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Socioeconomic adversity, maternal nutrition, and the prenatal programming of offspring cognition and language at two years of age through maternal inflammation.

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Abstract

Increasing rates of child neurodevelopmental vulnerability are a significant public health challenge. The adverse effect of socioeconomic adversity on offspring cognition may be mediated through elevated prenatal maternal systemic inflammation, but the role of modifiable antecedents such as maternal nutrition has not yet been clarified. This study aimed to examine (1) whether prenatal factors, with an emphasis on maternal nutrition, were associated with prenatal maternal systemic inflammation at 28 weeks' gestation, including the metabolomic marker glycoprotein acetyls (GlycA); (2) the extent to which

the association between prenatal maternal nutrition and child cognition and language at age two years was mediated by elevated maternal inflammation in pregnancy; (3) the extent to which the associations between prenatal socioeconomic adversity and child neurodevelopment were mediated through prenatal maternal nutrition and GlycA levels. We used a prospective population-derived pre-birth longitudinal cohort study, the Barwon Infant Study (Barwon region of Victoria, Australia), where 1074 mother-child pairs were recruited by 28 weeks' gestation using an unselected sampling frame. Exposures included prenatal factors such as maternal diet measured by a validated food frequency questionnaire at 28 weeks' gestation and dietary patterns determined by principal component analysis. The main outcome measures were maternal inflammatory biomarkers (GlycA and hsCRP levels) at 28 weeks' gestation, and offspring Bayley-III cognition and language scores at age two years. Results showed that the 'modern wholefoods' and 'processed' maternal dietary patterns were independently associated with reduced and elevated maternal inflammation respectively (GlycA or hsCRP $p<0.001$), and also with higher and reduced offspring Bayley-III scores respectively (cognition $p\leq0.004$, language $p\leq0.009$). Associations between dietary patterns and offspring cognition and language were partially mediated by higher maternal GlycA (indirect effect: cognition $p\leq0.036$, language $p\leq0.05$), but were less evident for hsCRP. The maternal dietary patterns mediated 22% of the association between socioeconomic adversity (lower maternal education and/or lower household income vs otherwise) and poorer offspring cognition (indirect effect $p=0.001$). Variation in prenatal GlycA levels that were independent of these dietary measures appeared less important. In conclusion, modifiable prenatal maternal dietary patterns were associated with adverse child neurocognitive outcomes through their effect on maternal inflammation (GlycA). Maternal diet may partially explain the association between socioeconomic adversity and child neurocognitive vulnerability. Maternal diet-by-inflammation pathways are an attractive target for future intervention studies.

Keywords: glycoprotein acetyls (GlycA); high-sensitivity C-reactive protein (hsCRP); mediation; mother-child dyads; cognition; language; modern wholefoods dietary pattern; processed dietary pattern

1 Introduction

Child neurodevelopmental problems, including cognitive dysfunction and language delay, are now a major health concern in both high and low income countries (Wang et al., 2023). Early life neurocognitive trajectories are shaped by prenatal environmental influences on the fetus *in utero* which program brain development. Maternal systemic inflammation has been proposed as one mechanism underlying the impact of adverse prenatal environmental factors on child neurocognition (Chen et al., 2016; Han et al., 2021a; Wang et al., 2023). The *in utero* period is critical for neurogenesis, synaptogenesis, and microglial development (Han et al., 2021a). Each of these processes may be impacted by the inflammatory milieu, and in this context, maternal systemic inflammation in pregnancy is emerging as a mechanism inducing adverse neurobiological embedding from an adverse prenatal environment, such as socioeconomic adversity (Girchenko et al., 2020; Gogos et al., 2020; Haddad et al., 2020; Han et al., 2021b; Marx et al., 2022; Pham et al., 2022; Sbisa et al., 2020; Wang et al., 2023). However, the role of associated modifiable factors such as maternal nutrition has not yet been clarified.

Advances in inflammatory biomarker measurement now allow a more precise investigation of the role of maternal chronic inflammation on infant outcomes. Glycoprotein acetyls (GlycA) is a metabolomic inflammatory marker comprising a composite signal of changes in multiple circulating glycosylated acute phase protein (Otvos et al., 2015; Ritchie et al., 2015). Increasing evidence indicates GlycA provides a better measure of cumulative inflammation than the acute phase reactant, high-sensitivity C-reactive protein (hsCRP) (Collier et al., 2019; Mansell et al., 2022; Otvos et al., 2015). Higher GlycA levels are associated with subsequent lower cognition in adulthood (Cohen-Manheim et al., 2015). Two birth cohorts now demonstrate that higher GlycA levels at 27-28 weeks of pregnancy are associated with reduced child cognition at age two years (Marx et al., 2022) and an increased risk of cognitive and motor delay until ten years of age (Girchenko et al., 2020). While the precise mechanism by which prenatal systemic inflammation leads to offspring cognitive impairment in humans is yet to be clarified, epigenetic changes in the brain and peripheral immune system during the critical *in utero* period of development, are likely to play a role (Han et al., 2021a). Animal models shed light on maternal immune activation-induced neurochemical changes that may underlie the association between maternal inflammation and offspring cognition, including dysregulation of microglia, lipids and neurotransmitter systems such as dopamine (Debs et al., 2024; Gogos et al., 2020; Han et al., 2021a; Reisinger et al., 2015; Sbisa et al., 2020; Vacy et al., 2024).

Prenatal maternal dietary variations have been associated with pro-inflammatory markers such as hsCRP, cytokines such as tumor necrosis factor-α, and newer metabolic markers such as GlycA (Yeh et al., 2021). A systematic review of observational studies concluded that diets characterised by higher intake of animal protein and cholesterol, and lower intake of fibre were associated with elevated inflammation, while higher adherence to the Mediterranean diet was associated with reduced inflammation (Yeh et al., 2021). Maternal diet has been demonstrated to be crucial for child neurocognitive development across observational and interventional studies, particularly in relation to micronutrients (Borge et al., 2017). A meta-analysis of 13 studies provided a summary effect of Hedges' g: 0.14, $p<0.001$ for higher maternal diet quality and benefit in offspring cognition (Borge et al., 2017). However, the underlying mechanisms remain to be clarified.

The effect of social adversity on child neurocognition has largely been intractable partly due to the lack of information on mechanisms and their timing, particularly in relation to factors

amenable to modification. Adversity exposure during early life is of great public health importance (Marmot and Bell, 2019; Nelson et al., 2020), as poorer cognitive outcomes in early life have adverse sequelae across the life course, perpetuating a cycle of disadvantage (Vinopal and Morrissey, 2020). Thus, identification of the underlying pathways has the potential to transform policy and interventions to reduce disparities across generations (Nelson et al., 2020; Yu et al., 2021). Socioeconomic adversity is associated with higher inflammation (Berger et al., 2019; O'Connor et al., 2020), lower prenatal maternal diet quality (Livingstone et al., 2017), and also reduced child neurocognition (Nelson et al., 2020; Yu et al., 2021). In our setting, elevated inflammation measured by maternal serum GlycA levels in the third trimester is a pathway underlying part of the adverse effects of socioeconomic adversity on offspring cognition (Marx et al., 2022). Other forms of adverse prenatal environments including maternal obesity, illness, and depression have also been shown to induce offspring neurodevelopmental harm through elevated prenatal maternal inflammation (Girchenko et al., 2020; Wang et al., 2023). An anti-inflammatory diet was shown to be associated with a reduction in adverse neurodevelopmental sequelae associated with prenatal environmental adversity (i.e. maternal obesity, diabetes, hypertensive disorders, mood disorders) (Wang et al., 2023). This raises the possibility that part of the transmission of socioeconomic adversity as a distal factor is occurring through prenatal maternal diet as a proximal factor, with maternal inflammation as the biological pathway to reduced child cognition and language delay (see graphical abstract). We aim to investigate such a developmental cascade (Yu et al., 2021) using highly dimensioned prenatal data from a birth cohort study, and advanced methods including modern causal inference and sequential mediation (VanderWeele and Vansteelandt, 2014). Here, in the Barwon Infant Study (BIS), we aim to examine (1) whether prenatal factors, with an emphasis on maternal nutrition, were associated with maternal systemic inflammation (i.e. GlycA and hsCRP levels measured at 28 weeks' gestation); (2) the extent to which the association between prenatal maternal nutrition and offspring cognition and language at age two years was mediated by higher maternal inflammation in pregnancy; and (3) the extent to which the associations between prenatal socioeconomic adversity and child neurodevelopment were mediated through prenatal maternal nutrition and GlycA levels.

2 Methods

2.1 Study design and participants

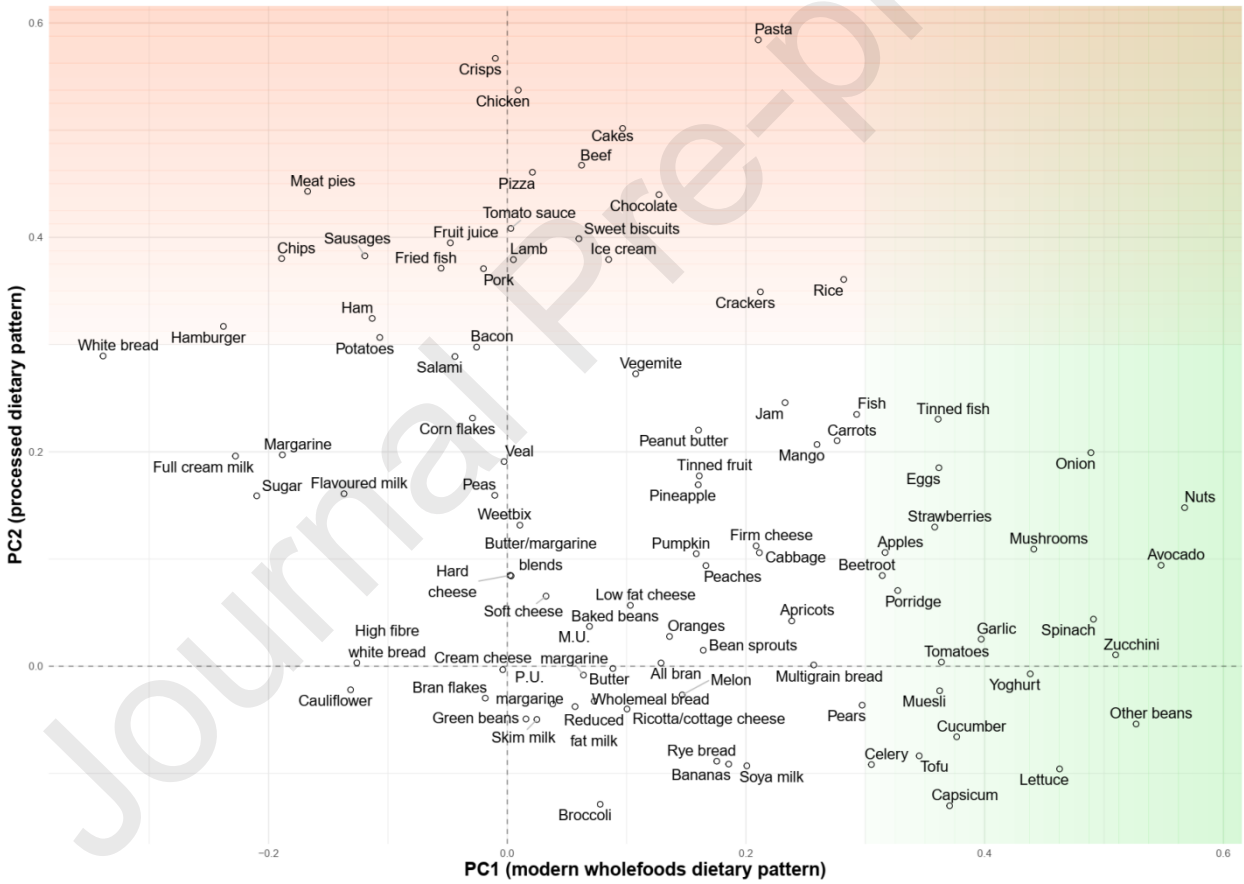
BIS is a pre-birth longitudinal cohort of mother-child pairs (mothers, n=1064; children, n=1074; 10 sets of twins). Antenatal recruitment was conducted using an unselected sampling frame within the Barwon region (south-western Victoria), Australia, by 28 weeks' gestation. Further details of this cohort, including inclusion/exclusion criteria, were reported previously (Marx et al., 2022; Vuillermin et al., 2015). Briefly, women attending either of the two main hospitals of the region during their antenatal appointment (~15 weeks' gestation) were invited to participate in the study. Inclusion criteria included women who were over 18 years, residents of the Barwon region, pregnant (<28 weeks), and English-speaking. Infants were excluded if born before 32 weeks' gestation. The BIS protocol was approved by the Barwon Health Human Research Ethics Committee (#10/24).

2.2 Prenatal factors

The primary exposure variables included 104 prenatal maternal factors grouped into nutrition, conditions/illnesses (self-reported inflammatory-related conditions, infections, mental health status), medication use during pregnancy (self-reported paracetamol and antibiotic use) and seasonality/vitamin D exposure, as well as parent/household demographic factors (Supp Data). A measure of socioeconomic adversity was derived as a composite measure of lower household income (under AU$100,000) and lower maternal education (not university educated) with children from families with one or both factors coded as yes, and neither factor coded as no, following the composite approach of Wang et al (2023). Comprehensive self-completed maternal questionnaires administered at the baseline 28-week visit (28-32 weeks' gestation) and medical record data were utilised.

Maternal nutrition over the preceding four weeks was based on the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES2 (Giles and Ireland, 1996) administered at 28 weeks' gestation; outputs included 10 macronutrients (e.g. fibre), 16 micronutrients (e.g. lutein/zeaxanthin), 18 individual fatty acids, glycemic index and fish intake (Supp Data). The Australian Recommended Food Score (Collins et al., 2008) and Dietary Inflammatory Index (Shivappa et al., 2014) were calculated from the DQES2 (Supp Data). As in our previous work, scores for two maternal dietary patterns derived by principal component (PC) analysis of the 99 food items from the DQES2 were used (Figure 1) (Dawson et al., 2021). PC1 is referred to as a 'modern wholefoods' dietary pattern that was characterised by higher intakes of nuts, green vegetables, and legumes and low intakes of white bread, full cream milk, and hamburgers. PC2 is referred to as a 'processed' dietary pattern that was characterised by higher intakes of pasta, chips, meat, and sweet or salty snack foods and lower intakes of vegetables, tofu, and rye bread. Omega-3 supplement use (g/day) was obtained from participant questionnaires.

Figure 1: Variable loadings for the first two components from a principal component (PC) analysis of 95 food items from the food frequency questionnaire completed at 28 weeks' gestation.



Note: PC1 was identified as a modern wholefoods dietary pattern (green shading) with high loadings on nuts, green vegetables, eggs, fish, legumes, and wholegrain or wholemeal bread and low loadings on white bread and non-core foods such as takeaway (proportion of variance explained was 6%). PC2 was identified as a processed dietary pattern (orange shading) with high loadings on pasta, chips, meat, and sweet or salty snack or takeaway foods and low loadings on vegetables and tofu/soy (proportion of

variance explained was 5%). Each participant had a centred and scaled z-score for the two dietary patterns. Reproduced based on (Dawson et al., 2021) with permission from Elsevier.

2.3 Inflammatory markers

Two inflammatory markers, GlycA and hsCRP, were assessed as previously described (Marx et al., 2022; Soininen et al., 2009). Non-fasting maternal blood samples were collected at approximately 28 weeks' gestation. The composite inflammatory biomarker, GlycA (mmol/l) was assessed by nuclear magnetic resonance (Nightingale Health Ltd., Helsinki, Finland). HsCRP (mg/l) was measured using an ELISA (Human C-Reactive Protein/hsCRP assay DY1707). The distribution of hsCRP was highly skewed, thus all hsCRP values were base-2 log-transformed prior to analysis (Pham et al., 2022).

2.4 Childhood cognitive and language development

The primary outcome measures were cognition and language (receptive and expressive communication) assessed using the Bayley Scales of Infant and Toddler Development, Third edition (Bayley-III (Bayley, 2006)). As described elsewhere (Senn et al., 2020; Symeonides et al., 2021), the Bayley-III was administered to children at age 2-3 years and scored by trained personnel. As in our previous work (Marx et al., 2022), the raw scores of the cognitive development, expressive communication, and receptive communication subscales of the Bayley-III were used. Two children were excluded from analysis: one child was measured outside the age criteria and one child had acquired a neurodisability before this assessment.

2.5 Statistical methods

The distribution of the variables was examined; variables were transformed as required. Complete cases were used for analyses. A Pearson's correlation matrix was created of the key variables. For Aim 1, separate multivariable linear regression models were used to estimate the extent to which prenatal maternal factors associate with i) maternal inflammatory markers (GlycA and hsCRP), and ii) child Bayley-III scores (cognition and language) (Stata v17.0, StataCorp LLC, TX, USA). Consistent with our past work (Marx et al., 2022), regression models were adjusted for child's assigned sex at birth and process factors relating to the outcome (for inflammatory outcomes: gestational age at blood collection; for child cognitive and language outcomes: postconceptional age at time of Bayley-III assessment, and experience of Bayley-III test administrator). Composite dietary variables (modern wholefoods dietary pattern, processed dietary pattern, and Dietary Inflammatory Index) were additionally adjusted for dietary energy intake (kJ/day) (Hodge et al., 2000). The Benjamini-Hochberg procedure was applied to the *p*-

values from regression sets to account for multiple hypothesis testing with discoveries reported using a false discovery rate of 5% (q<0.05).

To assess Aim 2, mediation analysis (Pearl, 2001; Tingley et al., 2014) was conducted for prenatal factors related to maternal nutrition that were associated with maternal inflammation and also with child cognitive or language scores with GlycA or hsCRP as the mediator (R v4.1.0, R Foundation for Statistical Computing). Counterfactual mediation analyses with single mediators were conducted with the R *mediation* package and confidence intervals were calculated using the quasi-Bayesian method with 1,000 replicates (Tingley et al., 2014). In addition to adjusting for child's sex and the process factors described above, ancestry (all grandparents of European descent vs otherwise; previously identified (Marx et al., 2022)) and socioeconomic adversity (lower maternal education and/or lower household income vs otherwise) were also included in the mediation models as confounders. We considered the role of over 50 other potential confounders (Supp Data) (Ponsonby, 2021), including across the following domains: socio-demographics (e.g. parity, household size), medical conditions during pregnancy (e.g. infections, inflammatory or metabolic conditions), maternal exposures (e.g. antibiotics, supplement use, tobacco smoke exposure), birth-related exposures (e.g. delivery, birth weight), and including previously published potential cognition determinants (Marx et al., 2022; Symeonides et al., 2021). We did not include maternal weight or antenatal depression as confounders because these can be a consequence of maternal diet (Baskin et al., 2017; International Weight Management in Pregnancy Collaborative, 2017) and thus do not fulfil the criteria of confounding (Lash et al., 2021).

For Aim 3, we considered both the role of dietary factors and also other, non-dietary factors that may be operating through elevated prenatal GlycA levels to mediate the association between socioeconomic adversity and child cognitive development. We utilised two approaches assessing the aggregate effect of the non-dietary factors, rather than considering each of the numerous factors operating individually. Joint mediation analyses (VanderWeele and Vansteelandt, 2014) using the R *medflex* package with bootstrapped standard errors and 1,000 replicates (Steen et al., 2017) assessed the extent to which multiple dietary factors (modern wholefoods and processed dietary patterns) mediated the association between the socioeconomic adversity composite measure and child cognition. We compared these joint mediation results to those where GlycA was also included as a mediator to assess the additional contribution of GlycA beyond dietary patterns alone (VanderWeele and Vansteelandt, 2014). For our second approach, we first endeavoured to remove variation in GlycA levels influenced by diet by regressing GlycA on modern wholefoods and processed dietary patterns and taking the residuals (Armitage et al., 2008). The residuals should largely then reflect variation in GlycA levels influenced by other, non-dietary factors, such as maternal smoking or genetic status. We then tested these residuals as mediators of the association between socioeconomic adversity and child cognition in mediation analyses. Both mediation approaches assume no exposure-induced mediator-outcome confounding (VanderWeele and Vansteelandt, 2014). HsCRP was not assessed in Aim 3 as it did not mediate the association between maternal dietary patterns and offspring cognition.

In sensitivity analyses, we investigated sex-specific effects by including in the model a product term for the interaction between the exposure and offspring sex (Buckley et al., 2017). Inverse probability weighting was used to assess the potential impact of any selection bias in the recruitment or retention of the cohort on the main findings. Variables used in the weighting were mother's age at conception, mother's asthma status (yes vs no), Socio-Economic Indexes

for Areas (Pink, 2011) in lowest tertile vs not, remoteness area (regional/remote vs major city (Pink, 2011)), and household size.

3 Results

3.1 General features

There were no major differences between the total cohort and the mediation sample (Table 1). 59% of mothers were university educated. Consistent with previous reports of Australian women (Collins et al., 2008), prenatal maternal diet was generally not optimal; the average Australian Recommended Food Score was 35 out of a maximum possible score of 74, with only 25% of women with a score over 40 (n=259), and 2% over 50 (n=22). During pregnancy, 71% of women had sufficient levels of vitamin D (>75 nmol/l), and 86% and 83% exhibited little depressive or anxiety symptoms, respectively, based on scores given on the Edinburgh Postnatal Depression Scale. During pregnancy, 64% of women reported at least one infection and 18% reported using antibiotics (Table 1). The two major maternal dietary patterns identified by principal component analysis are referred to as the modern wholefoods and the processed dietary patterns (Figure 1). These patterns are not correlated due to their derivation from PC analysis (Figure 2). Summarising the maternal inflammatory markers, the mean level of GlycA was 1.6 mmol/l (standard deviation = 0.2) and the median level of hsCRP was 3.6 mg/l (interquartile range = 1.9-5.9). Prenatal GlycA and hsCRP were moderately correlated (r=0.35, p<0.001) (Marx et al., 2022). Cognitive and language development scores were consistent with previous Australian data (Anderson et al., 2010).

Table 1: Key characteristics of participants in the Barwon Infant Study: comparison between the total birth cohort (used for regression analyses) and the mediation analyses cohort.

	Total birth cohort		**Mediation cohort**[A]	
	Mean (SD), % (n), or median [IQR]	**N**	Mean (SD), % (n), or median [IQR]	**N**
Children		1074		670

Parent and household factors

Mother's age at conception (years)	31.3 (4.8)	1074	32.0 (4.5)	670
Father's age at conception (years)	33.5 (5.9)	1024	33.8 (5.6)	637
Mother is university-educated	51.3% (548)	1068	59.2% (395)	667
Father is university-educated	35.2% (367)	1044	39.5% (258)	653
Mother born in Australia	90.2% (967)	1072	89.6% (600)	670
Grandparents are of European descent	73.0% (774)	1060	73.8% (491)	665
Household income under AU$100,000[B]	55.4% (577)	1041	51.7% (338)	654
SEIFA score in lowest tertile	33.6% (357)	1061	30.7% (203)	662
Birth order >1	55.3% (593)	1073	55.0% (368)	669
Environmental tobacco smoke exposure[C]	16.9% (177)	1049	13.1% (86)	656
Mother smoking throughout pregnancy	6.3% (67)	1061	3.3% (22)	667
Any alcohol consumption during pregnancy	52.7% (521)	989	51.2% (333)	650

Prenatal maternal factors

- Nutrition

Pre-pregnancy BMI (kg/m^2)	25.4 (5.5)	927	25.2 (5.3)	627
Mother's weight at 28-week interview (kg)	80.9 (15.5)	866	80.1 (14.9)	593
Australian Recommended Food Score	34.9 (8.0)	1016	35.3 (7.9)	642
Modern wholefoods dietary pattern	0.003 (1.0)	1016	0.113 (1.0)	642
Processed dietary pattern	-0.002 (1.0)	1016	-0.030 (0.9)	642
Dietary Inflammatory Index	3.6 (1.9)	1016	3.5 (1.9)	642
Dietary energy (kJ/day)	7442.1 (2346.0)	1016	7483.0 (2233.8)	642
Dietary sugars (g/day)	93.9 (32.5)	1016	93.9 (30.5)	642
Dietary fats (g/day)	74.3 (26.7)	1016	74.8 (25.7)	642
Dietary omega-3 (g/day)	1.4 (0.6)	1016	1.4 (0.6)	642
Dietary protein (g/day)	84.8 (27.4)	1016	85.9 (26.7)	642

Dietary fibre (g/day)	21.3 (7.4)	1016	21.8 (7.3)	642
- Medical conditions				
Fever during trimester 2	6.4% (68)	1064	6.0% (40)	666
Any infection during pregnancy[D]	67.0% (661)	987	64.3% (408)	635
Mother has an immuno-inflammatory illness[E]	59.3% (608)	1025	61.0% (389)	638
Mother has a metabolic-inflammatory condition[F]	8.4% (75)	891	7.3% (40)	551
EPDS depression score <10 (low symptoms)	82.9% (635)	766	85.9% (463)	539
EPDS anxiety subscale score <5 (low symptoms)	80.8% (619)	766	83.1% (448)	539
- Medications/supplements[G]				
Any paracetamol use	86.7% (896)	1033	84.7% (560)	661
Any antibiotics use	17.0% (180)	1061	17.5% (116)	663
Amoxicillin use during trimester 2	5.1% (54)	1061	4.7% (31)	663
Antidepressant use	4.7% (50)	1074	4.5% (30)	670

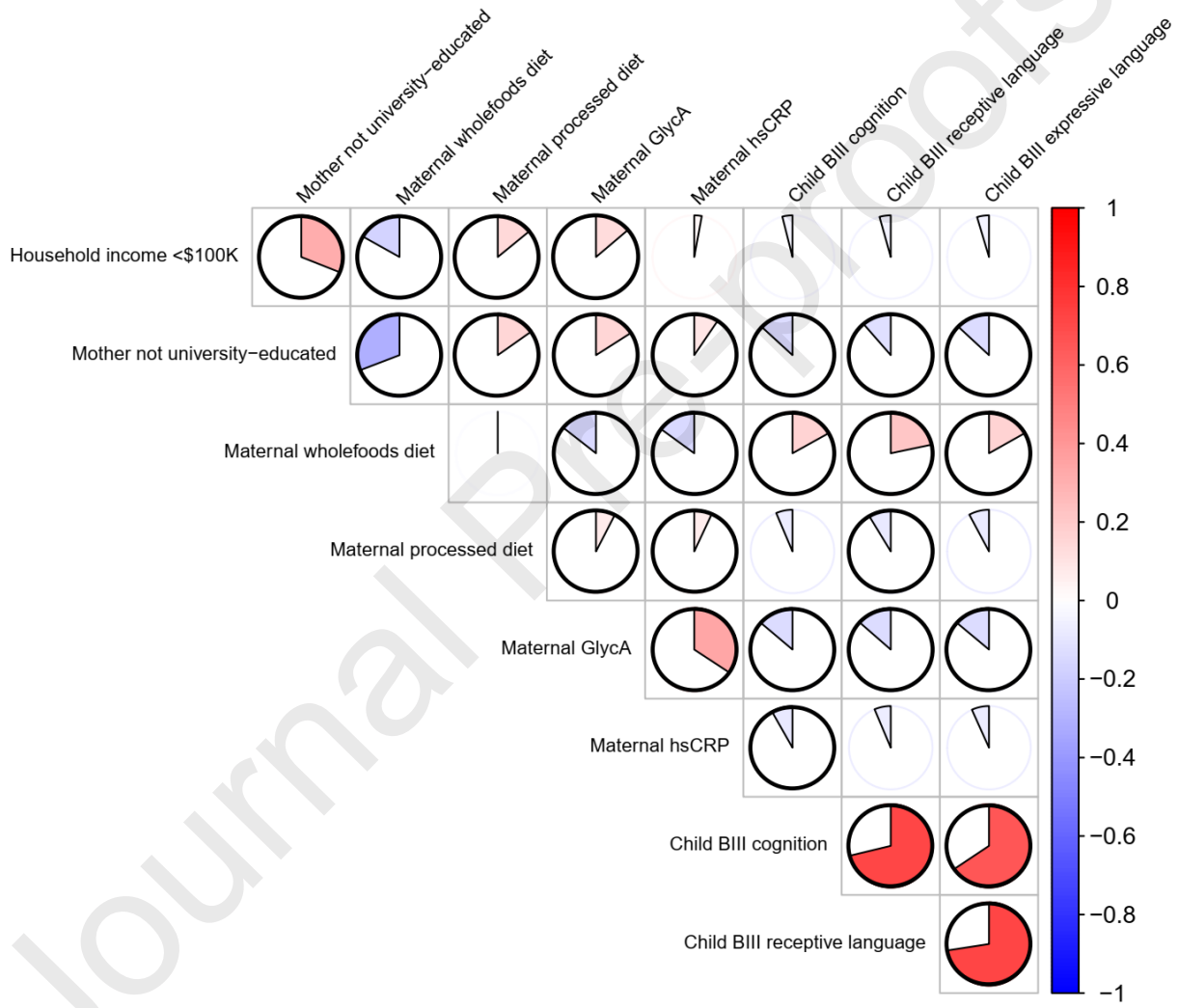
Folic acid supplement use	97.4% (1003)	1030	97.7% (640)	655
Fish oil supplement use	16.1% (98)	608	17.0% (74)	435
Vitamin D supplement use	89.9% (926)	1030	91.6% (600)	655
Vitamin D levels (nmol/l)	86.8 (25.5)	383	86.9 (24.7)	285
Child factors				
Sex at birth: Male	51.7% (555)	1074	53.7% (360)	670
Gestational age at birth (weeks)	39.4 (1.5)	1074	39.5 (1.5)	670
Caesarean birth[H]	31.2% (335)	1074	31.8% (213)	670
Winter birth	28.8% (309)	1074	33.4% (224)	670
Summer birth	25.5% (274)	1074	21.2% (142)	670
Birth weight: normal (2.5 - 4.2 kg)	88.3% (947)	1072	89.7% (601)	670
Breastfeeding for >6 months	60.7% (603)	993	65.4% (438)	670
Weeks since starting centre-based childcare at age 2	32.8 (33.6)	1006	36.1 (33.1)	670

Maternal inflammatory markers[I]				
Glycoprotein acetyls (GlycA; mmol/l)	1.6 (0.2)	1041	1.6 (0.2)	670
High-sensitivity C-reactive protein (hsCRP; mg/l)	3.6 [1.9-5.9]	1039	3.5 [1.9-5.6]	670
Gestational age at blood collection (weeks)	28.1 [27.6-28.7]	1047	28.0 [27.4-28.7]	670
Child development scales				
Bayley-III Cognition[J]	10.8 (2.0)	676	10.8 (2.0)	662
Bayley-III Language[J]				
Expressive Communication	12.1 (2.6)	569	12.1 (2.6)	559
Receptive Communication	11.9 (2.1)	579	11.9 (2.1)	569
Age at assessment (months)	29.3 [28.2-30.5]	701	29.3 [28.2-30.5]	670

[A] Includes only the participants who have Bayley-III cognition/language scores and GlycA measures; used in mediation analysis. [B] Annual household income during previous 12 months. [C] Exposure during the 3 months preconception or pregnancy. [D] Includes infections of teeth, gum, ear, eye, throat, urinary tract, kidney, cold sores, genital herpes, gastroenteritis, candidiasis, or pelvic inflammatory disease. [E] Includes current asthma/hayfever/ eczema/allergies, or a history of coeliac, lupus, autoimmune, multiple sclerosis, rheumatoid arthritis, or inflammatory bowel. [F] During pregnancy the mother had gestational diabetes, pre-eclampsia, or hypertension. [G] During pregnancy, unless otherwise stated. [H] Includes elective and emergency caesarean births vs vaginal (assisted or normal) birth. [I] Inflammatory markers measured from maternal blood. [J] Bayley-III scaled scores.

IQR = interquartile range; n = number; SD = standard deviation.

Figure 2: Pearson's correlation matrix of key variables used in this study.



Note: Maternal dietary patterns (modern wholefoods and processed) and inflammatory markers (GlycA and hsCRP) were assessed at 28 weeks' gestation. Child Bayley-III (BIII) cognition and language (receptive and expressive communication) scores assessed at 2 years of age. Red indicates a positive correlation; blue indicates a negative correlation. A larger coloured area in the circles indicates a stronger correlation between the row and column variable. A bold circle indicates a significant correlation (p<0.05).

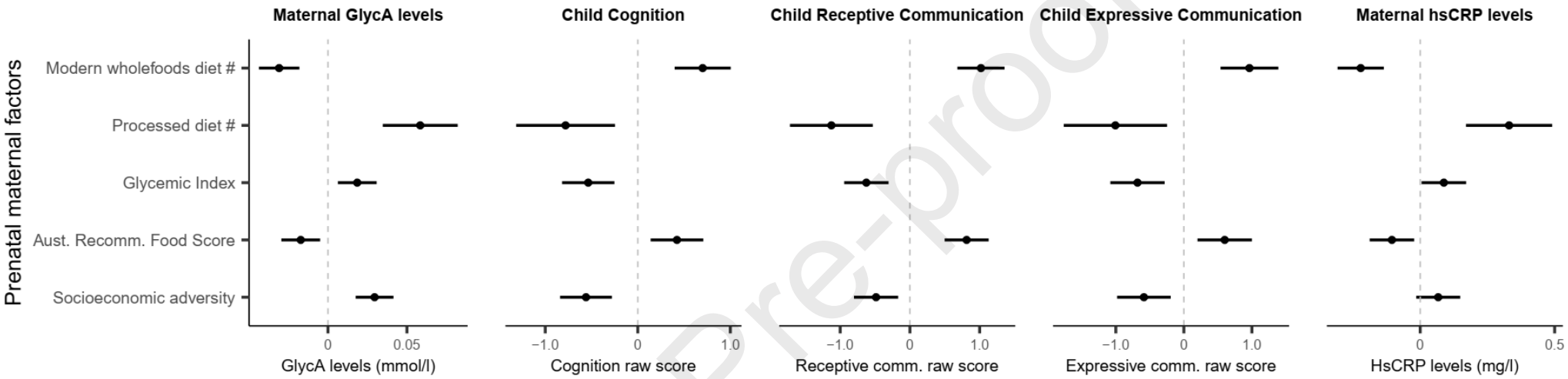
3.2 Linear regression analyses - Associations between prenatal factors, inflammatory markers, and child cognitive/language outcomes

A large number of prenatal factors were associated with maternal inflammatory markers or child cognition and/or language at age two years. See Table S1 for associations between all prenatal factors with inflammatory markers or child outcomes.

3.2.1 In the mother during pregnancy, a modern wholefoods dietary pattern was associated with lower inflammation, while a processed dietary pattern was associated with elevated inflammation.

Higher scores on the maternal wholefoods dietary pattern in pregnancy were associated with lower prenatal maternal GlycA levels (β=-0.03 mmol/l GlycA per SD increase in diet score, 95% CI -0.04 to -0.02, p<0.001, q<0.001; Figure 3, Table S1). A higher Australian Recommended Food Score and greater daily consumption of fibre and lutein/zeaxanthin were also associated with lower levels of maternal GlycA (Table S1). Conversely, higher scores for the processed dietary pattern in pregnancy were associated with higher prenatal maternal GlycA levels (β=0.06 mmol/l GlycA per SD increase in diet score, 95% CI 0.03 to 0.08, p<0.001, q<0.001). Similarly, higher maternal Dietary Inflammatory Index and glycemic index were associated with higher maternal GlycA levels (Table S1). Average dietary intake of four individual fatty acids was related to serum GlycA levels. These included eicosadienoic acid (C20:2 n-6) and dihomo-gamma-linolenic acid (C20:3 n-6; both omega-6 long-chain polyunsaturated fatty acids), which were negatively associated suggesting anti-inflammatory properties, while heptadecenoic acid (C17:1, a monounsaturated fatty acid) and elaidic/trans vaccenic acid (C18:1t, a trans-unsaturated fatty acid) were positively associated suggesting pro-inflammatory properties (Table S1). Other dietary variables (Table S1), such as the fatty acids, omega-3 and omega-6, and fish intake were each considered and were not strongly related to GlycA levels. None of the associations between the dietary factors mentioned above and GlycA, apart from a processed dietary pattern, persisted after adjustment for modern wholefoods dietary pattern score. Therefore, the two independent dietary factors, modern wholefoods and processed patterns, were then carried forward for further analysis. Similar patterns were observed in the associations between these maternal dietary factors and the maternal inflammatory marker, hsCRP (Figure 3, Table S1).

Figure 3: Associations between prenatal maternal factors and maternal GlycA levels at 28 weeks' gestation and child Bayley-III cognition and language scores at age 2 years: only prenatal factors that were significantly (p<0.05, q<0.05) associated with both maternal GlycA levels and child Bayley-III cognition scores are depicted.



Note: Forest plot shows the estimated change in maternal inflammatory markers (GlycA and hsCRP) levels and child Bayley-III cognition and language scores per one standard deviation increase of each prenatal maternal factor. Regression models minimally adjusted for: gestational age at blood collection and child's sex (for GlycA and hsCRP levels); and child's sex, child's post-conception age at time of Bayley-III testing, Bayley-III tester experience (for Bayley-III scores). To make the results comparable across factors in the forest plots, all variables were standardised to have a mean of 0 and a standard deviation of 1. # Model additionally adjusted for dietary energy. Aust. Recomm. Food Score = Australian Recommended Food Score; comm. = communication. Socioeconomic adversity refers to having at least one of lower maternal education (mother not university educated) and/or lower household income (<AU$100,000 annually) vs neither.

Note 2: After adjustment for modern wholefoods dietary pattern, glycemic index and Australian Recommended Food Score did not reach significance.

Note 3: We have previously reported associations between several parent and household factors and (i) maternal inflammatory markers (GlycA, hsCRP) and (ii) child cognition (Marx et al., 2022), thus these are not shown in the plot (see Table S1; significant associations include maternal education, paternal education, household size). Similar associations were found between these parent and household factors and child language outcomes, as for cognition (Table S1).

3.2.2 Maternal dietary patterns at 28 weeks' gestation were associated with offspring cognition and language scores at age 2 years.

Higher intakes of the prenatal maternal dietary patterns (modern wholefoods, processed) were associated with higher and lower offspring cognition scores, respectively (modern wholefoods: β =0.71 change in Bayley-III cognition raw score per SD increase in diet score, 95% CI 0.40 to 1.02, p<0.001, q<0.001; processed: β=-0.85 change in cognition raw score per SD increase in diet score, 95% CI -1.43 to -0.27, p=0.004, q=0.036; Figure 3, Table S1). Maternal dietary indices (Dietary Inflammatory Index, glycemic index, Australian Recommended Food Score) and other dietary components were also associated with offspring cognition (Table S1), but with the exception of fibre, these did not persist after modern wholefoods dietary pattern adjustment.

There were similar associations between these maternal dietary factors and offspring receptive and expressive communication scores (Figure 3, Table S1).

3.2.3 Prenatal maternal medical conditions were associated with elevated inflammation.

Women with gestational diabetes mellitus and pre-eclampsia had on average 0.15 (95% CI 0.09 to 0.21, p<0.001, q<0.001) and 0.12 (95% CI 0.05 to 0.18, p=0.001, q=0.013) mmol/l higher GlycA levels, respectively, compared to women without these conditions (Table S1). Other factors associated with higher GlycA levels included poor mental health (stress and depression), presence of any type of immuno-inflammatory illness, upper respiratory tract infection in the second trimester, and antibiotic use (Table S1).

Gestational diabetes mellitus, presence of any type of immuno-inflammatory illness, and infections and fever in the second trimester were associated with the maternal inflammatory marker, hsCRP (Table S1).

The prenatal maternal medical conditions listed above did not show evidence of an association with child cognitive/language outcomes (Table S1).

3.3 Mediation analyses

3.3.1 Prenatal maternal GlycA levels mediate the effect of maternal dietary patterns on child outcomes.

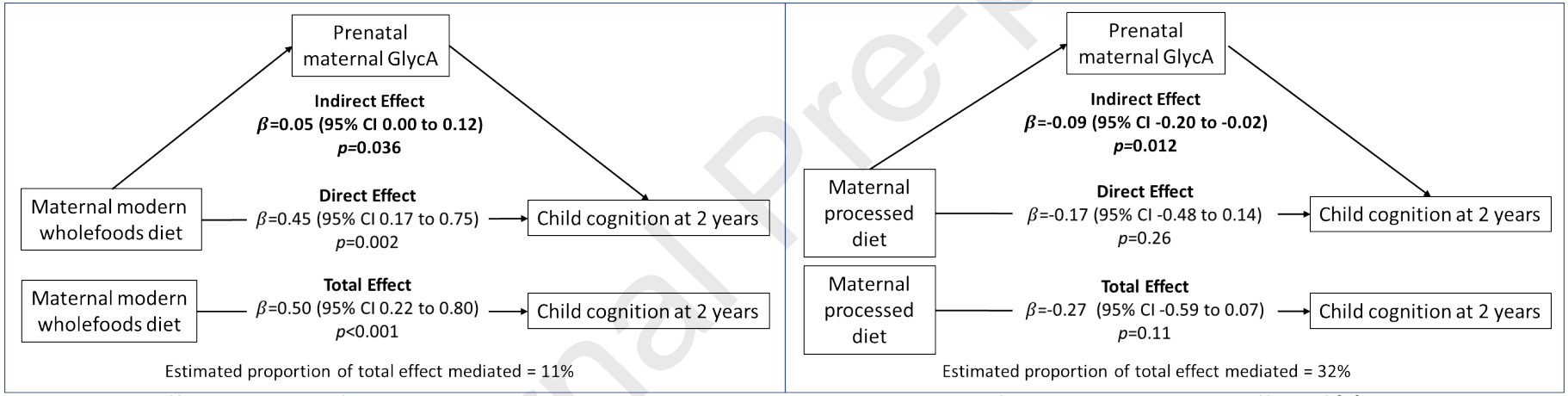
Maternal GlycA levels at 28 weeks' gestation partially mediated the association between each prenatal maternal dietary pattern and offspring outcomes measured at age 2 years (cognition, receptive and expressive communication) (Table 2, Figure 4). The estimated proportion of the effect of a modern wholefoods dietary pattern or a processed dietary pattern on the three child outcomes that was mediated by GlycA levels ranged from 7-13% and 27-36%, respectively (Table 2, Figure 4). There were weaker findings for hsCRP (Table 2).

Table 2: Mediation analyses of prenatal maternal dietary patterns at 28 weeks' gestation and child Bayley-III test scores at age 2 years, with consideration of maternal inflammatory markers as mediating factors.

		Child Bayley-III raw scores					
		Cognition		Receptive communication		Expressive communication	
Prenatal maternal factors	**Effects**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
GlycA as mediator							
Modern wholefoods dietary							
Processed dietary pattern							
Modern wholefoods dietary							
Processed dietary pattern							

Note: Models were adjusted for socioeconomic adversity (lower maternal education and/or lower household income vs otherwise), ancestry (European vs otherwise), dietary energy, gestational age at blood collection, child's sex, child's post-conception age at time of Bayley-III testing, Bayley-III tester experience. Bold values show $p<0.05$ for indirect effects only.

Figure 4: Prenatal maternal GlycA levels mediate the association between maternal dietary patterns at 28 weeks' gestation and child Bayley-III cognition scores at age 2 years.



Note: Indirect effect estimates of the extent to which prenatal maternal GlycA levels at 28 weeks' gestation mediate the effect of **(a)** a prenatal maternal modern wholefoods dietary pattern, and **(b)** a prenatal maternal processed dietary pattern on child cognition at 2 years. Models were adjusted for socioeconomic adversity (lower maternal education and/or lower household income), ancestry (European), dietary energy, gestational age at blood collection, child's sex, child's post-conception age at time of Bayley-III testing, Bayley-III tester experience.

3.3.2 A broader investigation taking socioeconomic adversity into consideration.

The indicators used in this study for socioeconomic adversity, i.e. lower maternal education and lower household income, associated with lower prenatal maternal intake of a modern wholefoods dietary pattern (r=-0.31, p<0.0001 and r=-0.17, p<0.0001, respectively) and higher intake of a processed dietary pattern (r=0.15, p<0.0001 and r=0.14, p<0.001, respectively) (Figure 2). We have previously reported that these socioeconomic adversity indicators were associated with reduced child cognition in the BIS cohort and part of their effect was mediated through higher prenatal GlycA levels (Marx et al., 2022). Here we report similar findings for child receptive and expressive communication scores (Table S2). That is, indirect effect estimates indicated that higher maternal GlycA levels partially mediate the association between the following factors and poorer child language indices: socioeconomic adversity, lower maternal education, lower paternal education, greater household size, prenatal maternal tobacco smoke exposure, and higher maternal body mass index (Table S2).

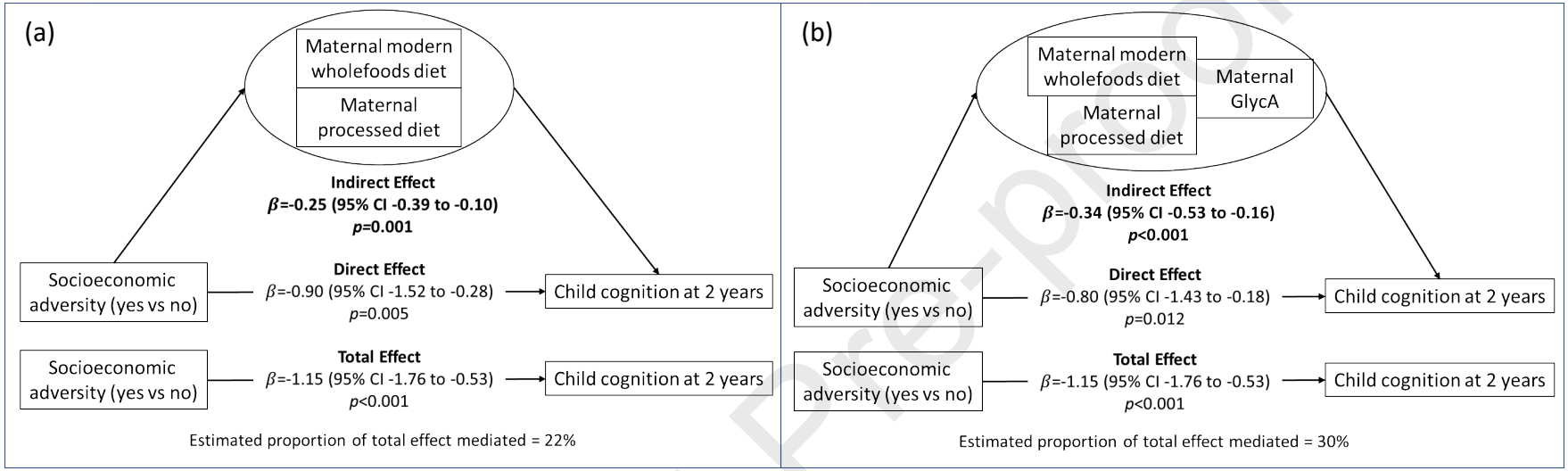
Further, we demonstrate that dietary patterns underly part of the effect of socioeconomic adversity on higher GlycA levels (Table S3). We then considered the interplay between socioeconomic adversity (distal exposure), prenatal maternal nutrition (proximal exposure), prenatal maternal GlycA levels (the biological indicator), and child cognitive and language outcomes. We utilised joint mediation considering multiple mediators (n=2-3), where the dietary patterns are independent of each other, and both are associated with GlycA levels (Figure 5). There was strong evidence that dietary patterns (n=2 mediators: maternal modern wholefoods and processed diet) were partial mediators of the effect of socioeconomic adversity on poorer child cognitive outcomes (indirect effect p=0.001; Figure 5a). We compared these mediation results to those where GlycA was also included as a mediator (n=3 mediators; Figure 5b) to assess the additional contribution of GlycA beyond dietary patterns alone. More of the effects of socioeconomic adversity were explained by dietary patterns (both through GlycA and non-GlycA pathways; estimated to be 22%) than through diet-independent differences in GlycA (estimated to be 8%), highlighting the importance of maternal diet during pregnancy (Figure 5).

Using an alternative approach, we conducted mediation analysis treating residuals of GlycA after regression on the two dietary patterns as the putative mediator. If the majority of the socioeconomic adversity effect were acting through non-dietarily sourced high inflammation, one would expect high magnitude and significant effects in Figure S1. By contrast, a low magnitude, insignificant indirect effect for socioeconomic adversity was found (β=-0.08, 95% CI -0.17 to 0.01, p=0.09; Figure S1). Consistent with this, higher residual GlycA levels (after the dietary contribution to GlycA had been removed) were only weakly associated with child cognition (β= -1.52, p=0.06).

3.4 Additional analyses - Inverse probability weighting and sex-specific analyses.

Regression and mediation analysis estimates for the two maternal dietary pattern exposures did not materially change after applying inverse probability weighting (data not shown). The key findings did not appear sex-specific, with no difference in effect by sex (p>0.2 for interaction) and effects evident in both males and females (data not shown).

Figure 5: **Prenatal maternal dietary patterns mediate the association between prenatal socioeconomic adversity and child Bayley-III cognition scores at age 2 years: joint mediation with consideration of maternal dietary patterns and GlycA levels as mediating factors.**



Note: Indirect effect estimates of the extent to which the association between prenatal socioeconomic adversity and child cognition at 2 years is mediated by **(a)** prenatal maternal dietary patterns (modern wholefoods and processed diets), or **(b)** prenatal maternal dietary patterns (modern wholefoods and processed diets) and prenatal maternal GlycA levels. Socioeconomic adversity refers to having at least one of lower maternal education (mother not university educated) and/or lower household income (<AU$100,000 annually) vs neither. Models were adjusted for ancestry (European), dietary energy, gestational age at blood collection, child's sex, child's post-conception age at time of Bayley-III testing, Bayley-III tester experience.

4 Discussion

In this large, population-based pre-birth cohort, we found compelling evidence that maternal diet impacts child cognitive and language outcomes through systemic inflammation during pregnancy. Furthermore, we found that this pathway contributes to higher rates of adverse neurocognitive outcomes for children with socioeconomic adversity in early life. Maternal dietary patterns (low modern wholefoods and high processed diets) accounted for 22% of the effect of socioeconomic adversity on reduced child cognition, with the maternal dietary pattern effect partly acting through higher inflammation at 28 weeks' gestation. These findings highlight the public health opportunity to improve child neurocognitive outcomes by improving maternal nutrition with the goal of reducing elevated GlycA levels during pregnancy.

We found that women with greater socioeconomic adversity were more likely to have lower quality diets and prenatal maternal dietary patterns were demonstrated to be one of the proximal factors through which socioeconomic adversity operates. Among a myriad of prenatal factors linked to higher inflammation, prenatal maternal dietary patterns were a key driver of inflammation-associated neurocognitive harm. When considering the combined effect of maternal diet and GlycA in mediating the effect of socioeconomic adversity on child cognition, more than half of the combined effect was accounted for by maternal dietary patterns alone during pregnancy. The association between socioeconomic adversity and child cognition did not appear to operate through non-dietary GlycA elevation. We then obtained a residual GlycA level where dietary patterns as a source had been extracted and found that other measured pro-inflammatory factors and unmeasured ones such as genetic inflammatory score (Bind et al., 2014; Heslop et al., 2012), had only low magnitude indirect effects of borderline significance. A meta-analysis of 13 studies has already reported that poor maternal diet quality during pregnancy was associated with lower subsequent cognitive and behavioural outcomes in children (Borge et al., 2017). The main contribution of the current study was to identify key underlying biological mechanisms, and to demonstrate that the impact of socioeconomic adversity on neurocognitive outcomes are partly mediated by maternal diet. Previous studies in pregnancy have found apparently separate effects of i) a high Dietary Inflammatory Index and glycemic index, and ii) low fibre intake, on elevated maternal inflammatory marker levels (Roytio et al., 2017; Yeh et al., 2021). Consistent with evidence that various dietary metrics may act independently of one another, we found that the effects of two independent dietary patterns, modern wholefoods and processed, suggest there are two potentials for dietary intervention.

GlycA appears to provide a better measure of chronic inflammation than hsCRP, which is highly impacted by recent inflammatory events (Collier et al., 2019; Mansell et al., 2022; Otvos et al., 2015; Ritchie et al., 2015). In previous studies, the associations between elevated GlycA levels and various diseases are generally independent of hsCRP levels (Duprez et al., 2016; Huckvale et al., 2020; Mansell et al., 2022; Mehta et al., 2020). Moreover, GlycA may be more biologically relevant than hsCRP because it is a composite marker that is highly correlated with metabolomic and microbiome variation (Mokkala et al., 2020). Consistent with these advantages, we were able to demonstrate compelling evidence of maternal diet-by-inflammation to adverse child neurocognitive pathways for GlycA, while findings were weaker for hsCRP. It is highly plausible that this is because GlycA is providing a better measure of cumulative inflammation over the course of prenatal brain development than hsCRP (Collier et al., 2019; Mansell et al., 2022; Otvos et al., 2015; Ritchie et al., 2015).

Strengths and weaknesses

Strengths include that this study used prospective longitudinal data from a large, well-characterised mother-child cohort with high retention, allowing us to comprehensively assess a diverse range of prenatal maternal factors, metabolic measures, and precise cognitive phenotyping at age 2 years. Prenatal diet was measured using a validated questionnaire which is correlates well with weighed food records (Hodge et al., 2000). The use of two inflammatory markers provides insight into the acute vs chronic inflammatory responses (Mansell et al., 2022) associated with child outcomes. Socioeconomic adversity is very difficult to measure and quantify properly (Yu et al., 2021). BIS includes families from a diverse range of backgrounds, including the very affluent, and those from some of the most disadvantaged communities in the country, where greater than 40% of children commence school with some neurocognitive vulnerability (2019; Vuillermin et al., 2015). Our markers of prenatal adversity (lower maternal education and lower household income) were chosen as they are less likely to reflect consequences of diet or inflammation, unlike other prenatal adversity scores based on maternal weight, depression, diabetes, or hypertension. However, we acknowledge that characterisation of early life adversity is difficult and would ideally include type, duration, critical period and interaction across adversities (Nelson et al., 2020).

Further limitations include that analysis was done in a single cohort without external replication, however comparable cohorts with an analogous depth of socioeconomic, nutrition, metabolomic, and cognition/language measures were not available. This Australian cohort was predominantly of European ancestry, limiting generalisation to other settings and populations. Misclassification of reported data, including dietary data (Yeh et al., 2021), may have occurred but this is likely non-differential and would have moved the findings towards the null, yet strong associations were nevertheless observed.

Several features improve our confidence in the potential causal nature of the results: (1) the direction of the effect is consistent with previous mechanistic understanding providing biological plausibility; (2) inflammatory markers were directly measured; (3) a modern causal inference technique, counterfactual mediation considering multiple mediators, was used, as well as an alternative residual approach - both provided similar findings for a key role of maternal dietary patterns in mediating socioeconomic adversity effects on inflammation-induced neurodevelopmental harm. In addition, selection bias was evaluated by inverse probability weighting and found to be minimal. While we did not find any major effects of sex, future studies with large power are needed given the known sex differences that exist in neurodevelopmental disorders (Breach and Lenz, 2022; Gogos et al., 2019).

Future research, implications, and conclusions

Further longitudinal studies are required to replicate the identified associations, ideally with multiple real-time diet assessments, serial GlycA measures, and cognition/language measures at later timepoints. Future studies should examine specific cognitive domains in more detail (Monthe-Dreze et al., 2019) as well as the mechanisms underlying the effects of GlycA on neurodevelopment. Possible inflammatory mechanisms underlying the association between prenatal nutrition and offspring cognition include metabolic regulation, genetic and cellular (microglia) programming, compromised blood-brain barrier integrity, gut microbiome changes, and placental dysfunction (Bordeleau et al., 2020; Dawson et al., 2021; Harris et al., 2022; Kendig et al., 2021; Khambadkone et al., 2020; Vuillermin et al., 2020).

Here, the findings of maternal dietary patterns mediating the association between socioeconomic adversity and poor offspring cognition, partly through maternal inflammatory mechanisms, have important implications for designing future intervention studies. Both the prenatal modern wholefoods and processed dietary patterns were differentially, independently, and strongly associated with both maternal inflammation and offspring cognitive outcomes. This knowledge can inform interventions aiming to support women to eat a healthy diet during pregnancy, with the proximal goal of reducing systemic inflammation. These results support evidence that prenatal diets rich in wholefoods may reduce pregnancy complications and adverse child health outcomes (Marshall et al., 2022). By contrast, consuming a processed diet depletes the diversity and function of the gut microbiome (Fernandes et al., 2023) and this diet is associated with long-term negative health outcomes (Lane et al., 2021; Lane et al., 2024; Mannino et al., 2023; Wang et al., 2022). It was recently reported that ultra-processed foods differ from unprocessed wholefoods in that they provide poorer nutrient profiles, displace wholefoods from the diet, have altered physical and chemical properties through industrial processing methods, and are more likely to contain additives and toxic contaminants from packaging/processing (Lane et al., 2024; Sugeng et al., 2020). Although mechanistic research is still in its infancy, such properties may act through physiological mechanisms including increased inflammation (Lane et al., 2024). Inflammation-targeted nutrition therapy (Yeh et al., 2021) and human microbiome manipulation (Sorboni et al., 2022), which show promise in reducing inflammation, are exciting future prospects and research should aim to identify the specific dietary changes needed to significantly alter inflammation. Importantly, GlycA appears to provide a proximal target measure for dietary interventions, which is likely to be better embraced than body mass index, which is impacted by a wide range of factors and is value laden. The field lacks dietary pattern interventions, especially those involving human participants (Koblinsky et al., 2022), highlighting the need for large randomised controlled trials evaluating novel interventions to improve prenatal nutrition and subsequently offspring neurodevelopment, with a focus on inflammation as a potential mediator.

The present findings, demonstrating sequential mediation, that is, socioeconomic adversity to prenatal maternal dietary patterns to maternal third trimester inflammation to child cognition and language, support the importance of an anti-inflammatory prenatal diet for optimal offspring neurocognitive development – a finding that is of substantial public health significance. The prenatal period is a critical window of neurodevelopment where increased inflammation may produce long-term cognitive changes. Intervening on modifiable maternal lifestyle factors during this prenatal window, particularly diet quality, with the proximal goal of lowering systemic inflammation, may improve early brain development and contribute to disrupting the intergenerational cycle of the negative impacts of socioeconomic adversity.

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Highlights

A pre-birth longitudinal cohort study with extensive molecular and early life data

Socioeconomic adversity operates through maternal diet

This elevates prenatal inflammation and reduces offspring cognition/language

Sequential mediation of socioeconomic adversity through prenatal maternal diet patterns to higher inflammation to reduced child cognition and language: linkage to study aims.