Gestational weight gain is associated with childhood height, weight, and BMI in the Peri/Postnatal Epigenetic Twins Study: Supplementary Material

Supplementary Table S1: Results from the adjusted linear regression models assessing the associations of GWG in kilograms with birth outcomes and early-life anthropometrics in the children. Results from the adjusted linear regression model using GWG z-scores have been included for ease of comparison (see also main text Table 2, column 2).

	GWG (kg)	GWG (kg)	GWG (z-score)
	Adjusted[a] β (95%CI)	Adjusted[b] β (95%CI)	Adjusted[c] β (95%CI)
Birth Outcomes			
Weight (z-score; n=304)	0.04 (0.02, 0.06)	0.05 (0.03, 0.01)	0.32 (0.19, 0.45)
BMI (z-score; n=279)	0.03 (0.01, 0.05)	0.04 (0.02, 0.06)	0.29 (0.14, 0.43)
Length (z-score; n=279)	0.03 (0.01, 0.05)	0.04 (0.01, 0.06)	0.23 (0.08, 0.38)
18-month Outcomes			
Height z-score (n=268)	*0.03 (0.01, 0.05)*	*0.01 (-0.02, 0.03)*	*0.08 (-0.07, 0.23)*
Weight (z-score; n=267)	*0.03 (0.01, 0.06)*	*0.02 (-0.004, 0.05)*	*0.18 (0.02, 0.34)*
BMI (z-score; n=267)	0.01 (-0.02, 0.03)	0.03 (-0.003, 0.06)	0.18 (-0.02, 0.37)
6-year Outcomes			
Height (z-score; n=194)	*0.03 (0.00, 0.06)*	*-0.01 (-0.04, 0.02)*	*-0.001 (-0.17, 0.16)*
BMI (z-score; n=194)	0.001 (-0.028, 0.030)	0.01 (-0.02, 0.03)	0.01 (-0.14, 0.15)
Weight (z-score; n=194)	*0.02 (0.00, 0.04)*	*0.00 (-0.03, 0.03)*	*-0.06 (-0.16, 0.14)*

[a]Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking status during pregnancy, socio-economic status, twin sex and chorionicity.
[b]Adjusted for variables in a, as well as for preterm birth. Preterm birth was used as an indicator of gestational age – since gestational age and GWG were moderately correlated (r: 0.201, 95%CI: 0.104 to 0.295), gestational age was not used.
[c]Adjusted for variables in a, as well as gestational age.
GWG=gestational weight gain; BMI=body mass index.
Italicised results indicate that inferences differ between the model with total GWG (kg) and no adjustment for gestational age, the model with GWG (kg) and preterm birth, or the model with GWG (z-scores).

Inverse Probability Weighting

Here we implement IPW for women with observed or missing gestational weight gain (GWG) in the Peri/Postnatal Epigenetic Twins Study (PETS).

Step 1: Determine whether IPW is necessary

First, we compare the demographic characteristics of women with observed GWG to women with missing GWG, Supplementary Table S2.

Supplementary Table S2: Differences in the demographic characteristics of the women and children in the PETS study, according to whether women had GWG observed or missing.

	Missing GWG	Observed GWG	P-value for difference
Mothers (n=250)	**n=78**	**n=172**	
Age at Delivery (years)	31.92 (5.33)	33.16 (4.88)	0.023
Smoked (at all) During Pregnancy	26 [13.68%]	46 [14.84%]	0.721
Alcohol (at all) During Pregnancy	48 [25.26%]	108 [34.84%]	0.025
Gestational Age (weeks)	34.32 (2.74)	36.78 (1.33)	<0.001
Pre-Pregnancy BMI (kg/m^2)	24.35 (5.69)	25.04 (5.36)	0.356
Delivery Type			0.002
Vaginal	50 [26.32%]	123 [39.94%]	
Caesarean	140 [73.68%]	185 [60.06%]	
Children (n=500)	**n=156**	**n=344**	
Zygosity			<0.001
Monozygotic	98 [51.58%]	108 [34.84%]	
Dizygotic	90 [47.37%]	198 [63.87%]	
Unknown	2 [1.05%]	4 [1.29%]	
Chorionicity			<0.001
Monochorionic	72 [37.89%]	68 [21.94%]	
Dichorionic	118 [62.11%]	242 [78.06%]	
Sex (Male)	87 [45.79%]	157 [50.65%]	0.292
Birthweight (grams)	2150.55 (578.49)	2663.77 (406.38)	<0.001
SGA	18 [9.47%]	16 [5.16%]	0.067
BMI (kg/m^2) at age six	15.88 (1.82)	15.88 (1.72)	0.999

GWG=gestational weight gain; BMI=body mass index; SGA=small for gestational age.

Mothers with observed GWG differed from mothers with missing GWG in age, alcohol consumption, gestational age, delivery type, zygosity and chorionicity of the twins, and birthweight. These differences indicate that women with missing GWG measurements may have had more complicated pregnancies than women with observed GWG. Therefore, the results of our regression models which assess the association of GWG with twin anthropometric outcomes may be biased. As such, IPW can be used to determine the effect of missing data on our regression results.

Step 2: Determine which method for calculating weights is appropriate for the data

Now that we have determined that implementing IPW is necessary, we need to compare weights calculated from a general IPW model to weights calculated from a stabilised IPW model. First, we generate a variable, *r*, to have the values 0 when GWG is missing, and 1 when GWG is observed. We then fit logistic regression models with robust standard errors to predict the probability of GWG being observed or missing, then use this probability to calculate weights. Weights are calculated as:[1] $w = \frac{1}{Pr}$ when $r = 1$, and $w = \frac{1}{1-Pr}$ when $r = 0$. To calculate stabilised weights:[1] $sw = \frac{1}{Pr}$ when $r = 1$, and $sw = \frac{1-Pr}{1-P}$ when $r = 0$ if data were missing, Pr is the probability of having complete data, when considering other covariates, and P is the probability of having complete data without considering other covariates. The covariates used in these calculations were the confounding variables used in the paper. We see that the general method for calculating weight leads to large weights, with a large standard deviation, whereas the stabilised method leads to a smaller standard deviation, mean weight closer to one, and a narrower range (Supplementary Table S3). This suggests that non-zero probability is less likely to influence the results when using stabilised IPW and that stabilised IPW is more appropriate to use for this data.[1-3]

Supplementary Table S3: Mean, standard deviation and range for the general and stabilised weights

	Mean	Standard Deviation	Range
Weights from general IPW	4.11	33.36	1.00 to 497.82
Weights from Stabilised IPW	2.00	0.58	1.36 to 4.53

Step 3: Determine whether weighting the observations balances the data

We now need to determine whether weighting the observations using the stabilised weights results in balanced data. In Supplementary Table S4, we show the means and standard deviations for women with observed or missing GWG, and present the standardised differences of these, from both the unweighted and weighted models. The differences in each variable between the missing and observed GWG reduce when using the weighted model. Importantly, the differences for variables identified as differing between women with observed and missing GWG reduce when using the IPW model, but variables which were similar for women with observed or missing GWG remain similar when using IPW. This indicates that implementing the IPW leads to balanced data.

Supplementary Table S4: Comparison of the standardised differences between the unweighted and weighted models for continuous demographic variables.

	Unweighted			Weighted		
	Missing	Observed	SD	Missing	Observed	SD
Maternal Age (years)	31.92 (5.33)	33.16 (4.88)	0.39	32.78	32.71	0.01
Gestational Age (weeks)	34.32 (2.74)	36.78 (1.33)	1.73	35.34 (0.27)	35.37 (0.21)	0.07
Maternal BMI (kg/m²)	24.35 (5.69)	25.04 (5.36)	0.30	21.25 (0.78)	21.27 (0.69)	0.03
Birthweight (grams)	2150.55 (578.49)	2663.77 (406.38)	23.13	2425.26 (60.17)	2429.71 (47.66)	0.61
BMI (kg/m²) at age 6	15.88 (1.82)	15.88 (1.72)	0.003	11.59 (0.60)	11.59 (0.65)	-0.001

SD=standardised difference; BMI=body mass index.

Step 4: Fit a weighted regression model

We have now determined that: there were differences in demographic characteristics of women with missing GWG compared to women with observed GWG (suggesting that our results may be biased, and that IPW is necessary); a stabilised IPW is more appropriate than a general IPW; and when stabilised IPW is applied to our data, our data are balanced. Now, we can apply the stabilised IPW to our regression models to determine whether missing data are biasing our results. In Supplementary Tables S5 and S6 we present the adjusted results from the unweighted and weighted regressions.

Supplementary Table S5: Results from the IPW adjusted linear regression models assessing the associations of GWG-for-gestational-age z-scores with birth outcomes and early-life anthropometrics in the children. Results from complete case analyses have been included for ease of comparison.

	Adjusted[a] β (95%CI)	IPW Adjusted[a] β (95%CI)
	Birth Outcomes	
Birthweight (grams; n=304)	117.30 (71.93, 162.68)	121.38 (71.62, 171.14)
Birthweight (z-score; n=304)	0.32 (0.19, 0.45)	0.33 (0.19, 0.48)
BMI (kg/m^2; n=279)	0.29 (0.14, 0.45)	0.28 (0.10, 0.45)
BMI (z-score; n=279)	0.29 (0.14, 0.43)	0.27 (0.11, 0.44)
Length (cm; n=279)	0.43 (0.15, 0.71)	0.45 (0.17, 0.74)
Length (z-score; n=279)	0.23 (0.08, 0.38)	0.24 (0.09, 0.39)
	18-month Outcomes	
Height (cm; n=268)	0.47 (0.08, 0.87)	0.48 (0.08, 0.88)
Height z-score (n=268)	0.08 (-0.07, 0.23)	0.09 (-0.05, 0.23)
Weight (kg; n=267)	0.26 (0.09, 0.43)	0.22 (0.05, 0.39)
Weight (z-score; n=267)	0.18 (0.02, 0.34)	0.14 (-0.02, 0.30)
BMI (kg/m^2; n=267)	0.20 (-0.05, 0.44)	0.13 (-0.11, 0.37)
BMI (z-score; n=267)	0.18 (-0.02, 0.37)	0.12 (-0.07, 0.32)
	6-year Outcomes	
Height (z-score; n=194)	-0.001 (-0.17, 0.16)	0.01 (-0.15, 0.16)
BMI (z-score; n=194)	0.01 (-0.14, 0.15)	-0.002 (-0.14, 0.14)
Weight (z-score; n=194)	-0.06 (-0.16, 0.14)	-0.005 (-0.14, 0.13)

[a]Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking status during pregnancy, socio-economic status, twin sex, chorionicity, and gestational age.
IPW=inverse probability weighting; GWG=gestational weight gain; BMI=body mass index.

Supplementary Table S6: Results from the IPW adjusted linear regression models assessing the associations of GWG-for-gestational-age z-scores with early-life growth[a]. Results from complete case analyses have been included for ease of comparison.

	Adjusted[b] β-coefficient (95%CI)	IPW Adjusted[b] β-coefficient (95%CI)
Growth Between Birth and 18-months		
Height (n=255)	-0.15 (-0.33, 0.03)	-0.14 (-0.31, 0.04)
Weight (n=267)	-0.16 (-0.38, 0.06)	-0.21 (-0.43, 0.01)
BMI (n=248)	-0.06 (-0.29, 0.17)	-0.12 (-0.36, 0.13)
Growth Between 18-months and 6-years		
Height (n=184)	-0.20 (-0.31, -0.09)	-0.22 (-0.33, -0.10)
Weight (n=184)	-0.26 (-0.44, -0.09)	-0.25 (-0.42, -0.07)
BMI (n=184)	-0.18 (-0.42, 0.05)	-0.14 (-0.39, 0.10)
Growth Between Birth and 6-years		
Height (n=178)	-0.23 (-0.42, -0.03)	-0.24 (-0.43, -0.04)
Weight (n=194)	-0.30 (-0.51, -0.09)	-0.33 (-0.53, -0.13)
BMI (n=178)	-0.19 (-0.42, 0.03)	-0.18 (-0.39, 0.02)

[a]Calculated as $\text{z-score}_{\text{time2}} - \text{z-score}_{\text{time1}}$
[b]adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking status during pregnancy, socio-economic status, twin sex, chorionicity, and gestational age
CI=confidence interval; BMI=body mass index. IPW=inverse probability weighting; GWG=gestational weight gain; BMI=body mass index.

References

1. Xu S, Ross C, Raebel MA, et al. Use of stabilized inverse propensity scores as weights to directly estimate relative risk and its confidence intervals. Value Health. 2010;13(2):273-277.

2. Austin PC, Stewart E. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. *Stat Med.* 2015;34:3661-3679.

3. Seaman SR, White I. Review of inverse probability weighting for dealing with missing data. *Stat Med.* 2011;22(3):278-295.

Subgroup Analyses

IOM Categories

In our main article, we examined the association of GWG z-scores with twin anthropometric measures at birth, 18-months, and six-years of age. Though we found an association of GWG with twin anthropometric measures overall, we recognise that the risk of adverse outcomes differs according to whether women gain too little or too much weight during pregnancy, which is why there are GWG recommendations. Although the current IOM recommendations are limited, they are currently the only recommendations for women pregnant with twins in Australia, so we were interested in understanding whether the association of GWG z-scores differed according to whether women gained weight within the recommendations, below the recommendations (considered to be 'inadequate' GWG), or above the recommendations (considered to be 'excessive' GWG).

Understanding whether the association of GWG with twin health outcomes differs according to these groups can provide further insight into whether the current recommendations for GWG in twin pregnancy are appropriate. For example, if the association of GWG z-scores with twin BMI is consistently positive for all IOM categories, then we might conclude that the current recommendations are not appropriate in mitigating adverse long-term health outcomes for the twins. Conversely, if the association of GWG z-scores with twin BMI is null for all IOM categories, then this might indicate that there is no benefit to gaining weight within compared to outside the recommendations, and call into question whether the recommendations are needed. If, however, the association of GWG z-scores with twin health differs according to whether women gain weight within or outside the recommendations, this indicates the GWG recommendations are necessary, though it does not address whether the current recommendations are appropriate or achievable.

To conduct this analysis, we fitted Model 1 (generalised estimating equations linear regression with GWG for gestational age z-score as a continuous exposure) for each of the subgroups 1) below IOM, 2) within IOM, and 3) above IOM. We have also included a comparison of the means and standard deviations of demographic characteristics (Table S7).

The association of GWG z-score with twin health outcomes was consistent for most outcomes (Fig S1). However, there was a positive association of GWG z-score with twin weight at 18-months for women who gained below the IOM recommendations but not for women who gained within or above the recommendations. At six-years, the association of GWG z-score was positive for women who gained above the recommendations but null for women who gained below or within the recommendations. However, this difference may not be significant. In contrast, the association of GWG z-score with six-year BMI appeared to be strongly positive for women who gained weight outside the recommendations, but this association was not observed for women who gained below or within the recommendations. This indicates that gaining an appropriate amount of weight during pregnancy may be important for childhood BMI. However, given the sample size limitations of the PETS, we recommend caution when interpreting these results. Furthermore, since we were limited by sample size, we cannot draw conclusions about the appropriateness of current GWG recommendations from these analyses.

Supplementary Table S2: Differences in demographic characteristics of women and children in the PETS, according to whether women had GWG below, within or above the IOM recommendations.

	Below IOM	Within IOM	Above IOM
Mothers (n=156)	**n=47**	**n=72**	**n=37**
Age at Delivery (years)	33.92 (23.86)	33.76 (4.66)	31.19 (5.04)
Gestational Age (weeks)	36.67 (1.86)	36.86 (0.92)	36.76 (1.20)
Pre-Pregnancy BMI (kg/m^2)	25.19 (18.62)	24.50 (4.51)	25.91 (5.95)
Children (n=312)	**n=94**	**n=144**	**n=74**
Birthweight (grams)	2534.73 (418.21)	2667.20 (380.34)	2816.60 (391.78)
SGA	8 [8.51%]	8 [5.56%]	0 [0.0%]
BMI (kg/m^2) at age six	15.65 (1.74)	16.11 (1.77)	15.68 (1.52)

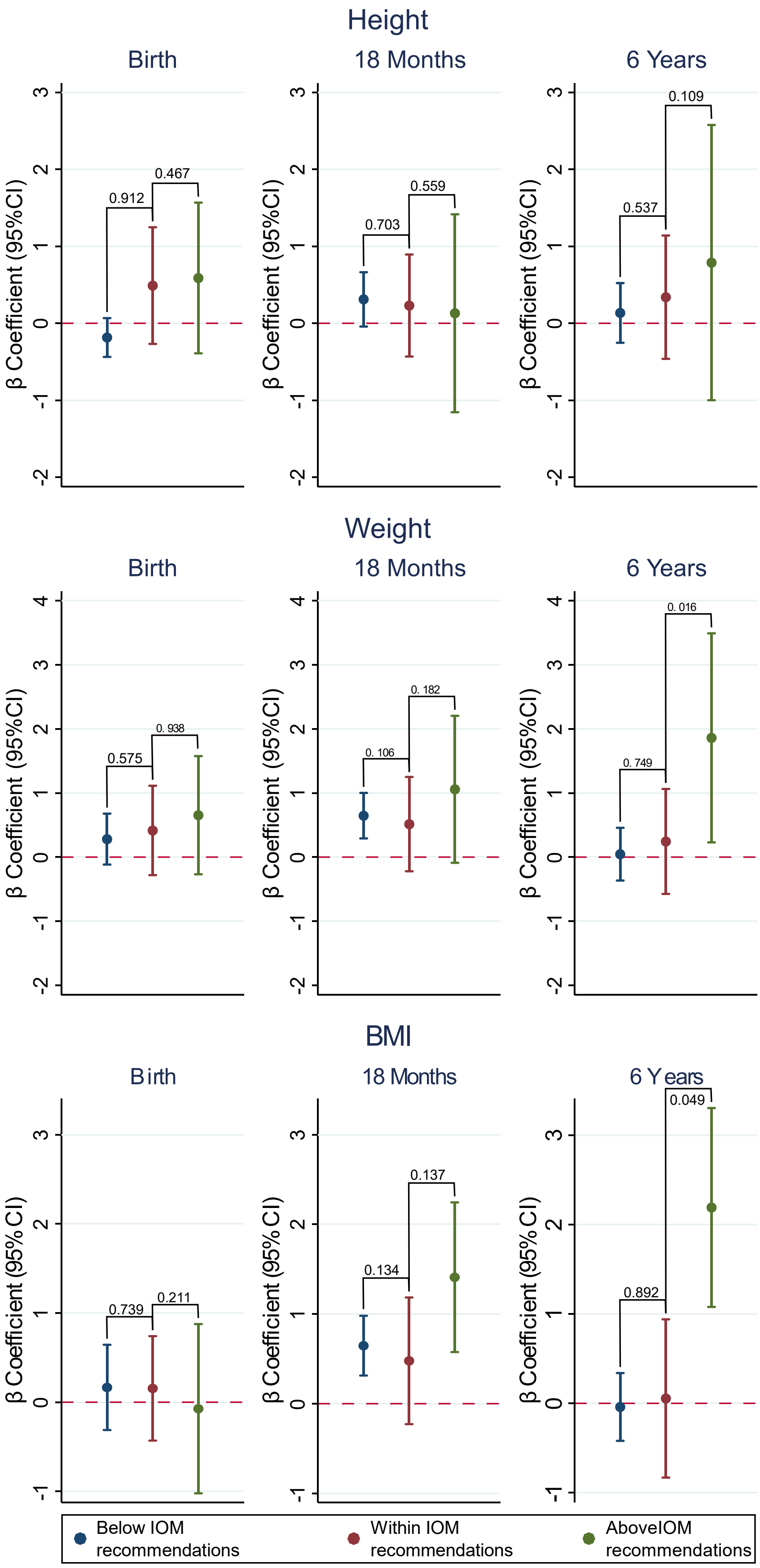
GWG=gestational weight gain; BMI=body mass index; SGA=small for gestational age.

BMI Categories

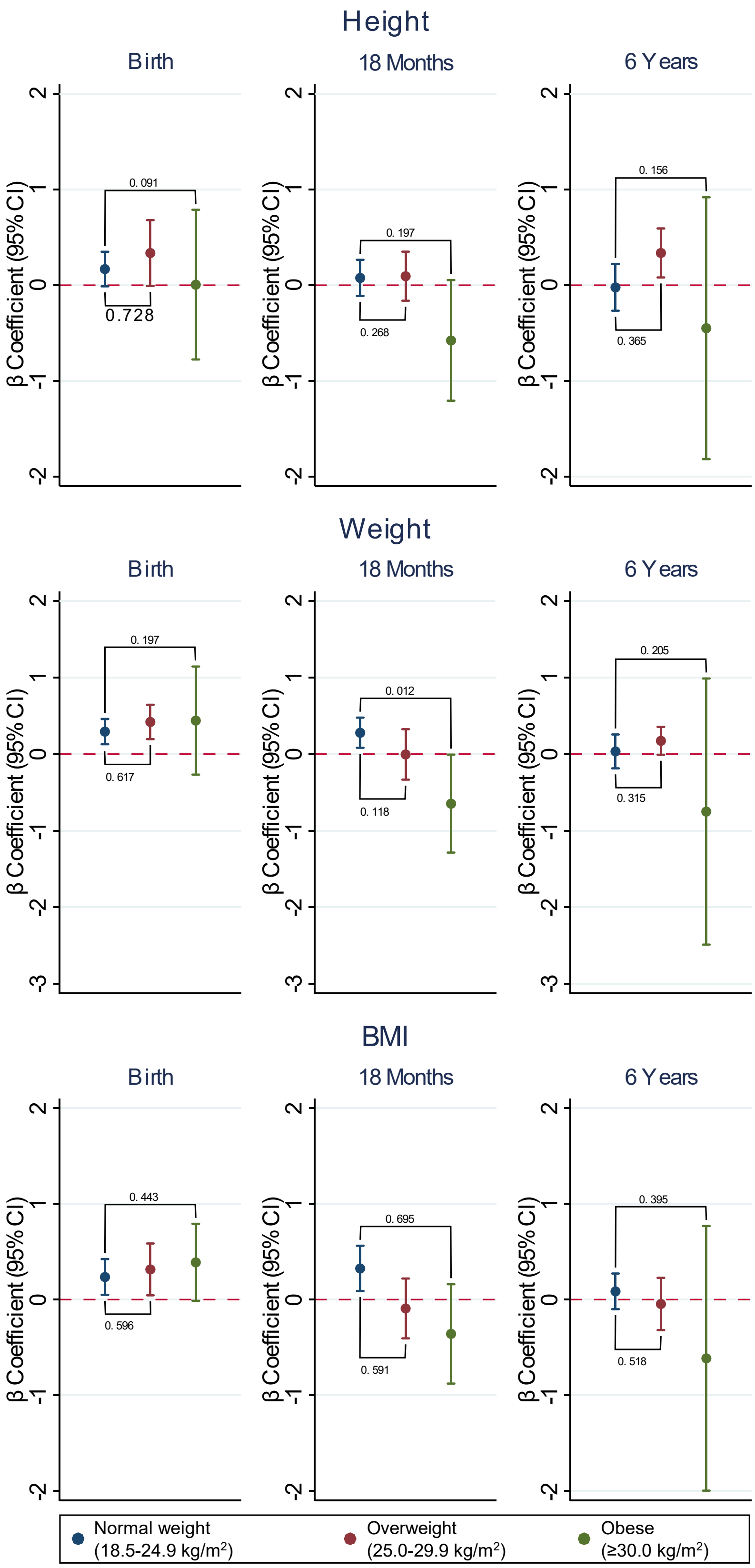
Previous studies have shown that birth outcomes for GWG differ according to pre-pregnancy BMI, so women in each BMI category should gain different amounts of weight. This is reflected in the IOM GWG recommendations, which are BMI-specific. Since outcomes are likely to differ according to BMI, we were interested in understanding whether associations of GWG with twin health outcomes differed for women in each pre-pregnancy BMI category.

To conduct this analysis, we fitted Model 1 for each of the subgroups 1) normal BMI prior to pregnancy 2) overweight BMI prior to pregnancy and 3) obese BMI prior to pregnancy. Since GWG growth charts are not available for women underweight prior to pregnancy, we were unable to include this as an additional subgroup.

There appeared to be no difference in the association of GWG z-score with twin anthropometric outcomes according to pre-pregnancy BMI (Fig S2). However, as with the IOM subgroup analyses, we were limited by sample size, and so are unable to conclusively determine whether the association of GWG z-score with twin health differs according to BMI. Though previous research indicates that GWG outcomes differ according to pre-pregnancy BMI, few studies have explored GWG with the long-term health outcomes in twins. As such, it is not yet clear whether these associations do differ according to pre-pregnancy BMI. If outcomes do not differ for each BMI category, then this raises an interesting discussion point as to whether the IOM recommendations need to be BMI-specific. Further research is therefore needed to determine whether the long-term associations of GWG with twin health differ according to pre-pregnancy BMI.



Supplementary Figure S1: Adjusted regression coefficients and 95% confidence intervals for the association of GWG z-scores with twin height, weight and BMI z-scores using IOM categories as subgroups. P-values above the black bars are for the difference between subgroups, with the within IOM group the reference group.



Supplementary Figure S2: Adjusted regression coefficients and 95% confidence intervals for the association of GWG z-scores with twin height, weight and BMI z-scores using maternal pre-pregnancy BMI categories as subgroups. Since few women underweight prior to pregnancy, and GWG growth charts are unavailable for these women, estimates cannot be provided for women underweight pre-pregnancy. P-values above or below the black bars are for the difference between subgroups, and the normal weight BMI category is the reference group.

Sex

The association of GWG z-scores with twin anthropometric measures differ by sex, and though our original analyses adjusted for sex, the main analyses did not explore differences in associations for male and female twins. Anthropometric measures are sexually dimorphic, so the associations of GWG with twin health outcomes may differ according to sex. As such, here we have included subgroup analyses to determine whether the association of GWG z-scores with twin anthropometric z-scores differed according to sex (Table S8). Further differences may be observed in same-sex or opposite-sex twin-pairs (male-male, female-female, or male-female), however, due to sample size limitations, we were underpowered to conduct these analyses. We found some differences in associations of GWG z-scores with 18-month weight and BMI for male and female twins – for female twins, higher GWG z-scores were associated with higher weight and BMI, but there were no associations for male twins. All other results appeared consisted regardless of sex. However, we did not formally test for differences, and the confidence intervals for male and female twins overlap, and so it is unclear from these analyses whether these differences represent true differences between male and female twins.

Supplementary Table S8: Results from the adjusted linear regression models assessing the associations of GWG-for-gestational-age z-scores with birth outcomes and early-life anthropometrics in the children for males and females separately.

	Males β (95%CI)	Females β (95%CI)
Birth Outcomes		
Birthweight (z-score; n=304)	0.38 (0.15, 0.61)	0.34 (0.17, 0.51)
Length (z-score; n=279)	0.43 (0.16, 0.69)	0.21 (0.06, 0.36)
BMI (z-score; n=279)	0.16 (-0.03, 0.35)	0.37 (0.15, 0.59)
18-month Outcomes		
Weight z-score (n=268)	-0.07 (-0.33, 0.18)	0.27 (0.10, 0.45)
Height (z-score; n=267)	0.07 (-0.18, 0.32)	0.09 (-0.11, 0.29)
BMI (z-score; n=267)	-0.16 (-0.40, 0.07)	0.31 (0.11, 0.51)
6-year Outcomes		
Weight (z-score; n=194)	0.14 (-0.16, 0.43)	0.02 (-0.15, 0.19)
Height (z-score; n=194)	0.17 (-0.14, 0.49)	0.01 (-0.17, 0.20)
BMI (z-score; n=194)	0.08 (-0,20, 0.37)	0.02 (-0.13, 0.18)

[a]Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking status during pregnancy, socio-economic status, twin sex, chorionicity, and gestational age.
IPW=inverse probability weighting; GWG=gestational weight gain; BMI=body mass index.

Early, mid, and late GWG

Since the timing of gestational weight gain may be important for twin health outcomes, we have included results from models assessing associations of early-pregnancy GWG z-score (until ~12 weeks' gestation), mid-pregnancy GWG (~12-24 weeks' gestation) and late-pregnancy GWG (~24-36 weeks' gestation) with twin anthropometric z-scores (Table S9). There are limitations in using GWG z-scores in this way, notably that many women experience gestational weight loss, at some point during the first trimester, and so these z-scores may not accurately reflect true patterns of GWG throughout each trimester. Additionally, given the power limitations of the PETS, these results should be interpreted with caution. However, higher GWG in late pregnancy appears to be associated with higher birthweight and birth BMI, and higher GWG in early pregnancy appears to be associated with lower weight and height at age six. These results indicate that the timing of GWG may have a differential effect on twin health outcomes and may have long-term associations with twin anthropometric outcomes. In contrast, results from our main model (which did not consider timing of GWG) indicated that GWG was only associated with birth anthropometric measures, and not childhood anthropometrics.

Supplementary Table S9: Results of associations of early, mid and late GWG (z-scores) with twin anthropometric z-scores at birth, 18 months and six years of age.

	Early GWG β (95%CI)	Mid GWG β (95%CI)	Late GWG β (95%CI)	Original results β (95%CI)
Birth Outcomes				
Birthweight (n=272)	0.08 (-0.03, 0.29)	0.13 (-0.05, 0.30)	0.10 (0.01, 0.19)	0.32 (0.19, 0.45)
Length (n=212)	-0.05 (-0.16,0.05)	0.25 (0.07, 0.44)	0.09 (-0.01, 0.18)	0.27 (0.09, 0.45)
BMI (n=210)	0.14 (0.02, 0.26)	0.01 (-0.19, 0.21)	0.09 (0.01, 0.17)	0.29 (0.14, 0.43)
18-month Outcomes				
Weight (n=251)	0.03 (-0.08, 0.13)	0.17 (-0.02, 0.36)	0.07 (-0.03, 0.17)	0.07 (-0.11, 0.25)[a]
Height (n=252)	-0.01 (-0.08, 0.06)	0.12 (-0.03, 0.28)	0.03 (-0.07, 0.13)	0.04 (-0.17, 025)
BMI (n=251)	0.05 (-0.08, 0.17)	0.13 (-0.09, 0.34)	0.08 (-0.02, 0.17)	0.03 (-0.14, 0.20)[a]
6-year Outcomes				
Weight (n=185)	-0.11 (-0.20, -0.02)	0.12 (-0.08, 0.32)	0.04 (-0.06, 0.14)	-0.06 (-0.16, 0.14)
Height (n=185)	-0.08 (-0.17, -0.00)	0.09 (-0.09, 0.28)	0.07 (-0.03, 0.17)	0.01 (-0.23, 0.25)
BMI (n=194)	-0.08 (-0.19, 0.04)	0.10 (-0.09, 0.29)	-0.01 (-0.12, 0.10)	0.01 (-0.14, 0.15)

Original results are from the adjusted model presented in main text Table 2. All results are adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking status during pregnancy, socio-economic status, twin sex, chorionicity, and gestational age.
GWG=gestational weight gain; BMI=body mass index. [a]Results presented here are from the linear component of a quadratic regression.