Physical activity during pregnancy and offspring cardiovascular risk factors: findings from a prospective cohort study

Supplementary Material

Louise A C Millard, MSc[1,2*], Debbie A Lawlor, PhD [1,2], Abigail Fraser, PhD [1,2], Laura D Howe, PhD[1,2]

1 – MRC Integrative Epidemiology Unit at the University of Bristol,
2 – School of Social and Community Medicine, University of Bristol

*Corresponding author.
Correspondence to: LAC Millard, School of Social and Community Medicine, University of Bristol, Oakfield House, Bristol, UK, BS8 2BN, [louise.millard@bristol.ac.uk], tel +44 117 331 0084, fax: +44 117 331 0123

SUPPLEMENTARY METHODS

Measures used in this study

Cardiovascular risk factors in the offspring Clinic participants were asked to fast overnight for those attending in the morning or for a minimum of six hours for those attending after lunch. Blood samples were immediately spun and frozen at −80^{o}C. Measurements were assayed three to nine months after samples were taken, with no previous freeze-thaw cycles. Plasma lipid concentrations (total cholesterol, triglycerides, and high density lipoprotein cholesterol (HDLc) were measured by modification of the standard Lipid Research Clinics Protocol by using enzymatic reagents for lipid determination. Low density lipoprotein cholesterol (LDLc) concentrations were determined from these with the Friedwald equation (LDLc=total cholesterol−HDLc+triglycerides×0.45 mmol/l)).

Assessment of potential mediators

Hypertensive disorder of pregnancy (HDP) was assessed using data abstracted from obstetric records that included every measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), and proteinuria recorded and the corresponding gestational age at the time of these measurements. We applied the International Society for the Study of Hypertension in Pregnancy [1] criteria to all of the clinic data in order to determine women with pre-eclampsia and those with gestational hypertension, and categorized the mothers into one of three mutually exclusive categories; no HDP, gestational hypertension, or pre-eclampsia.

Gestational weight gain was calculated from a linear spline multilevel model developed using all available pregnancy weight measurements (see [2] for further details). We use 3 gestational weight gain variables; 0-18 weeks, 18-28 weeks, 28 weeks onwards, where the 0-18 week variable is used as a confounder (see below) because it refers to the time before the exposure measurement (18 weeks antenatal) whereas the gestational weight gain variables from 18 weeks gestation onwards are used as potential mediators because they refer to the time between exposure and outcome measurement.

At recruitment, women were asked about existing diabetes and any previous history of gestational diabetes. Research midwives abstracted information on clinical diagnoses of gestational diabetes and glycosuria from the antenatal, pregnancy and postnatal medical records using a standard protocol. Using these data, we classified the women into one of four mutually exclusive categories: no evidence of glycosuria or diabetes; existing diabetes before the pregnancy; gestational diabetes (i.e. a diagnosis in the medical records of gestational diabetes in any woman with no history of existing diabetes); and glycosuria (i.e. ++ glycosuria on two occasions in women with no evidence of existing diabetes or gestational diabetes).

Child's physical activity was measured using an accelerometer (MTI Actigraph AM7164 2.2) worn over a period of 7 days. The accelerometer is worn around the waist and detects acceleration and deceleration in a vertical plane as a combined function of movement frequency and intensity. A measure of time spent in moderate and vigorous physical activity (MVPA, equivalent to brisk walking or running) was calculated and averaged over valid days. The accelerometer must have been worn for at least 10 hours a day for at least three days after deletion of missing data to be considered valid [3-5]. To maximize our sample size for this analysis, we used a multilevel model to predict MVPA at exactly 14 years for all participants who had accelerometer measures from at least one of the following time points: age 11, age 13, and age 15. The multilevel model (two levels: occasion and individual) uses all available data from all eligible participants (those with one or more measure) under a missing at random assumption to predict MVPA for all participants, assuming linear rates of change between 11-13 and 13-15 years. Although the model generates values of predicted MVPA between 11 and 15 years old, we included only predicted MVPA at 14 years in our model since there was a high correlation between predicted levels of MVPA at all ages. MLwiN [6] and the runmlwin [7] Stata command were used for the multilevel model.

Gestational age and infant birthweight were recorded in the delivery room and abstracted from obstetric records and/or birth notifications.

Potential confounders

Maternal weight and height At enrolment (questionnaire at approximately 12 weeks gestation), the mother was asked to record her height and pre-pregnancy weight. Maternal pre pregnancy body mass index (BMI) was calculated by dividing her weight in kilograms by her height in meters squared.

Child age & gender Child age was recorded in months as they arrived for the age 15 year clinic. Infant sex was

recorded in the delivery room or abstracted from obstetric records or birth notifications.

Household social class The mother recorded the occupation of both herself and her partner in a questionnaire at 32 weeks gestation, which were used to allocate them to social class groups (I, II, III manual, III non-manual, IV, V) using the 1991 Office of Population, Censuses and Surveys classification; the highest class of the mother and her partner was used in analysis.

Maternal education A questionnaire at 32 weeks gestation asked mothers to report their educational attainment. Maternal educational attainment was categorized as below O-level (ordinary level; exams taken in different subjects usually at age 15–16 at the completion of legally required school attendance, equivalent to today's General Certificate of Secondary Education), O-level only, A-level (advanced-level; exams taken in different subjects usually at age 18), or university degree or above.

Ethnicity A binary variable denoting offspring white or non-white ethnicity was used, where mixed was recoded as non-white due to the small numbers in the mixed category (n=3).

Parity Parity was obtained from obstetric records. Mothers with parity of two or more were grouped into a single category.

Maternal smoking during pregnancy The mother was asked about her smoking habits on two occasions during pregnancy (the 18 week and 32 week antenatal questionnaires), and a dichotomous variable was created for any smoking during pregnancy.

Previous hypertension Mothers were asked about their history of high blood pressure with a yes/no question in a self-administered questionnaire completed at 12 weeks gestation.

Missing data

We used multiple imputation in Stata as described by Royston [8]. We carried out 20 cycles of regression switching and generated 20 imputation data sets. All variables included in our analyses were used to impute the missing data. The multiple imputation approach creates a number of copies of the data (in this case, we generated 20 copies) in which missing values are imputed by chained equations [8]. The main analysis results are obtained by averaging across the results from each of these 20 data sets using Rubin's rules, which ensure that the standard errors for any regression coefficients take account of uncertainty in the imputations as well as uncertainty in the estimation [8].

To inform the imputation we included additional variables: cardiovascular disease (CVD) risk factors (BMI and waist circumference at age 11, and SBP, DBP, low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc), triglycerides at age 9), physical activity at age 12 and measures predictive of missingness of CVD risk factors (paternal education, child total fat mass (age 9), paternal height, maternal age at delivery and birth length). The following variables were log transformed to produce distributions that were approximately normally to improve the imputation: child BMI, waist circumference, LDLc, HDLc and triglycerides age 15, physical activity age 12 and 14, total fat mass and triglycerides age 9, BMI and waist circumference age 11 and maternal BMI.

SUPPLEMENTARY RESULTS

Supplemental Table 1 Physical activity MET scores

Activity	MET score	Number of hours equating to mean MET score [a]
Jogging	7.0	2.3
Brisk walking	3.8	4.2
Swimming	6.0	2.6
Antenatal exercise	2.0	7.9
Keep fit exercise	3.5	4.5
Cycling	5.5	2.9
Aerobics	6.5	2.4
Tennis	4.5	3.5
Yoga	2.5	6.3
Weight training	3.0	5.3
Squash	12.0	1.3
Other	3.5	4.5

[a] Average MET score for participants included in our analyses = 15.8. We have calculated what this equates to in terms of each activity, by dividing 15.8 by the MET score for each activity. Thus this column represents the average number of hours an individual would spend to reach the mean MET score solely through partaking in each particular activity.

Supplemental Table 2 Summary of variable distributions for observed and imputed data

		Number of participants with non-missing data	Observed data (all individuals) (n (%) or mean (standard deviation (SD)))	Observed data (individuals with complete data, N=1,570) (n (%) or mean (SD))	Imputed data [N= 4,665] (n (%) or mean (standard error)) [c]
Parity (n, %)	0	4633	2259 (48.8)	793 (50.5)	2277 (48.8)
	1		1598 (34.5)	553 (35.2)	1608 (34.5)
	2+		776 (16.8)	224 (14.3)	781 (16.7)
Household	i	4466	761 (17.0)	296 (18.9)	777 (16.7)
social class	ii		2058 (46.1)	766 (48.8)	2123 (45.5)
(n)	iiin		1074 (24.1)	354 (22.5)	1134 (24.3)
	iiim		400 (9.0)	108 (6.9)	438 (9.4)
	iv		173 (3.9)	46 (2.9)	194 (4.2)
Mothers	< o-level	4559	875 (19.2)	241 (15.4)	909 (19.5)
Education	o-level		1595 (35.0)	524 (33.4)	1633 (35.0)
(n)	a-level		1292 (28.3)	486 (31.0)	1315 (28.2)
	degree or above		797 (17.5)	319 (20.3)	808 (17.3)
Child Ethnicity white (n)		4649	4450 (95.7)	1516 (96.6)	4465 (95.7)
Maternal pre-pregnancy weight, kg (mean, SD)		4350	60.56 (11.91)	59.90 (11.18)	60.48 (0.18)
Maternal pre-pregnancy height, cm (mean)		4497	164.33 (6.68)	164.44 (6.72)	164.30 (0.10)
Maternal gestational weight gain 0 – 18 weeks, kg/week (mean)		4350	0.31 (0.17)	0.32 (0.17)	0.31 (0.00)
Mother previous hypertension, yes (n)		4087	190 (4.7)	60 (3.8)	216 (4.6)
Mother smokes in pregnancy, yes (n)		4653	762 (16.4)	238 (15.2)	764 (16.4)
Child gender, female (n)		4665	2436 (52.2)	801 (51.0)	2436 (52.2)
HDP (n)	None	4644	3885 (83.7)	1352 (86.1)	3903 (83.7)
	Pre-eclampsia		89 (1.9)	20 (1.3)	90 (1.9)
	Gestational hypertension		670 (14.4)	198 (12.6)	673 (14.4)
Maternal	None	4548	4358 (95.8)	1515 (96.5)	4468 (95.8)
diabetes	Existing diabetes		20 (0.4)	5 (0.3)	22 (0.5)
(n)	Gestational diabetes		27 (0.6)	8 (0.5)	28 (0.6)
	Glycosuria		143 (3.1)	42 (2.7)	147 (3.2)
Maternal physical activity, MET hours/week (mean) [a]		4665	16.41 (15.32)	17.02 (15.87)	16.41 (0.22)
Child BMI age 15, kg/m^2 (mean)		4603	21.44 (3.53)	21.19 (3.13)	21.45 (0.05)

Child waist circumference age 15, cm (mean)	3828	76.67 (8.92)	76.54 (8.58)	77.15 (0.14)
Child SBP age 15, mmHg (mean)	4345	123.21 (10.84)	123.52 (10.88)	123.28 (0.17)
Child DBP age 15, mmHg (mean)	4345	67.63 (8.74)	66.63 (8.45)	67.64 (0.13)
Child glucose age 15, mmol/L (mean)	2957	5.21 (0.39)	5.20 (0.37)	5.21 (0.01)
Child insulin age 15, IU/L (mean)	2953	10.32 (6.50)	9.60 (4.74)	10.34 (0.11)
Child LDLc age 15, mmol/L (mean)	2957	2.09 (0.56)	2.09 (0.54)	2.09 (0.01)
Child triglycerides age 15, mmol/L (mean)	2957	0.84 (0.39)	0.81 (0.33)	0.84 (0.01)
Child HDLc age 15, mmol/L (mean)	2957	1.28 (0.29)	1.30 (0.29)	1.29 (0.01)
Child age at 15 year clinic, weeks (mean)	4665	808.22 (17.45)	805.00 (12.49)	808.22 (0.26)
Child physical activity age 14 (mean) [b]	3938	1.45 (0.62)	1.48 (0.63)	1.45 (0.01)
Maternal gestational weight gain 18 - 28/40, kg/week (mean)	4350	0.54 (0.17)	0.53 (0.16)	0.53 (0.00)
Maternal gestational weight gain 28/40 onwards kg/week (mean)	4350	0.47 (0.19)	0.46 (0.19)	0.47 (0.00)
Birthweight, grams (mean)	4603	3440.06 (523.94)	3497.28 (472.43)	3439.64 (7.69)
Gestational age, weeks (mean)	4494	32.50 (1.21)	32.51 (1.12)	32.50 (0.02)

[a] Metabolic equivalent maternal physical activity
[b] Mean daily minutes of MVPA (predicted)
[c] Multivariate multiply imputed data over 20 imputation data sets of all mother-offspring pairs with a value of physical activity during pregnancy (exposure) and at least one offspring cardiovascular risk factor age 15.5 (outcomes)

Supplemental Table 3 Association of maternal physical activity with potential mediators of the relationship between physical activity during pregnancy and offspring cardiovascular risk factors: using multivariate multiply imputed data (N = 4,665) of individuals with data for maternal physical activity (exposure) and at least one outcome

Potential mediator	Mediator category	Change per 1 SD greater maternal metabolic equivalent physical activity during pregnancy
Odds ratio per SD greater maternal physical activity during pregnancy, null value = 1		
Hypertensive Disorders of Pregnancy	None	Reference
	Pre-eclampsia	-0.04 (-0.25 to 0.17)
	Gestational hypertension	-0.04 (-0.12 to 0.05)
Diabetes	None	Reference
	Existing diabetes	0.18 (-0.23 to 0.58)
	Gestational diabetes	-0.29 (-0.73 to 0.15)
	Glycosuria	-0.03 (-0.20 to 0.14)
Mean difference per SD greater maternal physical activity during pregnancy, null value = 0		
Gestational weight gain (SD)	18 - 28 weeks	-0.02 (-0.05 to 0.01)
	28 weeks onwards	-0.01 (-0.04 to 0.02)
Gestational age (SD)		-0.02 (-0.05 to 0.01)
Birthweight (SD)		-0.01 (-0.04 to 0.02)
Offspring moderate to vigorous physical activity age 14 (SD)		0.06 (0.03 to 0.09)

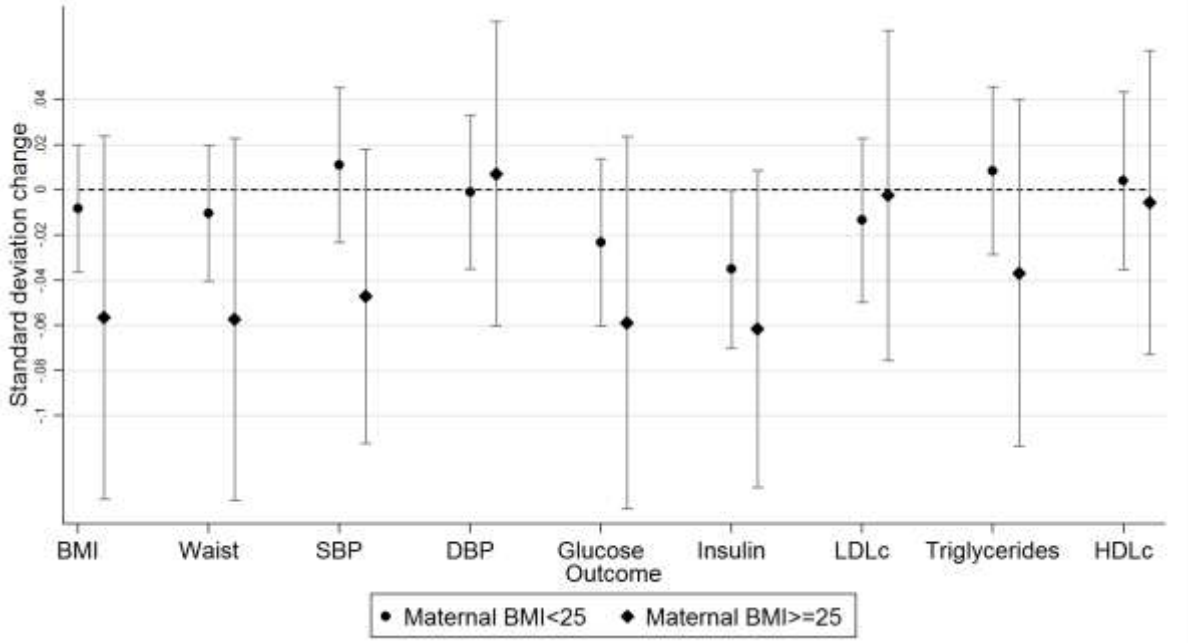
SD: Standard deviation

Supplemental Table 4 Association between maternal physical activity during pregnancy and offspring cardiovascular risk factors: using multivariate multiply imputed data (N = 4,665) of individuals with data for maternal physical activity (exposure) and at least one outcome

	Change per 1 standard deviation (SD) greater maternal metabolic equivalent physical activity (95% confidence intervals)					
Outcome	Minimal adjustment for child age and gender	Adjusted for confounders: household social class, maternal education, ethnicity, parity, maternal smoking during pregnancy, previous hypertension, maternal pre-pregnancy BMI and gestational weight gain 0-18 weeks	Adjusted for confounders and gestational diabetes, HDP, gestational weight gain	Adjusted for confounders, gestational diabetes, HDP, gestational weight gain, gestational age, and birthweight	Adjusted for confounders and offspring physical activity	Adjusted for all confounders and mediators
Mean difference (in units specified in first column) per 1 SD greater maternal physical activity during pregnancy						
BMI, kg/m^2	-0.089 (-0.190 to 0.011)	0.004 (-0.091 to 0.098)	0.004 (-0.090 to 0.099)	0.006 (-0.088 to 0.100)	0.021 (-0.073 to 0.115)	0.023 (-0.071 to 0.117)
Waist circumference, cm	-0.271 (-0.552 to 0.011)	-0.088 (-0.358 to 0.181)	-0.085 (-0.354 to 0.184)	-0.082 (-0.351 to 0.186)	-0.048 (-0.317 to 0.221)	-0.042 (-0.311 to 0.227)
SBP, mmHg	-0.044 (-0.360 to 0.272)	-0.014 (-0.332 to 0.303)	-0.012 (-0.329 to 0.305)	-0.008 (-0.325 to 0.309)	0.012 (-0.307 to 0.330)	0.017 (-0.301 to 0.334)
DBP, mmHg	0.015 (-0.251 to 0.280)	0.017 (-0.251 to 0.284)	0.019 (-0.248 to 0.287)	0.022 (-0.246 to 0.290)	0.014 (-0.254 to 0.282)	0.018 (-0.250 to 0.286)
Glucose, mmol/L	-0.013 (-0.027 to 0.001)	-0.013 (-0.027 to 0.001)	-0.013 (-0.026 to 0.001)	-0.013 (-0.027 to 0.001)	-0.013 (-0.026 to 0.001)	-0.012 (-0.026 to 0.001)
LDLc, mmol/L	-0.006 (-0.025 to 0.013)	-0.002 (-0.022 to 0.017)	-0.003 (-0.022 to 0.017)	-0.003 (-0.022 to 0.017)	-0.002 (-0.022 to 0.017)	-0.003 (-0.022 to 0.017)
HDLc, mmol/L	0.002 (-0.008 to 0.012)	0.000 (-0.010 to 0.010)	0.000 (-0.010 to 0.010)	0.000 (-0.010 to 0.010)	-0.001 (-0.011 to 0.009)	-0.001 (-0.011 to 0.009)
Percentage difference per 1 SD greater maternal physical activity during pregnancy						
Insulin, % difference	-1.90 (-3.50 to -0.30)	-1.20 (-2.80 to 0.40)	-1.20 (-2.80 to 0.40)	-1.20 (-2.80 to 0.40)	-1.00 (-2.60 to 0.60)	-1.00 (-2.60 to 0.60)
Triglycerides, %	0.00 (-1.20 to 1.30)	0.00 (-1.30 to 1.20)	0.00 (-1.30 to 1.30)	0.00 (-1.30 to 1.30)	0.00 (-1.20 to 1.30)	0.00 (-1.20 to 1.40)

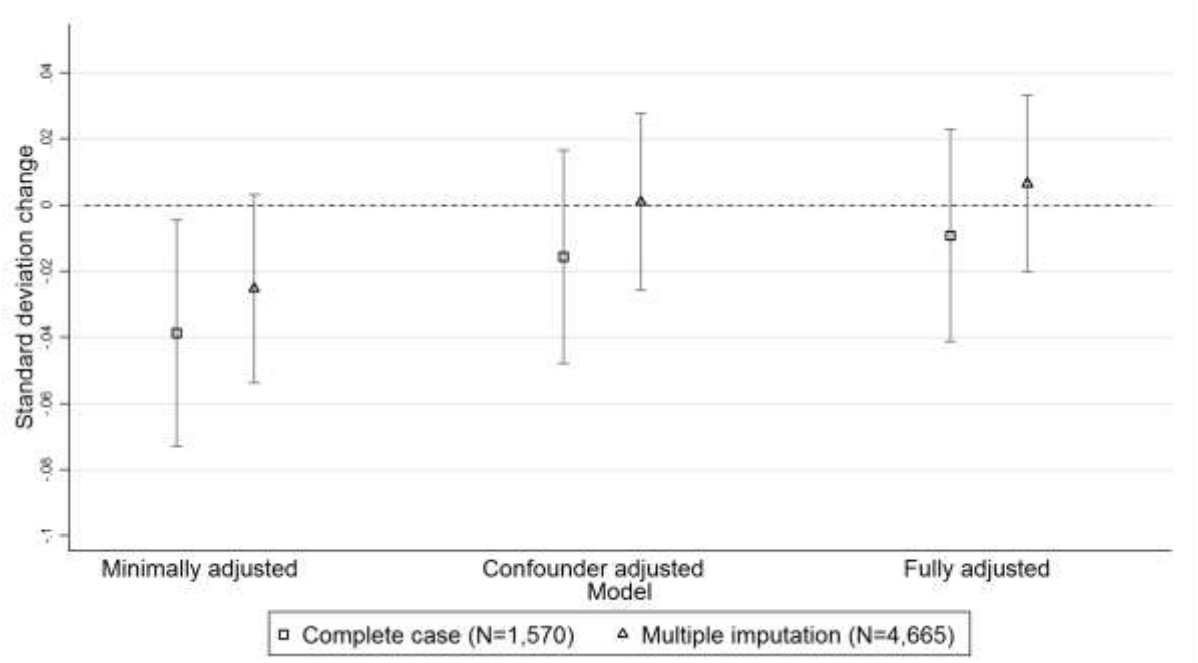
difference						

Supplemental Fig. 1 BMI interaction analysis showing associations of maternal physical activity with child offspring CVD outcomes for overweight and not overweight individuals using multiply imputed sample (N=4,665)



Mean standard deviation difference (and 95% confidence intervals) in offspring cardiovascular risk factors (or logged distribution for insulin and triglycerides) for a 1 standard deviation increase in metabolic equivalent maternal physical activity during pregnancy

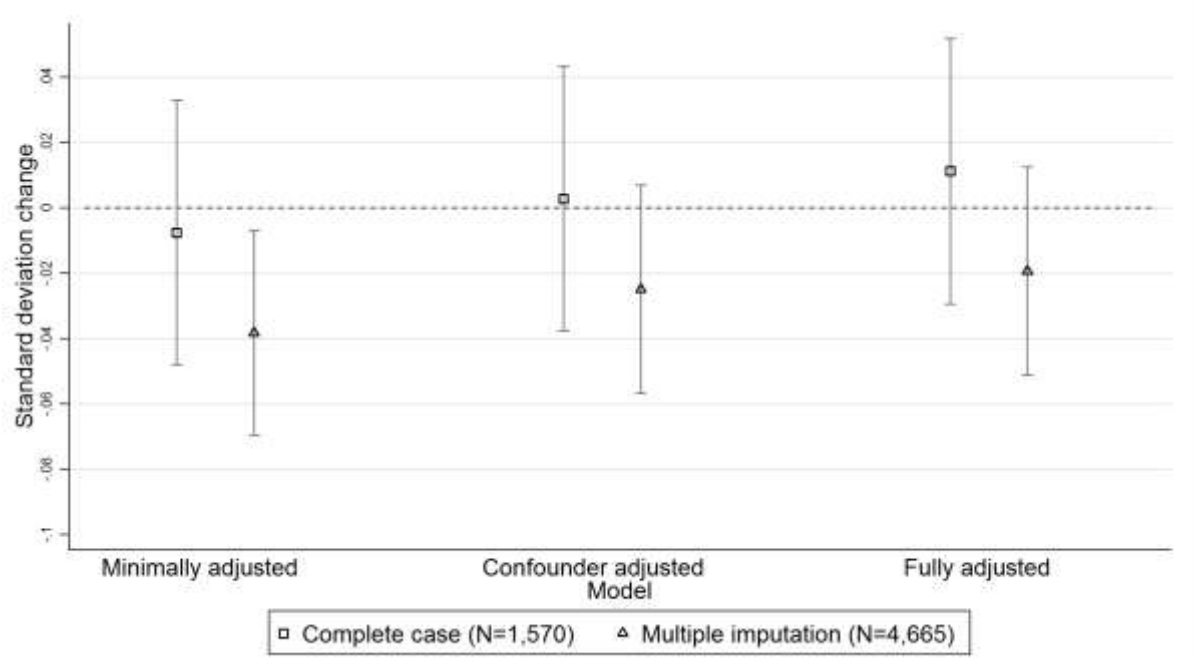
Supplemental Fig. 2 Associations of maternal metabolic equivalent maternal physical activity during pregnancy with child BMI using complete case and multiply imputed samples



Mean standard deviation difference (and 95% confidence intervals) in offspring BMI per 1 standard deviation increase in metabolic equivalent maternal physical activity during pregnancy

Multiple imputation: 20 imputations (Stata ice command)
Multiple imputation (N=4,665): all mother-offspring pairs with a value of physical activity during pregnancy and at least one offspring cardiovascular risk factor age 15.5
Complete case: Original data excluding individuals with any missing data for all variables per outcome

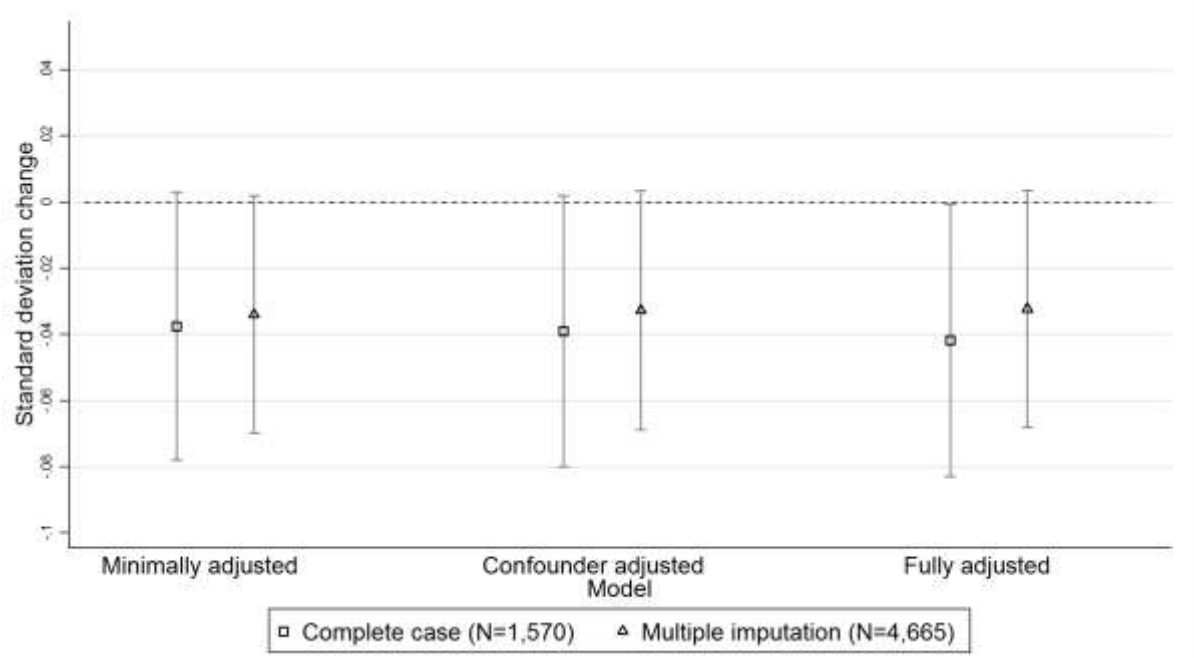
Supplemental Fig. 3 Associations of maternal metabolic equivalent maternal physical activity during pregnancy with child blood insulin levels using complete case and multiply imputed samples



Mean standard deviation difference (and 95% confidence intervals) in log transformed offspring insulin per 1 standard deviation increase in metabolic equivalent maternal physical activity during pregnancy

Multiple imputation: 20 imputations (Stata ice command)
Multiple imputation (N=4,665): all mother-offspring pairs with a value of physical activity during pregnancy and at least one offspring cardiovascular risk factor age 15.5
Complete case: Original data excluding individuals with any missing data for all variables per outcome

Supplemental Fig. 4 Associations of maternal metabolic equivalent maternal physical activity during pregnancy with child blood glucose levels using complete case and multiply imputed samples



Mean standard deviation difference (and 95% confidence intervals) in offspring glucose per 1 standard deviation increase in metabolic equivalent maternal physical activity during pregnancy

Multiple imputation: 20 imputations (Stata ice command)
Multiple imputation (N=4,665): all mother-offspring pairs with a value of physical activity during pregnancy and at least one offspring cardiovascular risk factor age 15.5
Complete case: Original data excluding individuals with any missing data for all variables per outcome

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