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ORIGINAL RESEARCH ARTICLE

Physical activity and circulating inflammatory markers and cytokines during pregnancy: A population-based cohort study

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Abstract

Introduction: Physical activity (PA) during pregnancy has numerous benefits, which may be mediated via effects on the immune system. However, supportive evidence is inconsistent and is mainly from studies in high-risk groups. We estimated the effect of PA during pregnancy on systemic inflammatory markers and cytokines in mothers recruited in the Barwon infant study.

Material and Methods: The Barwon infant study is a prebirth cohort of 1064 mothers recruited in the Barwon Region of Victoria, Australia. Participants reported their previous week's PA at their 28-week antenatal appointment using the International PA Questionnaire. Women were grouped into low, moderate, and high PA categories based on daily duration and weekly frequency of walking, moderate- or vigorous-intensity PA. Women reporting moderate levels of PA, consistent with current recommendations, served as the comparison group. Markers of systemic inflammation, high-sensitivity C-reactive protein (hsCRP), glycoprotein acetyls (GlycA), and 17 cytokines were measured at 28 weeks gestation and log transformed as appropriate. Regression analyses adjusted for maternal smoking, gestational diabetes mellitus, prepregnancy BMI, and household size were performed.

Results: Compared to women in the moderate group ($n = 371$, 42%), women reporting low PA ($n = 436$, 50%) had 10.1% higher hsCRP (95% CI (3.7% to 16.6%), $p < 0.01$) while women in high PA ($n = 76$, 9%) had a 14% higher hsCRP (95% CI (3.1% to 24.8%), $p = 0.01$). Women in the high PA category had higher interleukin (IL)-4 ($q = 0.03$) and IL-9 ($q = 0.03$) levels compared to those in moderate category. Each vigorous MET minute/week was associated with lower GlycA ($\beta = -0.004$, 95% CI (−0.044 to 0.035); $p = 0.03$).

Abbreviations: BIS, Barwon infant study; GlycA, glycoprotein acetyls; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; MET, metabolic equivalent of task; PA, physical activity.

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Conclusions: Low and high PA are each associated with higher hsCRP than moderate PA, suggesting that undertaking the recommended moderate PA during pregnancy decreases systemic inflammation. High PA affects T cell-associated cytokines during pregnancy. Evidence from our study suggests that PA can modulate the immune responses during pregnancy. Studies are now required to assess whether PA during pregnancy impacts maternal and infant clinical outcomes by modifying inflammatory responses.

KEYWORDS
chemokines, cytokines, gestation, GlycA, hsCRP, IL-4, IL-9, inflammation, physical activity, pregnancy

1 | INTRODUCTION

Engaging in physical activity (PA) confers numerous health benefits.[1] PA is particularly important during pregnancy; a window during which lifestyle modifications have long-term implications for both mother and infant.[2] Regular PA helps to prevent excessive gestational weight gain, reduces the risk of preeclampsia, preterm birth, and gestational diabetes, and improves maternal mental health.[3,4] In this context, the 2020 World Health Organization (WHO) guidelines on PA recommend that pregnant women (without contraindications) engage in regular PA to achieve at least 150 min of moderate-intensity PA/week.[1] The guidelines also state that women habitually engaged in vigorous-intensity PA before pregnancy should continue these activities during pregnancy. Despite these recommendations, women are less likely to engage in regular PA during pregnancy or continue to maintain levels of their prepregnancy activity due to physiological and psychological barriers such as pregnancy-related fatigue, discomfort, child care, and employment responsibilities.[5]

While PA during pregnancy appears to benefit both the mother and infant, the underlying mechanisms are unclear. One plausible pathway is via the effects of PA on the immune milieu during pregnancy.[6,7] Low-grade systemic inflammation in early pregnancy supports implantation and fetal growth.[8,9] However, as pregnancy progresses, excessive systemic inflammation is linked to complications including hypertension and preeclampsia,[10,11] and is a risk factor for chronic diseases later in the woman's life.[12]

Current evidence regarding PA on inflammatory markers and cytokines during pregnancy is limited. Findings from small studies among selected risk groups (such as those with gestational diabetes) are inconsistent, with some finding that PA interventions reduce high-sensitivity C-reactive protein (hsCRP), a marker of systemic inflammation, and various inflammatory cytokines, while others have found no effect.[13–19]

To address this gap, the aim of this study was to estimate the effect of the intensity and duration of PA during pregnancy on markers of systemic inflammation in a large and representative cohort of pregnant women. We measured two key markers of systemic inflammation, hsCRP and glycoprotein acetyls (GlycA).[20] GlycA is a composite nuclear magnetic resonance (NMR) marker of cumulative inflammation. It reflects glycosylation and concentration of five major acute-phase proteins (haptoglobin, α1-acid glycoprotein, α1-antitrypsin, α1-antichymotrypsin, and transferrin).[20–22] Previous studies show that GlycA predicts maternal[23] and infant inflammatory outcomes.[24–26] We also investigated the effect of PA on levels of classic pro-inflammatory cytokines, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α, IL-8, along with cytokines involved in cell growth (such as IL-9, G-CSF) and those implicated in lowering inflammation (such as IL-1ra).[27] We hypothesized that women engaging in moderate PA during pregnancy will have reduced systemic inflammation in comparison to women engaged in low PA.

Key message

A U-shaped relationship exists between physical activity and systemic inflammation, as low and high physical activity are each associated with higher hsCRP. The long-term benefits of physical activity-induced effects on inflammation on maternal and fetal clinical outcomes need to be evaluated.

2 | MATERIAL AND METHODS

2.1 | Participants

The Barwon infant study (BIS) is a prebirth cohort study ($n=1064$ mothers; $n=1074$ infants) recruited using an unselected antenatal sampling frame in the Barwon Region of Victoria, Australia.[28] In brief, women were recruited during their antenatal appointments between June 2010 and June 2013. Inclusion criteria: being an Australian permanent resident based in the Barwon region, less than 32 weeks pregnant at the time of enrolment, and planning to give birth at the University Hospital Geelong or St John of God Geelong Hospital, Victoria, Australia. The study protocol was approved by

the Barwon Health Human Research Ethics Committee (Project Number: 10/24; Approval Date: June 22, 2010), and the participants provided written informed consent.

Following enrolment, women completed comprehensive questionnaires to collect sociodemographic and lifestyle information, including age at conception, education level, self-reported pre-pregnancy weight and smoking status, and maternal psychological stress. Pregnancy height and weight were measured at enrolment. Preeclampsia and gestational diabetes were confirmed from medical records. The final analysis subcohort included women with measures of PA (walking days/week) as well as either of the systemic inflammatory markers hsCRP or GlycA ($n=972$).

2.2 | Exposures: measures of PA during pregnancy

At recruitment between 28 and 32 weeks of gestation, women were provided with the self-administered diet and PA questionnaire in which they reported their PA in the preceding week. PA questionnaire measures were based on the International Physical Activity Questionnaire (IPAQ) short form.[29] Women reported the duration (hours/day) and the frequency (number of days/week) of walking, moderate and vigorous intensity, and time spent sitting. Following IPAQ processing guidelines, each type of PA was converted to energy requirements defined in multiples of the resting metabolic rate or metabolic equivalent of task (MET) to generate walking MET minutes/week, moderate MET minutes/week, vigorous MET minutes/week, and total PA MET minutes/week (derived based on[30]). MET minutes/week were obtained by multiplying the minutes each PA was performed by their MET equivalent. Using this approach, walking MET minutes/week (3.3 × walking minutes x walking days), moderate MET minutes/week (4.0 × moderate-intensity activity minutes × moderate days), vigorous MET minutes/week (8.0 × vigorous-intensity activity minutes × vigorous-intensity days), and total PA MET minutes/week (sum of walking, moderate and vigorous MET minutes/week) were generated.

PA categories of low, moderate, and high were derived based on the frequency and duration of PA and their MET values.[30] If participants had not recorded a response for any of the continuous PA measures, they were excluded from the analysis estimating the effect of PA categories on maternal inflammation, resulting in a cohort of 883 participants for estimating the effect of PA categories on inflammatory outcomes. Participants were categorized as moderate PA if they engaged in 3 or more days of vigorous-intensity PA of at least 20 min per day; 5 or more days of moderate-intensity PA and/or walking of at least 30 min per day; or 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity physical activities, achieving a minimum total PA of at least 600 MET minutes/week. Participants were classified into the high PA category if they engaged in vigorous-intensity PA on at least 3 days, achieving a minimum total PA of at least 1500 MET minutes/week; or 7 days of any combination of walking, moderate-intensity or vigorous-intensity physical activities, achieving a minimum total PA of at least 3000 MET minutes/week. The moderate physical activity group, which consists of participants who meet the WHO recommendation for pregnant women to engage in 150 min/week of moderate-intensity physical activity served as the comparison group.

2.3 | Outcomes: measures of maternal systemic immune status

Nonfasting blood samples were collected at approximately 28 weeks gestation into serum-clotting or EDTA-coated tubes.[25] Samples were centrifuged within 3 h of collection, and serum and plasma were separated and stored at −80°C, until processing. Serum hsCRP was measured in 981/1009 participants using an enzyme-linked immunosorbent assay (hsCRP assay, DY1707, R&D Systems, Minneapolis, USA) as described previously.[25] Serum GlycA was measured in 965/1009 participants by Nightingale Nuclear Magnetic Resonance Platform (Nightingale Health, Helsinki, Finland), as reported in.[25]

Multiplex immunoassay was used to measure levels of circulating cytokines and chemokines in EDTA plasma samples in a subset of participants ($n=839/1009$) using a Bio-Plex Pro Human Cytokine 27-plex Assay (M500KCAF0Y, Bio-Rad Laboratories, Hercules, USA). The details of processing, inclusion criteria, and cut-offs have been described in.[31] Briefly, each plate was assayed as per manufacturer's instructions; however, reagents and samples were run in the half the volume. On each plate, quality control and cytokine standards were assayed in duplicate. Absolute concentrations of cytokines were obtained and for cytokines where the concentrations were below the lower limit of detection, values were replaced by an amount equal to the smallest observation divided by the square root of 2. Cytokines were removed from further analysis if more than 30% of samples were below the limit of detection, which resulted in 17 analytes. The final list of cytokines and chemokines included pro-inflammatory cytokines: interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF) α; chemokines: IL-8, macrophage inflammatory protein (MIP) 1α, MIP-1β, eotaxin, interferon- γ inducible protein 10 (IP-10), monocyte chemoattractant protein/monocyte chemotactic and activating factor (MCP/MCAF), regulated upon activation, and normal T cell expressed and presumably secreted (RANTES); growth factors: IL-7, IL-9, granulocyte colony-stimulating factor (G-CSF), and platelet-derived growth factor (PDGF-BB); and anti-inflammatory cytokines: IL-1 receptor antagonist (IL-1ra) and IL-4.

hsCRP and cytokine measurements were skewed and were log transformed to approximate a normal distribution before regression analysis.

2.4 | Covariates

Directed acyclic graphs (constructed using dagitty.net) were developed a priori based on published literature regarding factors that affect both maternal PA (exposure) and systemic inflammation (outcome; Figure S1). The identified potential confounders

were prepregnancy BMI (calculated from self-reported weight and height), age at conception, household income (annual), PSS score (measure of perceived psychosocial stress[31]), family size, gestational diabetes mellitus (yes vs no), preeclampsia (yes vs no), and maternal smoking (any during pregnancy, yes or no). Covariates were retained in the regression model if they made greater than a 10% difference to the point estimate. PSS score was not included in the final adjustment set due to the risk of reverse causation in its association with maternal PA and maternal inflammation.[32]

2.5 | Statistical analysis

All analyses were conducted using RStudio (version 4.2.1). A base-10 logarithm transformation was applied to the absolute concentrations of hsCRP (μg/mL) and cytokines and chemokines (pg/mL) to approximate a normal distribution. GlycA (nmol/L) had a normal distribution and was not log-transformed. Where cytokine values were below lower limit of detection, the value was replaced by an amount equal to the smallest observation divided by the square root of 2.[31]

Regression analyses were undertaken to determine the estimated effect of maternal PA measures on each maternal immune variable in unadjusted and adjusted models. Where the outcome was maternal cytokines, the Benjamini–Hochberg adjustment, which provides false discovery rates (*q*-values) was applied to account for multiple testing. We also fitted models with a quadratic dependence of outcome on exposure, but on assessing these via ANOVA found that with only three exceptions, this did not improve the linear fit. Thus and to retain the interpretability afforded by the linear fits, we did not further pursue nonlinear approaches.

Mediation analyses were performed using the R package medflex version 0.6–8[33] using the weighting-based approach and robust standard error estimation (except when a multinomial mediator model was fitted and when bootstrap standard errors were estimated).

3 | RESULTS

3.1 | Participant characteristics

The baseline characteristics of the participants included in this study were similar to those of the complete cohort (Table 1). Of note, 56% of women were in the healthy weight range before pregnancy, less than 15% of women smoked cigarettes, and 52% of women had completed tertiary education. Mean age of participants at conception was 31 years. Mean gestational age at delivery was 277 days. Participants gained a mean of 11 kg weight during their pregnancy. Participants spent a mean of 5.3 h/day sitting, walked a mean of 4 days/week, and participated in moderate- and vigorous-intensity PA on 1.5 and 0.25 days/week, respectively. They spent a mean of 0.7 h walking/day and engaged in moderate-intensity activities for a mean of 0.5 h/day. Mean time spent on vigorous-intensity activity

TABLE 1 Description of study participants in the subcohort included in the analysis in comparison to the inception birth cohort.

	Analysis subcohort		Inception cohort	
	n	Mean (SD) or *n* (%)	*n*	Mean (SD) or *n* (%)
Prenatal and demographic factors				
Maternal age at conception	972	31.4 (4.7)	1064	31.3 (4.8)
Preeclampsia (yes vs. no)	970	31 (3.2%)	1060	35 (3.3%)
Gestational hypertension (without preeclampsia)	970	26 (2.7%)	1060	27 (2.5%)
Family size	970	2.9 (1)	1059	2.9 (1)
Maternal smoking (any during pregnancy)	995	140 (14.5%)	1063	169 (16%)
Maternal university (university education vs. no)	997	520 (52.1%)	1058	541 (51.1%)
Household income	943		1061	
< AU$50000		107 (11.3%)		125 (11.8%)
AU$50000–AU$150000		723 (76.7%)		795 (74.9%)
>AU$150000		113 (12.0%)		121 (11.4%)
Gestational diabetes mellitus (yes vs. no)	855	41 (4.8%)	908	44 (4.9%)
Perceived stress scale score	754	18.5 (6.9)	801	18.7 (6.9%)
Prepregnancy BMI (kg/m^2)	871		917	
Underweight (<18.5)		21 (2.4%)		24 (2.6%)
Normal weight (18.5–24.9)		490 (56.2%)		512 (55.8%)
Overweight (25–29.9)		210 (24.1%)		227 (24.8%)
Obese (≥ 30)		150 (17.2%)		154 (16.8%)
Gestational weight gain (kg)	774	11.1 (5.0)	846	11.0 (5.1)
Gestational age at delivery (days)	972	276.6 (10.0)	1064	276.3 (10.4)
Maternal physical activity				
Sitting hours/day	885	5.3 (4.2)	894	5.3 (4.2)
Walking days/week	972	3.9 (2.2)	1009	3.9 (2.2)
Walking hours/day	912	0.7 (1.0)	914	0.7 (1.0)
Moderate-intensity activity days/week	957	1.5 (2.0)	1003	1.5 (1.9)
Moderate-intensity activity hours/day	920	0.5 (1.0)	961	0.5 (1.0)
Vigorous-intensity activity days/week	959	0.2 (0.8)	1004	0.2 (0.8)
Vigorous-intensity activity hours/day	954	0.1 (0.5)	999	0.1 (0.5)
Maternal inflammatory markers				
Markers of systemic inflammation				
• High-sensitivity C-reactive protein (mg/L)	972	4.3 (3.3)	1039	4.3 (3.5)

(Continues)

	Analysis subcohort		Inception cohort	
	n	Mean (SD) or *n* (%)	*n*	Mean (SD) or *n* (%)
• Glycoprotein acetyls (nmol/L)	965	1.6 (0.2)	1021	1.6 (0.2)
Pro-inflammatory cytokines[a]				
• IL-6 (pg/mL)	839	0.5 (0.4)	892	0.5 (0.4)
• IL-1β (pg/mL)	839	0.7 (0.6)	892	0.8 (0.6)
• TNF-α (pg/mL)	839	2 (0.2)	892	2 (0.2)
Chemokines[a]				
• IL-8 (pg/mL)	839	0.7 (0.9)	892	0.7 (0.9)
• MIP-1α (pg/mL)	839	0 (0.7)	892	0.1 (0.7)
• MIP-1β (pg/mL)	839	2.1 (0.3)	892	2.1 (0.3)
• Eotaxin (pg/mL)	839	1.8 (0.2)	892	1.8 (0.2)
• IP-10 (pg/mL)	839	3 (0.2)	892	3 (0.2)
• MCP/MCAF (pg/mL)	839	1.5 (0.3)	892	1.5 (0.3)
• RANTES (pg/mL)	839	4.1 (0.3)	892	4.1 (0.3)
Growth factors, incl cell survival[a]				
• IL-7 (pg/mL)	839	1.7 (0.6)	892	1.7 (0.5)
• IL-9 (pg/mL)	839	2.2 (0.8)	892	2.2 (0.8)
• G-CSF (pg/mL)	839	1.7 (0.8)	892	1.8 (0.8)
• PDGF-β (pg/mL)	839	2.9 (0.7)	892	2.9 (0.7)
Anti-inflammatory cytokines[a]				
• IL-1ra (pg/mL)	839	2.6 (0.6)	892	2.6 (0.6)
• IL-4 (pg/mL)	839	0.6 (0.5)	892	0.6 (0.5)

[a]Analyte concentrations have been log10 transformed. Interleukin (IL), tumor necrosis factor (TNF), macrophage inflammatory protein (MIP), interferon-γ inducible protein (IP), regulated upon activation normal T cell expressed and presumably secreted (RANTES), monocyte chemoattractant protein/monocyte chemotactic and activating factor (MCP/MCAF), granulocyte colony-stimulating factor (G-CSF), platelet-derived growth factor (PDGF), and receptor antagonist (ra).

was 0.1 h/day. Of 972 women, 213 (21.3%) reported walking every day. Fifty percent of participants reported not undertaking any PA at moderate- or higher-intensity PA in the past week and 12% reported engaging in vigorous-intensity physical activities in the previous week.

Mean levels of hsCRP and GlycA were 4.3 mg/L and 1.6 nmol/L, respectively (Table 1). Cytokine and chemokine measurements were available from 839 participants and mean values are presented in Table 1.

3.2 | Duration and intensity of PA

MET equivalents of each PA are represented in Table 2. The mean total PA was 1078 MET minutes/week (Table 2). Of this, mean levels for walking were 588 MET minutes/week, followed by moderate-intensity PA with a mean of 366 MET minutes/week. Participants spent 128 MET minutes/week on vigorous-intensity PA (Table 2). The mean number of days spent doing any form of PA, including

TABLE 2 Maternal physical activity (PA) variables derived from BIS Diet and Exercise Questionnaire administered at the 28-week antenatal appointment based on IPAQ processing guidelines.

Maternal physical activity (continuous)	*n*	Mean (SD)
Walking MET minutes/week	912	587.5 (713.4)
Moderate MET minutes/week	918	360.3 (800.1)
Vigorous MET minutes/week	954	126.8 (540.9)
Total MET minutes/week	863	1077.5 (1406.2)
Total days of activity/week	946	4.8 (2.3)
Total activity minutes/week	867	73.0 (72.8)
Maternal physical activity (category)		***n* (%)**
Low		436 (49.4%)
Moderate		371 (42%)
High		76 (8.6%)

Note: Women reported the duration (hours/day) and the frequency (number of days/week) of walking, moderate and vigorous intensity, and time spent sitting. These continuous PA measures were converted to energy requirements defined in metabolic equivalent of task (MET) to generate MET equivalents of each PA measure and total PA MET minutes/week. PA categories of low, moderate, and high were derived based on the frequency and duration of continuous PA measures and their MET values.

time spent on walking, moderate- and vigorous-intensity PA, was 4.8 days/week; and the mean for the total activity was 73 min/week (Table 2).

Nearly half of the cohort 436/883 (49%) were in the low PA group, while 371/883 participants (42%) and 76/883 (9%) were in moderate and high PA groups, respectively. Thus, 447/883 (51%) of the cohort met the WHO recommendations regarding PA during pregnancy (Table 2).

The baseline characteristics of the participants in the three PA categories have been presented in Table 3. Notably, women in the moderate PA group reported a lower average perceived stress score (16.9) compared to women in the low (19.5) or high groups (18.3). A higher percentage of women in the low PA group (40%) were in the overweight and obese prepregnancy BMI compared to moderate (30%) or high PA group (26.3%).

3.3 | Effect of PA during pregnancy on systemic markers of inflammation

After adjustment for prepregnancy BMI, family size, maternal smoking, and gestational diabetes mellitus, women in the low PA group had 10.1% higher hsCRP (95% CI (3.7% to 16.6%)) compared to mothers in moderate PA group (Figure 1, Table S1). Similarly, mothers in the high PA group had 14% higher hsCRP 95% CI (3.1% to 24.8%) compared to mothers in the moderate PA group.

Women in the low PA category had higher GlycA compared to women who engaged in moderate PA; however, this was attenuated after adjustment (Figure 1, Table S1, Figure S5). Following adjustment,

TABLE 3 Description of study participants in the low, moderate, and high physical activity groups.

	Low physical activity group $n=436$	Moderate physical activity group $n=371$	High physical activity group $n=76$
	Mean (SD) or *n* (%)	Mean (SD) or *n* (%)	Mean (SD) or *n* (%)
Prenatal and demographic factors			
Maternal age at conception	31.3 (4.7)	31.6 (4.4)	31.5 (5.0)
Preeclampsia (yes vs. no)	12 (2.8%)	11 (3.0%)	3 (3.9%)
Gestational hypertension (without preeclampsia)	9 (2.1%)	10 (2.7%)	0 (0.0%)
Family size	3.0 (1.0)	2.7 (1)	2.9 (1.1)
Maternal smoking (any during pregnancy)	76 (17.8%)	37 (10.2%)	16 (21.1%)
Maternal university (university education vs. no)	217 (50.5%)	232 (63.2%)	30 (39.5%)
Household income			
<AU$50000	48 (11.2%)	28 (7.9%)	13 (17.8%)
AU$50000–AU$150000	339 (79.4%)	270 (76.1%)	51 (69.9%)
>AU$150000	40 (9.4%)	57 (16.1%)	9 (12.3%)
Gestational diabetes mellitus (yes vs. no)	19 (5.1%)	14 (4.6%)	2 (3.2%)
Perceived stress scale score	19.5 (7.2)	16.9 (6.0)	18.3 (8.6)
Prepregnancy BMI (kg/m^2)			
Underweight (<18.5)	11 (2.9%)	4 (1.2%)	5 (7.7%)
Normal weight (18.5–24.9)	190 (50.4%)	204 (63.7%)	40 (61.5%)
Overweight (25–29.9)	105 (27.9%)	71 (22.2%)	13 (20.0%)
Obese (≥30)	71 (18.8%)	41 (12.8%)	7 (10.8%)
Gestational weight gain (kg)	11.0 (5.4)	11.1 (4.4)	11.1 (4.9)
Gestational age at delivery (days)	276.1 (9.7)	277.1 (9.8)	276 (11.0)

Note: Women reported the duration (hours/day) and the frequency (number of days/week) of walking, moderate and vigorous intensity, and time spent sitting. These continuous PA measures were converted to energy requirements defined in metabolic equivalent of task (MET) to generate MET equivalents of each PA measure and total PA MET minutes/week. Participants were divided into PA categories of low (did not meet the criteria of moderate or high), moderate (≥3 days/week of 20 min/day of vigorous-intensity PA, ≥5 days/week of 30 min/day of walking or moderate-intensity PA, or 5 days of any PA achieving 600 MET minutes/week), and high (≥3 days/week vigorous-intensity PA achieving 1500 MET minutes/week or 7 days of any PA achieving 3000 MET minutes/week).

each vigorous MET minute/week PA was associated with lower GlycA levels ($\beta=-0.004$, 95% CI (−0.044 to 0.035 (Figure 1, Table S1))).

In univariate models, each day of walking, moderate-intensity PA/week, and extra days of PA/week were each associated with lower hsCRP (Figure 1, Table S1), but these associations were no longer evident following adjustment (Figure 1, Table S1). In univariate models, each day of walking, extra days of PA, and total activity minutes/week were each associated with lower GlycA, but once again, these associations were no longer evident following adjustment (Figure 1, Table S1). Exploratory analysis revealed that the attenuation of associations in the multivariate models was primarily driven by adjusting for prepregnancy BMI. We considered whether greater prepregnancy BMI may be increasing maternal systemic inflammation via decreased PA, that is, prepregnancy BMI may be an instrumental variable operating via PA rather than a confounding factor. However, we found no evidence to support this possibility in mediation analyses (Figure S2). There was no evidence that time spent seated daily influenced either maternal GlycA or hsCRP levels at 28 weeks gestation (Figure 1, Table S1).

We next assessed whether the association between PA and systemic inflammation is modified by the women's weight at 28 weeks of pregnancy. Overall, the pattern of association between maternal total days of activity/week and systemic inflammation was similar within the different BMI categories, although the association between low PA and higher systemic inflammation was most evident in the normal-weight BMI category (Figure S3). We also considered whether gestational weight gain mediates the association between physical activity and systemic inflammation and found no evidence of mediation (Figure S4).

3.4 | Effect of maternal PA on maternal cytokines and chemokines

There was no effect of PA on the classic pro-inflammatory cytokines and chemokines, IL-6, IL-1β, TNF- α, IL-8, and MIP-1β (Figure 2, Table S2). However, following adjustment, each day/week of vigorous-intensity PA was associated with 8.6% (95% CI (4.1% to 13.1%) higher MIP-1α levels (Figure 2, Table S2)).

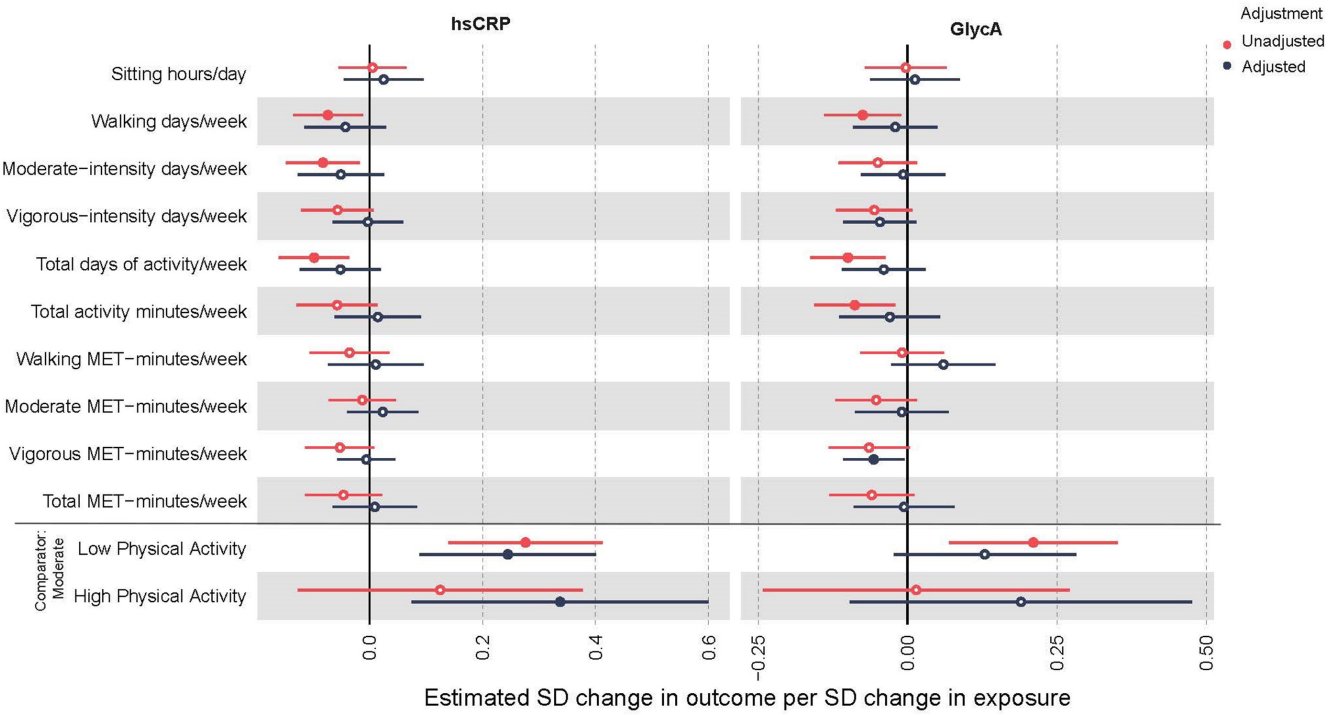


FIGURE 1 Forest plots representing regression effect estimates of maternal physical activity on maternal inflammatory markers high-sensitivity C-reactive protein (hsCRP) and glycoprotein acetyls (GlycA) at 28 weeks of gestation. Women reported the duration (hours/day) and the frequency (number of days/week) of walking, moderate and vigorous intensity, and time spent sitting. These continuous PA measures were converted to energy requirements defined in metabolic equivalent of task (MET) to generate MET equivalents of each PA measure and total PA MET minutes/week. Participants were divided into PA categories of low (did not meet the criteria of moderate or high; $n = 436$), moderate (≥3 days/week of 20 min/day of vigorous-intensity PA, ≥5 days/week of 30 min/day of walking, or moderate-intensity PA or 5 days of any PA achieving 600 MET minutes/week; $n = 371$), and high (≥3 days/week vigorous-intensity PA achieving 1500 MET minutes/week or 7 days of any PA achieving 3000 MET minutes/week; $n = 76$). Points indicate estimates and horizontal lines represent 95% confidence intervals. Red points indicate results of unadjusted regression model and black points represent regression model adjusted for maternal smoking, gestational diabetes mellitus, prepregnancy BMI, and family size. Closed points represent adjusted p-value <0.05. To make the results comparable across factors in the forest plots, all variables were standardized to have a mean of 0 and a standard deviation of 1. Standard deviation (SD).

IL-4 and IL-9 are cytokines predominantly produced by T helper 2 (Th2) cells and Th9 that are associated with adaptive immune cell function.[34] Women in the high PA category had 16% higher IL-4 levels (95% CI (5.6% to 26.4%)) and 27.3% higher IL-9 levels (95% CI (8.3% to 46.4%)) compared to women in moderate PA group (Figure 2, Table S2). Following adjustment, each day of vigorous-intensity PA and each vigorous MET minute/week were both associated with higher IL-9. Each total MET minute/week was associated with higher IL-9 as well (Figure 2, Table S2).

4 | DISCUSSION

We investigated the impact of maternal PA during pregnancy on maternal circulating inflammatory markers and a range of cytokines and found a U-shaped curve relationship between PA and systemic inflammatory marker hsCRP, with women reporting low or high levels of PA having higher levels of hsCRP than women reporting moderate levels of activity. These findings suggest that undertaking the recommended 150 min of moderate-intensity PA during pregnancy is associated with decreased systemic inflammation. An inverse association was observed between vigorous-intensity activity and GlycA, indicating a role of vigorous-intensity PA in lowering cumulative systemic inflammation. High PA was associated with increased levels of Th2 cytokines, IL-4 and IL-9. Overall, these findings suggest that vigorous-intensity PA has the potential to lower systemic inflammation, as indicated by lower GlycA. However, the elevation of IL-9 and IL-4 in high PA group suggests the immune effects of prolonged PA during pregnancy are complex.

Given that immune regulation is critical to a healthy pregnancy,[8,9] with consistent evidence that excessive systemic inflammation increases the risk of miscarriage, preeclampsia, and preterm birth,[10,11] and may also impact inflammatory[35] and neurocognitive outcomes in infants,[23] ensuring women achieve the recommended 150 min of moderate-intensity activity will likely be beneficial and is very unlikely to cause harm.

Previous studies regarding the effect of moderate-intensity physical activities on CRP levels in pregnant women have found conflicting results.[13,14] One study reports a lack of correlation between moderate-intensity activity and CRP, while low-intensity activities

(eg walking) negatively correlated with maternal plasma CRP in late pregnancy.[13] We found no effect of days spent on walking or moderate-intensity PA on hsCRP, however, the effect of moderate-intensity PA on hsCRP became evident in analysis based on PA categories. Here, we report a positive association between low PA and hsCRP as compared to women engaged in moderate PA.

In BIS, women who were less active, had higher levels of systemic inflammation than women in moderate PA group. This supports the findings from a randomized controlled trial to prevent the development of gestational diabetes mellitus that reports that increasing light-intensity PA by 30 min reduced CRP level by 0.4 mg/L[14] and thus highlights anti-inflammatory benefits of women adhering to the current WHO guidelines regarding PA.

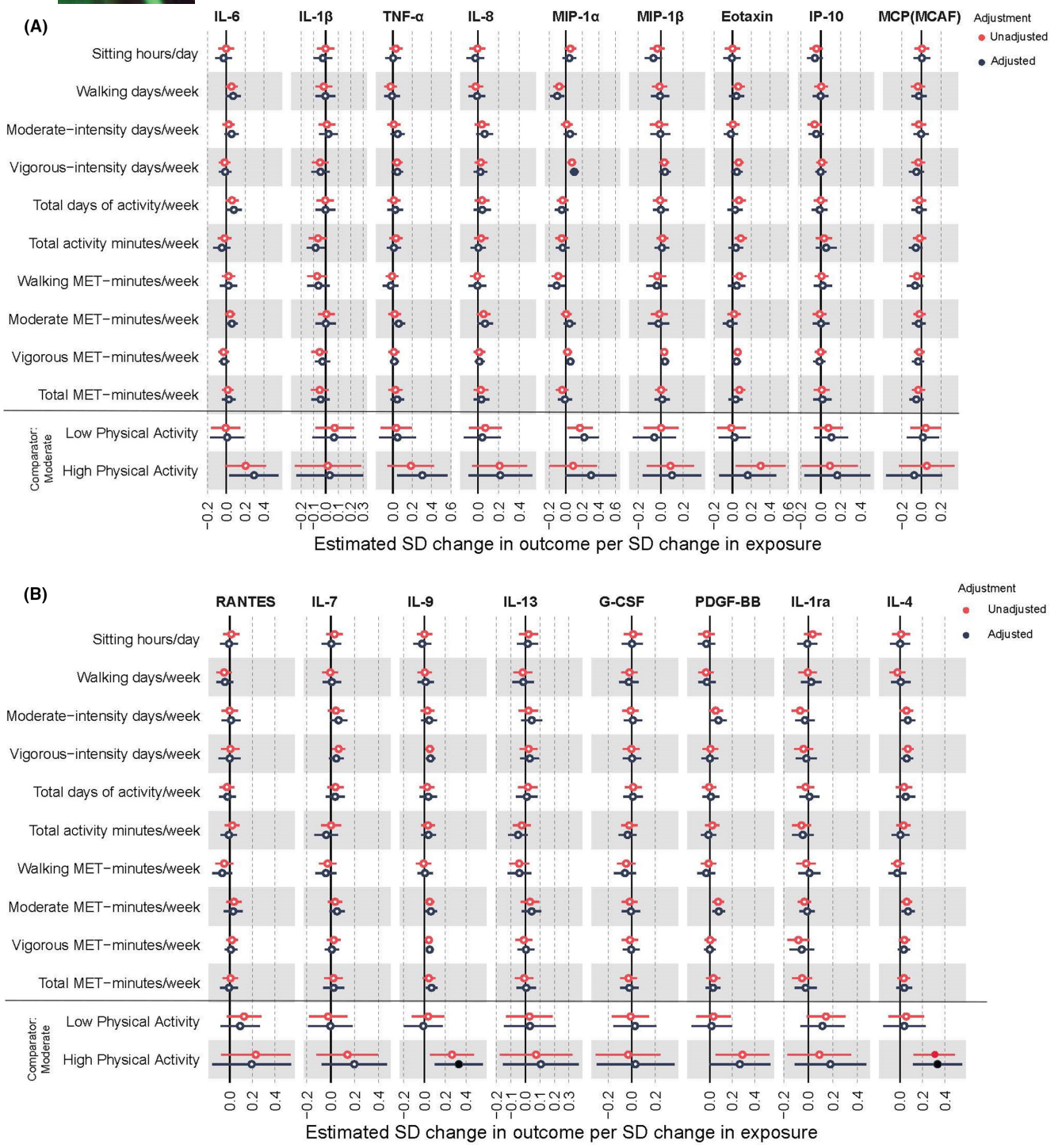
In the Norwegian mother and child cohort study (MoBa), only PA before (and not during) pregnancy was associated with reduced CRP during pregnancy.[15] Exercise before pregnancy was adjusted for in MoBA. PA prepregnancy and during pregnancy may or may not be correlated, and as we lack measures of physical activity levels prepregnancy, we cannot ascertain if this explains the discrepancy between our results.

This is the first large-scale study investigating the impact of PA during pregnancy on a multiplex panel of cytokines, all of which are known to play diverse roles in immune responses and homeostasis during gestation.[9] Burgeoning evidence from studies in animals and nonpregnant populations shows physical activity-induced production of cytokines (classified as exerkines) by skeletal muscle, adipose tissues, heart, liver, and immune cells enters the systemic circulation and affects glucose metabolism, insulin secretion, and fatty acid uptake.[6,7] This is responsible for the crosstalk between physical activity and cardiovascular and metabolic systems. PA appears to reduce the levels of IL-6 and TNF-α, while increasing anti-inflammatory cytokines IL-10, IL4, and IL-1ra.[6,7] However, we found no evidence of an effect of PA on the levels of pro-inflammatory cytokines and chemokines IL-6, IL-1β, IL-8, TNF-α, or MIP-1β during pregnancy. This is consistent with evidence from the GESTAFFIT, where PA appeared to have no effect on circulating IL-6.[16,18] By contrast, one study in women with high BMI (≥25 kg/m^2) and increased risk of gestational diabetes found that moderate to vigorous, compared to low–moderate PA is associated with higher circulating IL-6.[36] Similarly, the evidence regarding maternal PA and anti-inflammatory cytokines, IL-1ra and IL-10, is conflicting.[16,19] In BIS, there was no effect of moderate-intensity PA on levels of IL-1ra. This indicates that the immune-suppressive effects of moderate- or higher-intensity PA may not be driven by its effect on modulating the circulating levels of this anti-inflammatory cytokine—acknowledging the limitations of single-measure cytokine concentrations, which are inherently variable, at a single time point.

We found that vigorous-intensity PA was associated with higher levels of MIP-1α. MIP-1α is a chemotactic cytokine that recruits other immune cells (macrophages and dendritic cells) to the site of injury or infection. Vigorous- or moderate-intensity exercise induces the production of pro-inflammatory cytokines in response to exercise-induced muscle contraction and damage, which is necessary for normal physiological function and necessary for tissue homeostasis.[37] It is therefore unsurprising to find increased MIP-1α in participants who spent a greater number of days doing high-intensity exercise.

More days spent on vigorous-intensity/week and high PA week were each associated with higher level of Th2-associated cytokines, IL-4 and IL-9. This is consistent with evidence that longer periods of repeated exercise are associated with upregulation of IL-4 in muscle tissue.[38] IL-4 and IL-9 have been implicated in allergic responses but may also reduce inflammation by suppressing IL-1β production.[39,40] The role of IL-4 during pregnancy is, however, highly complex and incompletely understood (reviewed in[41]), and thus interpretation of the implications of our IL-4-related findings remains speculative. IL-9 levels decrease throughout pregnancy[9] and while IL-9 plays a role in resolution of inflammation and immunological tolerance,[42] its function during pregnancy is unclear. Nevertheless, a high Th1/Th2 cytokine ratio is observed in women who experience recurrent miscarriages and infertility issues,[41,43] highlighting the likely contribution of maintaining adequate IL-9 levels to immune homeostasis during pregnancy. Taken together, our findings regarding PA and circulating cytokines suggest PA likely contributes to maintaining a balanced immune status in pregnancy.

FIGURE 2 Forest plots representing regression estimates of effect of maternal physical activity on levels of circulating maternal cytokines and chemokines (A) IL-6, IL-1β, TNF-α, IL-8, MIP-1α, MIP-1β, eotaxin, IP-10, and MCP (MCAF) and (B) RANTES, IL-7, IL-9, IL-13, G-CSF, PDGF-BB, IL-1ra, and IL-4, at 28 weeks of gestation. Women reported the duration (hours/day) and the frequency (number of days/week) of walking, moderate and vigorous intensity, and time spent sitting. These continuous PA measures were converted to energy requirements defined in metabolic equivalent of task (MET) to generate MET equivalents of each PA measure and total PA MET minutes/week. Participants were divided into PA categories of low (did not meet the criteria of moderate or high; $n = 436$), moderate (≥3 days/week of 20 min/day of vigorous-intensity PA, ≥5 days/week of 30 min/day of walking or moderate-intensity PA, or 5 days of any PA achieving 600 MET minutes/week; $n = 371$), and high (≥3 days/week vigorous-intensity PA achieving 1500 MET minutes/week or 7 days of any PA achieving 3000 MET minutes/week; $n = 76$). Points indicate estimates and horizontal lines represent 95% confidence intervals. Red points indicate results of unadjusted regression model and black points represent regression model adjusted for maternal smoking, gestational diabetes mellitus, prepregnancy BMI, and family size. Closed points represent adjusted q-value <0.05 (i.e., adjusted for multiple-hypothesis testing). To make the results comparable across factors in the forest plots, all variables were standardized to have a mean of 0 and a standard deviation of 1. Granulocyte colony-stimulating factor (G-CSF) interleukin (IL)-1β, IL-1ra, IL-4, IL-6, IL-7, IL-8, IL-9, interferon-γ inducible protein (IP)-10, monocyte chemoattractant protein/monocyte chemotactic and activating factor (MCP/MCAF), macrophage inflammatory protein (MIP)-1α, MIP-1b, platelet-derived growth factor (PDGF), regulated upon activation, tumor necrosis factor (TNF)-α, normal T cell expressed and presumably secreted (RANTES), and standard deviation (SD).



In our analysis, the univariate association between increased physical activity and decreased systemic inflammation was substantially attenuated by the inclusion of prepregnancy BMI in the regression model. On formal mediation analysis, we found no evidence that prepregnancy BMI is acting via physical activity during pregnancy to decrease systemic inflammation. Previous studies investigating the relationship between PA and inflammation during pregnancy have been restricted to women who were overweight/obese and/or were at risk of gestational diabetes.[13,17,19,36] Given that obesity and gestational diabetes are strongly associated with increased inflammation,[44] sampling on the presence of obesity and gestational diabetes is likely to introduce significant selection. Interestingly, in our study, the anti-inflammatory effect of PA was most apparent among women in the healthy weight category. Thus, our findings extend the generalizability of previous work in this space and confirm that adherence with WHO guidelines regarding PA during pregnancy is associated with benefits to the systemic immune status.

The strengths of our study include a relatively large cohort size, collection and analysis of highly dimensioned covariate data, and assessment of a range of clinical and inflammatory markers. As

noted, the unselected sampling frame mitigates selection bias and aids generalizability. Limitations include the lack of independent and objective measures of PA. Importantly, since diet and physical activity questionnaires were administered only at recruitment at approximately 28 weeks gestation, there are no measures of diet and physical activity during the first or third trimesters of pregnancy in this cohort. Another consideration is that women reporting vigorous-intensity activity during pregnancy are likely to have also been undertaking vigorous-intensity activity prepregnancy, although we lack data to confirm this. While we have considered confounders based on published literature regarding factors that affect both maternal and maternal inflammation, other potential confounders such as allergic and autoimmune disorders, or other conditions that may impact both physical activity and immune status should also be considered in future studies. We measured immune parameters at a single time point, and given cytokines and chemokines have high intrinsic variability, more frequent sampling may reveal longitudinal patterns that are not apparent with a single measure.

5 | CONCLUSION

From a public health perspective, our findings in large cohort provide support for current clinical recommendations to undertake 150 min of moderate-intensity PA per week during pregnancy.[1] Evidence from our study suggests that PA affects levels of immune markers hsCRP, IL-4, and IL-9 during pregnancy. To assess the clinical relevance of these findings, further studies are required to determine if these PA-by-inflammation effects impact clinical outcomes during pregnancy. Nevertheless, as inflammation is a trigger for pregnancy-related complications, our study has important implications for clinical practice, encouraging a simple lifestyle change. For pregnant women, adopting simple strategies to incorporate PA in their day-to-day lives, such as taking the stairs instead of a lift, is likely to be of benefit and unlikely to cause harm. Future studies should investigate the interplay among PA, diet, mental health, and body composition during pregnancy with the ultimate goal of optimizing immune homeostasis to promote both maternal and infant outcomes.

AUTHOR CONTRIBUTIONS

Poshmaal Dhar and Peter Vuillermin were involved in conceptualization, analysis, writing, and reviewing. Luba Sominsky, Fiona Collier, Toby Mansell, and David Burgner were involved in sample processing, methodology, writing, and reviewing. Martin O'Hely, Samantha Dawson, Anne-Louise Ponsonby, Kylie D Hesketh, and Mimi LK. Tang were involved in analysis, writing, and reviewing. Craig Smith, Natalie Hyde, and Katherine Downing assisted with writing and reviewing of this manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

ETHICS STATEMENT

The BIS study protocol was approved by the Barwon Health Human Research Ethics Committee (Project Number: 10/24; Approval Date: June 22, 2010), and the participants provided written informed consent to participate. All participants provided consent for publication as part of the approved ethics protocol. All information was de-identified prior to analysis and incorporation in this publication.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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