust for the residual confounding effect of unmeasured variables related to child demography and developmental status.[48] However, we would like to acknowledge that this study did not measure other novel CVD biomarkers such as interleukins, C-reactive protein and tumour necrosis factor[8] as these were not collected in the LSAC child health checkpoint survey. The use of semi-fasting blood samples might not alter the estimated value of lipid profiles as current evidence shows a similar result for the fasting and a non-fasting blood sample.[49] Finally, our model was not adjusted for the prenatal exposure to an antibiotic that could have some impact on the observed association. Basic research suggests that antibiotic exposure to mother wombs hampers the biodiversity of children's guts with an additional risk of poor CVD outcomes.[50]

This study provides important insight into the health care policy and strategic direction towards chronic disease risk reduction. The observed independent association of C-section birth with increased CVD risk has positioned the Australian adolescents in multiple chronic disease risks in their life course. The growing rates of C-sections conducted for non-clinical reasons is a major public health concern that calls for a reduction in the rate of unnecessary C-sections and their associated human and economic costs.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Supplementary File 1: STROBE Statement to report study quality of an observational study on association between caesarean section birth and CVD risk indicators in adolescence from LSAC study population.

Supplementary Table 1: Comparing covariates by mode of birth in between wave one, and check point survey of LSAC.

Supplementary Table 2: Sensitivity analysis on the distribution of categorical variables across imputed and complete data.

Supplementary Table 3: Sensitivity analysis on the association between C-section and CVD risk indicators across three different data set: Multivariable regression findings from final adjusted model.

Supplementary Table 4: Association between C-section and CVD risk indicators across five different models: multivariable findings from imputed data.

Supplementary Figure 1: Sensitivity analysis on the distribution of continuous variables across imputed and complete data.

Supplementary Figure 2: Joint association of mode of birth and BMI trajectory on the 4 components metabolic syndrome score.