Perinatal microbial exposure may influence aortic intima–media thickness in early infancy

Article *in* International Journal of Epidemiology · April 2016

DOI: 10.1093/ije/dyw042

13 authors, including:

Peter Vuillermin
Deakin University
66 PUBLICATIONS **656** CITATIONS
SEE PROFILE

Mimi L K Tang
The Royal Children's Hospital
342 PUBLICATIONS **8,129** CITATIONS
SEE PROFILE

Fiona Collier
University Hospital, Barwon Health
69 PUBLICATIONS **1,100** CITATIONS
SEE PROFILE

Anne-Louise Ponsonby
Murdoch Childrens Research Institute
262 PUBLICATIONS **7,399** CITATIONS
SEE PROFILE

International Journal of Epidemiology, 2016, 1–10
doi: 10.1093/ije/dyw042
Original article

Original article

Perinatal microbial exposure may influence aortic intima-media thickness in early infancy

Kate McCloskey,[1,2,3] Peter Vuillermin,[1,2,4†] John B. Carlin,[1,3] Michael Cheung,[1,3] Michael R. Skilton,[5] Mimi L.K. Tang,[1,3] Katie Allen,[1,3] Gwendolyn L. Gilbert,[6] Sarath Ranganathan,[1,3] Fiona Collier,[2,4] Terence Dwyer,[1,3] Anne-Louise Ponsonby[1,3†] and David Burgner;[1,3,7*†] on behalf of the BIS Investigator Group

[1]Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, VIC, Australia, [2]Child Health Research Unit, University Hospital Barwon Health, Geelong, VIC, Australia, [3]Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia, [4]Department of Medicine, Deakin University, Waurn Ponds, VIC, Australia, [5]Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, [6]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, WA, Australia and [7]Department of Paediatrics, Monash University, Melbourne, VIC, Australia

*Corresponding author. Murdoch Childrens Research Institute, Royal Children's Hospital, 50 Flemington Road, Parkville, VIC, Australia. E-mail: david.burgner@mcri.edu.au

[†]Contributed equally as senior authors.

Accepted 5 February 2016

Abstract

Background: The maternal and infant microbiome may influence infant cardiovascular risk through immune programming. The maternal vagino-enteric microbiome is often sampled for group B streptococcus (GBS) colonization during pregnancy. Our aim was to investigate the association between maternal GBS colonization, intrapartum antibiotics, antenatal pet exposure and infant aortic intima-media thickness (aIMT), an intermediate vascular phenotype, and whether this association varied by mode of delivery.

Methods: The Barwon Infant Study is a population-derived pre-birth cohort. Perinatal data were collected on participants. Women were tested for vagino-enteric group B streptococcus (GBS) colonization during third trimester. Six-week infant aIMT was measured by trans-abdominal ultrasound. Adjustment for confounders included maternal age, pre-pregnancy body mass index (BMI), smoking, socioeconomic status, gestational diabetes, length of gestation, infant sex, birthweight and aortic internal diameter.

Results: Data were available on 835 mother-infant pairs. Of these, 574 (69%) women delivered vaginally; of those, 129 (22%) were GBS-colonized; and of these women, 111 (86%) received prophylactic intrapartum antibiotics. An association between maternal GBS colonization and infant aIMT was observed among those delivered vaginally ($\beta = 19.5\,\mu$m, 95% CI 9.5, 29.4; $P < 0.0001$) but not by Caesarean section (P for interaction $= 0.02$). A similar pattern was seen for intrapartum antibiotics. There was a negative association between antenatal pet exposure and aIMT observed in those delivered vaginally.

Conclusion: Maternal GBS colonization and intrapartum antibiotics were associated with increased infant aIMT in those delivered vaginally, whereas antenatal pet exposure was associated with decreased aIMT. These data suggest that differences in early life microbial experience may contribute to an increased cardiovascular risk.

Key words: maternal, microbiome, infant, intima-media thickness, cardiovascular

Key Messages

- Among vaginally born infants, maternal GBS colonization is associated with increased infant aIMT.
- Among vaginally born infants, antenatal pet and livestock ownership are associated with decreased infant aIMT.
- These associations were not evident in infants born by caesarean section.

Background

Atherosclerotic cardiovascular disease, a leading cause of morbidity and mortality, has its origins in early life.[1] Studies have implicated the ante- and perinatal periods as a developmental window that influences long-term cardiometabolic health.[1,2] The underlying mechanisms are unclear, partly because there have been few in-depth studies of early life programming and cardiovascular risk markers.

Perinatal microbial colonization of the infant represents a critical window in early life immune programming, that may have long-term cardiometabolic consequences.[3–5] The infant gastrointestinal tract is rapidly colonized after birth, leading to the establishment of the microbiome which plays an important role in the education and maturation of immune responses,[6,7] maintenance of gastrointestinal epithelial integrity[8] and metabolic signalling and energy harvesting.[5,7,8] Perturbation of the infant microbiome (dysbiosis) may lead to low-grade inflammation and increased metabolic risk,[3–5] but there is a relative paucity of longitudinal human data. Environmental factors associated with the establishment and diversity of the infant microbiome include the maternal microbiome,[9] pet and livestock exposure in the prenatal and early postnatal periods, family size, antibiotic use during pregnancy or delivery[10] and mode of delivery.[11] Initial bacterial colonization is determined by the maternal vagino-enteric microbiome in vaginal deliveries, and by the environmental and maternal skin microbiome in caesarean deliveries.[9] These pioneer bacteria strongly influence the infant's subsequent microbial signature.[10,12,13]

The maternal vagino-enteric microbiome, a key determinant of the initial colonization of the vaginally delivered infant,[14–16] undergoes significant change throughout pregnancy; in particular there is a relative increase in the abundance of streptococcal species as gestation proceeds.[9,17] In Australia, screening for maternal group B streptococcus (GBS) colonization occurs during the third trimester of pregnancy, as a recommended strategy to inform the prevention of GBS sepsis in the newborn. In Australia, approximately 25% of pregnant women are GBS-colonized.[18] It is routine practice for GBS-colonized women who have a vaginal delivery to receive intrapartum antibiotics to minimize transmission of GBS to their infant.[18] Antibiotics given in the perinatal and neonatal periods may permanently alter infant microbiota, in particular decreasing the abundance of *Bifidobacteria* and *Bacteroides* species;[19] animal models suggest these species may protect against later obesity.[20–22] Maternal GBS colonization may therefore influence infant microbial colonization either through exposure to a specific maternal vagino-enteric microbiome, or alternatively through the effect of intrapartum antibiotics on maternal-infant microbial transfer.

Measurement of aortic intima-media thickness (aIMT) has become established as an early marker of cardiovascular risk.[23] In the early stages of atherosclerosis, there is diffuse thickening of the intima-media layers of the vessel wall, and formation of 'fatty streaks'[24] that may be evident in the infant aorta from early infancy.[25] Aortic IMT is an ultrasonic measurement of this diffuse thickening and a widely used putative marker of early cardiovascular risk.[26,27] In previous small exposure-specific studies, maternal and infant variables, such as maternal obesity, smoking and infant intrauterine growth restriction, are associated with increased aIMT.[28–36] Furthermore, there is evidence that carotid IMT is increased in early childhood following acute infection,[37] and by other inflammatory conditions of childhood such as inflammatory bowel disease and Kawasaki Disease.[38] Although the association between aIMT in infancy and cardiovascular disease in adulthood has yet to be established, increased aIMT is a

promising marker that may reflect sub-acute inflammation associated with early atherosclerotic change.[23,26] To date no study has investigated the association between perinatal exposures and aIMT in early life.

The aim of this study was to characterize the association between environmental exposures affecting infant microbial colonization, particularly maternal GBS colonization, intrapartum antibiotics, pet and livestock exposure and mode of delivery with infant aIMT.

Methods

The Barwon Infant Study (BIS) is a population-derived prebirth cohort ($n = 1074$) of mother-infant pairs in south-eastern Australia.[39] Women were recruited at their first antenatal hospital visit and were subsequently excluded if their infants were delivered before 32 weeks, developed a serious illness in the first week of life or had major congenital abnormalities. The Barwon Health Human Research Ethics committee approved the study.

Data on maternal age, parity, GBS colonization, antibiotic use in pregnancy, delivery type, intrapartum antibiotic use and antenatal comorbidities were collected from questionnaires and hospital databases. Questionnaires specific to pre-pregnancy, trimester 1 and trimester 2 were administered at recruitment.[28–32] Data relating to the third trimester and post-delivery were obtained by questionnaires self-completed before the 6-week visit. Maternal pre-pregnancy BMI was calculated from pre-pregnancy weight (self-report) and directly measured maternal height. Results of glucose tolerance testing were obtained from pathology databases, and gestational diabetes was defined on diagnosis and treatment using the consensus criteria applicable at the time of recruitment.[40,41] In Australia, GBS screening is one of two recommended strategies to identify pregnant women whose infants are at risk from GBS sepsis, the other being risk stratification of the infant post-delivery.[42] Screening involves a combined low vaginal and ano-rectal swab at 35–37 weeks of gestation.[42] GBS screening was the strategy recommended by Barwon Health. Australian guidelines recommend all women undergoing caesarean section receive prophylactic antibiotics before first incision,[43] and that GBS-colonized women having a vaginal delivery should also receive antibiotics before delivery.[42] Questionnaire data provided information on variables that may affect the maternal and infant microbiome, including sibling numbers, pet ownership, livestock exposure and maternal smoking. Birth-related data were recorded within 48 h of delivery. The Socio-economic Index for Areas (SEIFA) was calculated using the home address of participants.[44]

Aortic IMT was measured at a study centre visit at 6 weeks of age by trans-abdominal ultrasound. Images of the distal aorta were captured using a GE Vivid I with a 4–13 MHz linear array vascular transducer, as previously described.[45] The ultrasound settings were standardized by using presets and images acquired with simultaneous three-lead electrocardiogram (ECG) gating. The abdominal aorta was first identified in cross section, just above the umbilicus. A longitudinal, straight, unbranched 1-cm segment of abdominal aorta proximal to the abdominal bifurcation was captured between the umbilicus and xiphisternum, using a standard protocol.[27,35,45] Mean aIMT was measured offline using edge-detection software (Carotid Analyzer for Research, version 6, Medical Imaging Applications LLC, Iowa), by one of two readers. The between-reader intra-class coefficient was 0.92.[45]

Statistical analysis

Associations between antenatal and postnatal variables and aIMT were investigated using Pearson's correlation and linear regression analysis and categorical variables were investigated using logistic regression. The relationship between maternal GBS colonization and infant aIMT was investigated by linear regression modelling. Infant aIMT approximated a normal distribution. Models were investigated for the effect of outliers and extreme values and there was no substantive change in the findings. In addition to infant sex, potential confounding variables for associations with the outcome (aIMT) were chosen based on previous evidence that they affected maternal or infant microbiota or infant aIMT. They were then included in the model and retained if they made greater than a 10% difference to the magnitude of the effect estimate. As discussed in detail elsewhere, aIMT increases in proportion to vessel size in the growing infant; thus to investigate any further increase above this physiological phenomenon, minimal vessel diameter was controlled for in all regression analyses.[46] Interactions between exposure variables GBS colonization and mode of delivery were assessed by the significance of the Wald test associated with the relevant product term in multiple regression models.[47] GBS colonization and antibiotic use were highly correlated and therefore additional models were fitted including both exposure variables, in order to assess the independent association of each with aIMT.

In the cohort of 835, data regarding socioeconomic status (SEIFA), pet ownership, livestock exposure during pregnancy, maternal pre-pregnancy BMI, GBS colonization and minimal vessel diameter were infrequently missing for some individuals (Table 1). Sensitivity analyses for the effect of these missing data (assuming missing at random) were performed using multiple imputation by chained equations (50 imputed datasets). Analysis of association with GBS colonization only included women for whom

Table 1. Characteristics of infants who had aIMT measured at 6 weeks of age ($N = 835$)

Characteristic	*n* (%)	Missing data[a] *n*/835 (%)
Twins	8 (1.0%)	0
Sex of child: male	434 (52.0%)	0
Socio-Economic Indexes for Areas (SEIFA):		
Low	197 (24%)	11 (<0.1%)
Mid	165 (20%)	
High	459 (55%)	
Maternal age, years (mean, SD)	32.3 (4.6)	0
Maternal cigarette smoking (any during pregnancy):	68 (8.7%)	0
Family pet ownership during pregnancy	620 (74%)	3 (<1%)
Family livestock exposure during pregnancy	59 (7%)	9 (1%)
Maternal prenatal BMI kg/m^2 (median IQR)	24.3 (21.8–28.3)	106 (13%)
Maternal gestational diabetes	38 (4.6%)	0
Maternal GBS colonization in third trimester	156 (21%)	78 (9%)[c]
Antibiotic use in pregnancy		0
Trimester 1	35 (4%)	
Trimester 2	73 (9%)	
Trimester 3	34 (4%)	
Total[b]	130 (16%)	
Delivery via caesarean section	261 (31%)	0
Intrapartum antibiotic exposure	389 (46%)	0
Gestational age at birth:		0
32 to 36 completed weeks	23 (2.8%)	
37 to 42 completed weeks	812 (97.2%)	
>42 completed weeks	0 (0.0%)	
Birthweight in kilograms (mean, SD)	3.6 (0.5)	0
Age in weeks at scan (mean, SD)	6.2 (1.5)	0
Mean aIMT μm (mean, SD)	616 (50)	0
Minimal diameter μm (mean, SD)	4980 (535)	35 (4%)

[a]Indicates the number of participants within the cohort of 835 who had specific data missing.
[b]Some participants had antibiotics in more than one trimester.
[c]Women who were not screened in pregnancy.

this exposure was recorded. All statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, TX).

Results

Baseline characteristics of the cohort:

Of the 1158 women who were recruited, 53 withdrew and 41 were ineligible, leaving a total of 1074 women in the study and[39] 984 infants attended the first postnatal visit at a median age of 5.9 weeks, [interquartile range (IQR) 5.14–6.86]. Of these, 835 (85%) had aIMT successfully measured.[45] The characteristics of these 835 participants are summarized in Table 1. There was minimal difference in characteristics between the 835 participants and the rest of the cohort (Supplementary Table 5, available as Supplementary data at *IJE* online).

Maternal GBS colonization

Of the 835 women, 757 (91%) were screened for GBS colonization; 156 of those screened (21%) were colonized. Of the women who did not have screening ($n = 78$), 20 had infants born at <37 weeks of gestation and 43 (55%) had caesarean deliveries. There was no obvious difference in the other maternal characteristics when compared with women who underwent GBS screening. Among women who delivered vaginally, only 35 of 574 (6%) did not have GBS screening.

Maternal antibiotic use in pregnancy

In all, 130 women (16%) had a total of 205 antibiotic courses prescribed during pregnancy, of which 71 courses (35%) were either amoxicillin or penicillin, and 35 (17%) were cephalexin. Broad-spectrum antibiotics, such as co-amoxyclav and ceftriaxone, were prescribed in fewer than 5% of courses. The prevalence of maternal GBS colonization was lower among those prescribed antibiotics in pregnancy (143/640, 22% vs 13/117, 11%, $P = 0.006$). The magnitude of this difference was greatest for women prescribed antibiotics in the second and third trimester (trimester 1: 5/31, 16% vs 151/726, 21%; trimester 2: 6/65, 9% vs 150/692, 22%; trimester 3: 3/32, 9% vs 153/725, 21%).

Intrapartum antibiotics

Intrapartum antibiotics were administered due to either caesarean section or maternal GBS colonization. All women who delivered by caesarean section received intrapartum antibiotics, predominantly as surgical prophylaxis. Of women who delivered vaginally, 128/574 (22%) received antibiotics, including 111/129 (86%) who were GBS-colonized and 5/410 (1%) who were GBS-negative (35 women had unknown GBS colonization).

Associations between antenatal factors, GBS colonization and intrapartum antibiotics

Other than antibiotic use in pregnancy, there was no evidence that other antenatal or maternal variables, including maternal age, parity, socioeconomic status or pet exposure, were asscociated with GBS colonization (Table 2).

Table 2. Associations of maternal and perinatal covariates with (i) odds of maternal GBS colonization, (ii) odds of maternal intrapartum antibiotics use and (iii) mean aIMT

Variable	Odds ratio (OR) for GBS colonization			Odds ratio for intrapartum antibiotics			Difference in mean aIMT[a] (μm)		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	β	95% CI	*P*-value
GBS colonization	N/A			15.84	9.42, 26.54	<0.001	14.1	5.2, 22.9	0.002
SEIFA	1.00	0.81, 1.25	0.95	0.90	0.76, 1.06	0.19	−1.8	−5.9, 2.4	0.40
Pet ownership	0.84	0.57, 1.24	0.38	1.01	0.74, 1.37	0.97	−6.2	−14.0, 1.6	0.12
Livestock exposure	0.97	0.49, 1.93	0.93	0.96	0.57, 1.64	0.96	−3.2	−16.3, 10.0	0.64
Maternal age	0.99	0.95, 1.03	0.56	1.05	1.02, 1.08	0.003	−0.6	−1.3, 0.2	0.14
Pre-pregnancy BMI (kg/m^2)	1.01	0.98, 1.05	0.41	1.07	1.04, 1.10	<0.001	0.1	−0.6, 0.7	0.79
Antibiotics in pregnancy	0.43	0.24, 0.80	0.007	0.56	0.38, 0.83	0.003	−5.3	−14.7, 4.1	0.27
Intrapartum antibiotics	15.84	0.42, 26.64	<0.001	N/A			5.7	−1.2, 12.6	0.11
Mode of delivery (caesarean)	0.45	0.29, 0.70	<0.001	N/A			0.4	−7.1, 7.7	0.93
Gestational age (weeks)	1.10	0.96, 1.27	0.19	0.69	0.62, 0.78	<0.001	4.2	1.8, 6.7	<0.001
Sex (male)	1.04	0.73, 1.48	0.87	1.23	0.94, 1.62	0.13	2.6	−4.6, 9.5	0.47
Birthweight (kg)	1.20	0.85, 1.74	0.28	0.56	0.45,0.77	<0.001	20.7	13.7, 27.6	<0.001
Minimal vessel diameter (μm)	1.00	1.00, 1.00	0.67	1.00	1.00, 1.00	0.002[b]	N/A		
Age at aIMT (weeks)	1.03	0.93, 1.17	0.60	1.05	0.96, 1.15	0.25	4.7	2.5, 7.0	<0.001
Gestational diabetes	1.44	0.65, 3.17	0.36	2.4	1.21,4.78	0.01	0.0	−16.2, 16.3	1.00
Smoking in pregnancy	0.98	0.52, 1.87	0.96	0.83	0.50, 1.37	0.47	4.1	−8.2, 16.5	0.51
Sibling number	1.05	0.86, 1.29	0.62	0.88	0.75, 1.03	0.11	1.4	−2.6, 5.3	0.50

[a]Adjusted for minimal vessel diameter.
[b]OR = 0.9996, 95% CI 0.9993 to 0.9998, $P = 0.002$.

Table 3. The association between GBS colonization, pet ownership, livestock, sibling number and aIMT, stratified by mode of delivery

	Complete cohort			Vaginal delivery			Caesarean section		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
GBS colonization	14.1	5.2, 22.9	0.002	19.5	9.5, 29.4	<0.001	−6.6	−26.5, 13.3	0.51
Siblings	3.0	−4.0, 9.9	0.40	0.8	−7.8, 9.3	0.86	7.7	−4.2, 19.6	0.20
Pet ownership	−6.2	−14.0, 1.6	0.12	−9.1	−18.6, 0.4	0.06	0.3	−13.3, 13.9	0.97
Dog ownership	−7.4	−15.6, 0.9	0.08	−11.6	−21.8, −1.4	0.03	1.7	−12.2,15.5	0.82
Livestock exposure	−3.2	−16.3, 10.0	0.64	−18.5	−34.3, −2.6	0.02	33.7	10.3, 57.1	0.005

The odds of intrapartum antibiotics increased with Caesarean delivery, GBS colonization, higher pre-pregnancy BMI, prematurity and reduced birthweight.

Maternal GBS colonization and infant aIMT

There was a strong positive association between maternal GBS colonization and mean aIMT (mean increase = 14.1 µm, 95%, CI 5.6, 23.0, $P = 0.002$). When stratified by mode of delivery, the association between maternal GBS and offspring aIMT was observed in infants born by vaginal delivery ($\beta = 19.5$ µm, 95% CI 9.5, 29.4, $P < 0.001$) but not in those born by caesarean section ($\beta = -6.6$, 95% CI −26.5, 13.3, $P = 0.51$; *P* for interaction = 0.02) (Table 3).

In infants born by vaginal delivery, the association between maternal GBS colonization and infant mean aIMT persisted after adjustment for socioeconomic status, pet ownership, livestock ownership, maternal age, pre-pregnancy BMI, antibiotic exposure in pregnancy, length of gestation, sex, birthweight, age at aIMT measurement and minimal vessel diameter ($\beta = 22.6$ µm, 95% CI 11.9, 33.3, $P < 0.001$). Sensitivity analysis using multiple imputation for missing data produced largely similar results for the association between GBS colonization and aIMT (overall $\beta = 13.5$ µm, 95% CI 4.9, 22.1, $P = 0.002$; vaginal delivery $\beta = 18.6$ µm, 95% CI 8.9, 28.3, $P < 0.001$).

Infants were then classified considering both GBS status and mode of delivery, with those born by caesarean section

to GBS-negative mothers as the reference category. Infants born by vaginal delivery to GBS-positive mothers were more likely to have a higher mean aIMT ($\beta = 13.4$, 95% CI 2.2 to 24.4, $P = 0.02$), but infants born by vaginal delivery to GBS-negative mothers were not ($\beta = -6.0$, 95% CI -24.7 to 2.6, $P = 0.17$). The pattern of associations persisted following both adjustment for confounders and multiple imputation (Table 4).

Association between intrapartum antibiotics and infant aIMT

In women who had a caesarean delivery, there were no infants unexposed to intrapartum antibiotics. In vaginal deliveries, however, there was an association between intrapartum antibiotics and infant aIMT ($\beta = 11.3\,\mu m$, 95% CI 1.3, 21.4, $P = 0.03$).

Maternal GBS colonization, intrapartum antibiotics and infant aIMT in vaginal deliveries

In vaginal deliveries, maternal GBS colonization and intrapartum antibiotics were highly correlated: 111/129 (86%) GBS-colonized women received intrapartum antibiotics, whereas only 5/410 (1%) GBS-negative women received antibiotics. In a regression model for aIMT that includes both GBS colonization and intrapartum antibiotics, the (adjusted) effect of GBS colonization remained substantial ($\beta = 20.5\,\mu m$, 95% CI −0.1, 41.3, $P = 0.05$), whereas the effect of intrapartum antibiotics was strongly null ($\beta = -1.3\,\mu m$, 95% CI −22.8, 20.3, $P = 0.91$). Because of the small number of GBS-negative and antibiotic-positive women, the adjusted GBS effect here is determined primarily within the subgroup of women who did not receive antibiotics (18/423 of whom were GBS-positive), whereas the adjusted effect of intrapartum antibiotics is driven mainly by the sub-group of GBS-positive women (111/129 of whom received antibiotics).

Other antenatal microbial exposures and infant aIMT

In this cohort, 620 of 835 women (74%) had a household pet and 59 (7%) had exposure to livestock. Excluding missing data regarding the type of pet owned, 69% of women owned dogs (473 of 688). There was an association between exposure to livestock ($\beta = -18.5\,\mu m$, 95% CI −34.3 to −2.6, $P = 0.02$) and pet ownership ($\beta = -9.1\,\mu m$, 95% CI −18.6 to 0.43, $P = 0.06$) and reduced aIMT in infants born via vaginal deliveries, whereas there was a positive association between livestock exposure and infant aIMT following caesarean delivery (Table 3). Specifically, there was a negative association between dog ownership in pregnancy and infant aIMT, ($\beta = -11.5\,\mu m$, 95% CI −21.5 to −1.6, $P = 0.02$), but not following caesarean delivery ($\beta = 1.7\,\mu m$, 95% CI −12.2 to 15.5, $P = 0.82$). There was no association between sibling number and aIMT before or after stratification by mode of delivery.

Discussion

This study is the first to investigate the association between antenatal and perinatal factors that may affect the maternal-infant microbiome and aIMT at 6 weeks of age. Here we observe both markers of maternal microbial exposure and microbial transfer are associated with infant aIMT in vaginal, but not caesarean section, deliveries. This difference by mode of delivery also highlights effects on maternal-infant microbial transfer. In vaginal deliveries there was a positive association between maternal GBS colonization and increased infant aIMT, independent of birthweight and gestational age. Similarly, intrapartum antibiotics were associated with increased aIMT, whereas both pet ownership and livestock exposure were associated with reduced aIMT. Together these findings suggest that the very early microbial experience among vaginally born infants may have implications for cardiovascular health.

Table 4. A comparison of how combinations of both GBS colonization and mode of delivery are associated with mean aIMT

Stratification	Model 1[a]			Model 2[b]			Model 2MI[c]		
	β	95% CI	P	β	95% CI	P	β	95% CI	P
GBS negative and born by caesarean section	0	(reference)	–	0	(reference)	–	0	(reference)	–
GBS negative and born by vaginal delivery	−6.0	−14.7, 2.6	0.17	−7.8	−17.5, 1.9	0.12	−6.8	−15.5, 1.9	0.13
GBS colonized and born by caesarean section	−6.6	−26.7, 13.5	0.52	−11.2	−32.2, 10.8	0.42	−10.0	−29.6, 9.6	0.32
GBS colonized and born by vaginal delivery	13.4	2.2, 24.6	0.02	15.2	2.6, 27.4	0.02	12.0	1.0, 23.0	0.03

[a]Adjusted for minimal vessel diameter.

[b]Adjusted for socioeconomic status, pet ownership, livestock ownership, maternal age, pre-pregnancy BMI, antibiotic exposure in pregnancy, length of gestation, gender, birthweight, age at aIMT measurement and minimal vessel diameter.

[c]As for model 2 following multiple imputation for missing variables (50 per variable).

Infants born by vaginal delivery are known to be colonized with microbiota that resemble maternal vaginal and enteric flora, whereas those born by caesarean section have microbiota that resemble maternal skin flora.[48] It is therefore plausible that the association between GBS colonization and infant aIMT is mediated by infant exposure to maternal vaginal and enteric microbiota during delivery. Maternal GBS colonization may indicate an altered maternal microbiome that then colonizes the infant in vaginally-born infants. Recent evidence suggests that maternal GBS colonization in pregnancy is associated with enrichment of the infant gastrointestinal microbiota with specific bacterial families (*Clostridiaceae, Ruminococcoceae* and *Enterococcaceae*) that is independent of maternal intrapartum antibiotic exposure.[49] In Australia, GBS screening is performed frequently, but not universally, by sampling both vaginal and enteric flora in late pregnancy.[42] Asymptomatic maternal GBS colonization occurs in 18–27% of Australian women,[18] compared with a varying global incidence of 6.5–36%.[50–52] Risk factors that predict GBS colonization remain poorly defined.[18] Inconsistently reported risk factors include younger maternal age and parity,[18,53] reduced socioeconomic status,[51,54] and, in contrast, increased maternal professional employment.[55] In our study, antibiotic use during pregnancy was the only antenatal exposure associated with GBS colonization.

Antibiotic administration is known to alter the intestinal microbiome markedly. The most significant effect is within days of administration, but it may be months before the microbiome returns to its pre-treatment state.[56–58] In keeping with this, women in our cohort who received antibiotics during their second and third trimester had a lower incidence of GBS colonization than those receiving antibiotics in the first trimester. Previous studies have suggested an association between prenatal and early life antibiotic exposure and childhood obesity.[5,59,60] To date there are few data on the association between prenatal antibiotics and other cardiovascular risk factors. Here, there was no association between prenatal antibiotics and infant aIMT, either overall or after stratification by delivery.

Consistent with the relevance of the maternal and maternal-infant microbial transfer, antenatal pet ownership and livestock exposure were associated with reduced aIMT, but only among infants delivered vaginally. Dog ownership is a key determinant of human microbiota,[61] including maternal vaginal colonization during pregnancy.[62] Pet ownership has been shown to both protect against offspring allergy[63,64] and modify the association between caesarean delivery and offspring obesity.[65] It is considered likely that these findings are mediated by microbial factors. Here, we have shown that pet ownership has a similarly potentially protective effect on aIMT. Further characterization of maternal and infant microbiota would elucidate the relationship between maternal GBS colonization, intrapartum antibiotics and animal exposures, and microbial composition and metabolic profile. The finding of a positive association between livestock exposure and aIMT in those delivering by caesarean section is unexpected and requires replication in cohorts with a higher frequency of livestock exposure.

Infant colonization is influenced not only by the maternal microbiome, but also by the mode of microbial transfer from mother to newborn.[48] For GBS-colonized women having vaginal deliveries, the role of intrapartum antibiotics is to reduce maternal-infant microbial transfer.[66] Due to the high correlation of maternal GBS colonization and intrapartum antibiotics in vaginal deliveries, we are unable to confidently differentiate between the two exposures in this study. However, the magnitude of association between maternal GBS and aIMT was essentially unchanged following adjustment for intrapartum antibiotics, whereas the association between intrapartum antibiotics and aIMT was no longer evident following adjustment for maternal GBS status. Further characterization of the maternal microbiome and the effect of GBS colonization would help clarify this relationship.

Infant aIMT measurement allows the timely investigation of relationships between perinatal exposures and cardiovascular disease risk. Increased aIMT is a putative marker of cardiovascular disease risk, but there are no longitudinal data linking neonatal aIMT and cardiovascular disease in adulthood.[23] Carotid IMT has been shown to closely correlate with histological findings,[67] and is widely used in adults to predict cardiovascular events.[68,69] In children, both carotid and aIMT have been associated with established risk factors for cardiovascular disease, with evidence that aIMT is a more discriminating measure.[70] As the aorta is the first site of atherosclerotic change,[25] aIMT has become an established early life marker of cardiovascular disease risk.[23]

It is not yet known whether differences in infant aIMT persist through childhood. The magnitude of the observed changes in aIMT is similar to largely unadjusted data comparing other putative perinatal cardiovascular risk factors, such as gestational age,[32] smoking, gestational diabetes[29] and pre-eclampsia,[30] but somewhat less than that demonstrated with small exposure-specific studies of newborn intrauterine growth restriction (IUGR).[34,35] Previous studies have demonstrated increased IMT following hospitalization with childhood infection.[37] In addition, severe childhood infection has been associated with subclinical atherosclerosis,[71] and with cardiovascular events in adulthood.[72] Our finding of an association between maternal

GBS colonization and neonatal aIMT may be indicative of either a transitory inflammatory effect and/or early changes of atherosclerosis; further longitudinal studies are under way.

The strengths of this study include a population-derived sample, the largest study of infant aIMT to date, multiple measures of microbial exposure, a high rate of GBS screening and a rate of GBS colonization in keeping with previous Australian data.[18] Aortic IMT was successfully measured in 85% of infants in our cohort, with no differences identified in participants where measurement was unsuccessful.[45] The main limitations of our study are that we so far only have a single measure of aIMT, and that we have not yet characterized the maternal and infant microbiota beyond GBS colonization. Intrapartum antibiotic administration was infrequent in non-GBS colonized women who had a vaginal delivery. This limited our ability to investigate the independent effect of specific antibiotic classes on GBS colonization or the effect of antibiotics on maternal infant microbial transfer in vaginal delivery.

Maternal GBS colonization and intrapartum antibiotic administration are common, therefore any association with infant cardiovascular health may have significant public health implications. There is evidence that intrapartum antibiotics reduce early onset GBS disease in the neonate,[66] but the long-term implications for the infant microbiome are unknown. Given the importance of preventing neonatal GBS sepsis, it is likely that interventions aimed at supporting the infant microbiome are more appropriate than withholding intrapartum antibiotics.

Conclusion

Maternal GBS colonization is associated with increased infant aIMT, modified by mode of delivery. This is consistent with neonatal colonization with maternal vaginal and enteric microbiota during vaginal delivery, but not caesarean section. Furthermore, in these same infants, pet ownership and livestock exposure are associated with reduced aIMT. Together, these data raise the possibility that differences in early life microbial experience are involved in establishment of early cardiovascular risk. Further studies to quantify differences in microbiota associated with GBS colonization are warranted. Given the prevalence of both GBS colonization and cardiovascular disease, any association may have wide-reaching public health implications.

Supplementary Data

Supplementary data are available at *IJE* online.

Funding

The initial establishment work and infrastructure for the BIS was supported by the Murdoch Childrens Research Institute and Barwon Health. Deakin University is now a partner organization and has provided funding and infrastructure. Funding has been provided by the National Health and Medical Research Council of Australia (APP607370, APP1009044, APP102997, APP1082037, APP1076667, APP1030701 and APP1084017), the Jack Brockhoff Foundation, the Scobie Trust, the Shane O'Brien Memorial Asthma Foundation, Our Women's Our Children's Fundraising Committee Barwon Health, the Rotary Club of Geelong, the Shepherd Foundation and the Ilhan Foundation. K.M. is funded by a Sidney Myer Scholarship. D.B. is supported by a National Health and Medical Research Council (Australia) Fellowship [APP1064629] and an Honorary Future Leader Fellowship of the National Heart Foundation of Australia (100369). M.S. is supported by a Future Leader Fellowship from the National Heart Foundation of Australia (#100419). Research at Murdoch Childrens Research Institute is supported by the Victorian Government's Operational Infrastructure Support Program. The Heart Research Group at Murdoch Childrens Research Institute is supported by Big W, RCH 1000 and the RCH Foundation.

Conflict of interest: K.A. has received speakers' honoraria from Nutricia, Wyeth, Danone, Nestle and Abbott. M.T. is a member of the medical advisory boards for Nestle Nutrition Institute Oceania and Nutricia Australia New Zealand, and a member of the Danone Nutricia Global Scientific Advisory Board Allergy and Immunity. She has received speaker fees for speaking at symposia sponsored by Danone Nutricia.

References

1. Barker DJ. The fetal and infant origins of adult disease. *BMJ* 1990;**301:**1111.
2. Eriksson JG, Kajantie E, Thornburg KL, Osmond C, Barker DJ. Mother's body size and placental size predict coronary heart disease in men. *Eur Heart J* 2011;**32:**2297–303.
3. Cox LM, Yamanishi S, Sohn J *et al*. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014;**15:**705–21.
4. Blustein J, Attina T, Liu M *et al*. Association of caesarean delivery with child adiposity from age 6 weeks to 15 years. *Int J Obes (Lond)* 2013;**37:**900–06.
5. Ajslev TA, Andersen CS, Gamborg M, Sorensen TI, Jess T. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond)* 2011;**35:**522–29.
6. Sjogren YM, Tomicic S, Lundberg A *et al*. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. *Clin Exp Allergy*2009;**39:**1842–51.
7. Cahenzli J, Koller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* 2013;**14:**559–70.
8. Camp JG, Frank CL, Lickwar CR *et al*. Microbiota modulate transcription in the intestinal epithelium without remodeling the accessible chromatin landscape. *Genome Res* 2014;**24:**1504–16.
9. Koren O, Goodrich JK, Cullender TC *et al*. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;**150:**470–80.

10. Koenig JE, Spor A, Scalfone N *et al*. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* 2011;**108(Suppl 1):**4578–85.
11. Dominguez-Bello MG, Blaser MJ, Ley RE, Knight R. Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. *Gastroenterology* 2011;**140:**1713–19.
12. Biasucci G, Rubini M, Riboni S, Morelli L, Bessi E, Retetangos C. Mode of delivery affects the bacterial community in the newborn gut. *Early Hum Dev* 2010;**86(Suppl 1):**13–35.
13. Dominguez-Bello MG, Costello EK, Contreras M *et al*. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010;**107:**11971–75.
14. Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. *J Exp Med* 1965;**122:**59–66.
15. Tourneur E, Chassin C. Neonatal immune adaptation of the gut and its role during infections. *Clin Dev Immunol* 2013;**2013:**270301.
16. MDogra S, Sakwinska O, Soh SE, Ngom-Bru C, Bruck WM, Berger B, *et al*. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. *MBio* 2015;**6**. doi:10.1128/mBio.02419-14.
17. Jost T, Lacroix C, Braegger C, Chassard C. Stability of the maternal gut microbiota during late pregnancy and early lactation. *Curr Microbiol* 2014;**68:**419–27.
18. Gilbert GL, Hewitt MC, Turner CM, Leeder SR. Epidemiology and predictive values of risk factors for neonatal group B streptococcal sepsis. *Aust N Z J Obstet Gynaecol* 2002;**42:**497–503.
19. Penders J, Thijs C, Vink C *et al*. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006;**118:**511–21.
20. Million M, Maraninchi M, Henry M *et al*. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int J Obes (Lond)* 2012;**36:**817–25.
21. Kondo S, Xiao JZ, Satoh T *et al*. Antiobesity effects of Bifidobacterium breve strain B-3 supplementation in a mouse model with high-fat diet-induced obesity. *Biosci Biotechnol Biochem* 2010;**74:**1656–61.
22. An HM, Park SY, Lee do K *et al*. Antiobesity and lipid-lowering effects of Bifidobacterium spp. in high fat diet-induced obese rats. *Lipids Health Dis* 2011;**10:**116.
23. McCloskey K, Vuillermin P, Ponsonby AL, Cheung M, Skilton MR, Burgner D. Aortic intima-media thickness measured by trans-abdominal ultrasound as an early life marker of subclinical atherosclerosis. *Acta Paediatr* 2014;**103:**124–30.
24. Nakashima Y, Chen YX, Kinukawa N, Sueishi K. Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age. *Virchows Arch* 2002;**441:**279–88.
25. Stary HC. Lipid and macrophage accumulations in arteries of children and the development of atherosclerosis. *Am J Clin Nutr* 2000;**72(Suppl 5):**1297–306S.
26. Urbina EM, Williams RV, Alpert BS *et al*. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension* 2009;**54:**919–50.
27. Jarvisalo MJ, Jartti L, Nanto-Salonen K *et al*. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. *Circulation* 2001;**104:**2943–47.
28. Gunes T, Koklu E, Yikilmaz A, *et al*. Influence of maternal smoking on neonatal aortic intima-media thickness, serum IGF-I and IGFBP-3 levels. *Eur J Pediatr* 2007;**166:**1039–44.
29. Akcakus M, Koklu E, Baykan A *et al*. Macrosomic newborns of diabetic mothers are associated with increased aortic intima-media thickness and lipid concentrations. *Horm Res* 2007;**67:**277–83.
30. Akcakus M, Altunay L, Yikilmaz A, Yazici C, Koklu E. The relationship between abdominal aortic intima-media thickness and lipid profile in neonates born to mothers with preeclampsia. *J Pediatr Endocrinol Metab* 2010;**23:**1143–39.
31. Begg LM, Palma-Dias R, Wang J, Chin-Dusting JP, Skilton MR. Maternal adiposity and newborn vascular health. *Arch Dis Child Fetal Neonatal Ed* 2013;**98:**F279–80.
32. Koklu E, Kurtoglu S, Akcakus M, Yikilmaz A, Coskun A, Gunes T. Intima-media thickness of the abdominal aorta of neonate with different gestational ages. *J Clin Ultrasound* 2007;**35:**491–97.
33. Koklu E, Akcakus M, Kurtoglu S *et al*. Aortic intima-media thickness and lipid profile in macrosomic newborns. *Eur J Pediatr* 2007;**166:**333–38.
34. Koklu E, Kurtoglu S, Akcakus M *et al*. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. *Horm Res* 2006;**65:**269–75.
35. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS. Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet* 2005;**365:**1484–86.
36. Cosmi E, Visentin S, Fanelli T, Mautone AJ, Zanardo V. Aortic intima media thickness in fetuses and children with intrauterine growth restriction. *Obstet Gynecol* 2009;**114:**1109–14.
37. Liuba P, Persson J, Luoma J, Yla-Herttuala S, Pesonen E. Acute infections in children are accompanied by oxidative modification of LDL and decrease of HDL cholesterol, and are followed by thickening of carotid intima-media. *Eur Heart J* 2003;**24:**515–21.
38. Aloi M, Tromba L, Rizzo V *et al*. Aortic intima-media thickness as an early marker of atherosclerosis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2015;**61:**41–46.
39. Vuillermin P, Saffery R, Allen KJ *et al*. Cohort Profile: The Barwon Infant Study. *Int J Epidemiol* 2015;**44:**1048–60.
40. Martin FI. The diagnosis of gestational diabetes. Ad Hoc Working Party. *Med J Aust* 1991;**155:**112.
41. Nankervis A, Conn J. Gestational diabetes mellitus - negotiating the confusion. *Aust Fam Physician* 2013;**42:**528–31.
42. Royal College of Obstetricians and Gynaecologists. *Maternal Group B Streptococcus in Pregnancy: Screening and Management*. London: RCOG, 2012.
43. Royal College of Obstetricians and Gynaecologists. *Prophylactic Antibiotics in Obstetrics and Gynaecology*. London: RCOG, 2013.
44. Pink B. *Socio-economic Indexes for Areas (SEIFA)*. Canberra: Australian Bureau of Statistics, 2011.
45. McCloskey K, Ponsonby AL, Carlin JB *et al*. Reproducibility of aortic intima-media thickness in infants using edge-detection

software and manual caliper measurements. *Cardiovasc Ultrasound* 2014;**12:**18.
46. McCloskey KV, Carlin P, Skilton J *et al*. Early life markers of atherosclerosis using aortic and carotid intima-media thickness; an assessment of methods to account for child size. *J Vasc Ultrasound* 2015;**39:**119–26.
47. Wang X, Elston RC, Zhu X. The meaning of interaction. *Hum Hered* 2010;**70:**269–77.
48. Miniello VL, Colasanto A, Cristofori F *et al*. Gut microbiota biomodulators, when the stork comes by the scalpel. *Clin Chim Acta* 2015;**451(Pt A):**88–96.
49. Cassidy-Bushrow AE, Sitarik A *et al*. Maternal group B Streptococcus and the infant gut microbiota. *J Dev Orig Health Dis* 2016;**7:**45–53.
50. Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal group B streptococcal colonization in European countries. *Acta Obstet Gynecol* 2008;**87:**260–71.
51. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonization of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* 2009;**9:**437.
52. Dillon HC Jr, Gray E, Pass MA, Gray BM. Anorectal and vaginal carriage of group B streptococci during pregnancy. *J Infect Dis* 1982;**145:**794–99.
53. Colicchia LC, Lauderdale DS, Du H, Adams M, Hirsch E. Recurrence of group B streptococcus colonization in successive pregnancies. *J Perinatol* 2015;**35:**173–76.
54. Mavenyengwa RT, Masunga P, Meque E *et al*. Streptococcus agalactiae (group B streptococcus (GBS)) colonization and persistence, in pregnancy; a comparison of two diverse communities (rural and urban). *Cent Afr J Med* 2006;**52:**38–43.
55. Tsui MH, Ip M, Ng PC, Sahota DS, Leung TN, Lau TK. Change in prevalence of group B Streptococcus maternal colonization in Hong Kong. *Hong Kong Med J* 2009;**15:**41419.
56. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2011;**108(Suppl 1):**4554–61.
57. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J* 2007;**1:**56–66.
58. De La Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP, Dore J. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J Clin Microbiol* 2005;**43:**5588–92.
59. Mueller NT, Whyatt R, Hoepner L *et al*. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int J Obes (Lond)* 2015;**39:**665–70.
60. Azad MB, Bridgman SL, Becker AB, Kozyrskyj AL. Infant antibiotic exposure and the development of childhood overweight and central adiposity. *Int J Obes (Lond)* 2014;**38:**1290–98.
61. Kettleson EM, Adhikari A, Vesper S, Coombs K, Indugula R, Reponen T. Key determinants of the fungal and bacterial microbiomes in homes. *Environ Res* 2015;**138:**130–35.
62. Stokholm J, Schjorring S, Pedersen L *et al*. Living with cat and dog increases vaginal colonization with E. coli in pregnant women. *PloS One* 2012;**7:**e46226.
63. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002;**288:**963–72.
64. Hesselmar B, Aberg N, Aberg B, Eriksson B, Bjorksten B. Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999; 61–17.
65. Cassidy-Bushrow AE, Wegienka G, Havstad S *et al*. Does pet-keeping modify the association of delivery mode with offspring body size? *Matern Child Health J* 2015;**19:**1426–33.
66. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. *Cochrane Database Syst Rev* 2014;**6:**CD007467.
67. Wong M, Edelstein J, Wollman J, Bond MG. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. *Arterioscler Thromb* 1993;**13:**482–86.
68. Chambless LE, Heiss G, Folsom AR *et al*. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors:the Atherosclerosis Risk in Communities (ARIC) Study,1987–1993. *Am J Epidemiol* 1997;**146:**483–94.
69. Salonen JT, Salonen R. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. *Arterioscler Thromb* 1991;**11:**1245–49.
70. Dawson JD, Sonka M, Blecha MB, Lin W, Davis PH. Risk factors associated with aortic and carotid intima-media thickness in adolescents and young adults: the Muscatine Offspring Study. *J Am Coll Cardiol* 2009;**53:**2273–79.
71. Burgner DP, Sabin MA, Magnussen CG *et al*. Early childhood hospitalisation with infection and subclinical atherosclerosis in adulthood: the Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 2015;**239:**496–502.
72. Burgner DP, Cooper MN, Moore HC *et al*. Childhood hospitalisation with infection and cardiovascular disease in early-mid adulthood: a longitudinal population-based study. *PLoS One* 2015;**10:**e0125342.