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BMJ Open

Changing levels of maternal exercise during pregnancy and neonatal adiposity: secondary analysis of the SCOPE/BASELINE birth cohort

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017987
Article Type:	Research
Date Submitted by the Author:	31-May-2017
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	adiposity, exercise, pregnancy, PEAPOD

Changing levels of maternal exercise during pregnancy and neonatal adiposity: secondary analysis of the SCOPE/BASELINE birth cohort

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on behalf of the SCOPE Ireland Cohort study and the Cork BASELINE Birth Cohort Study

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Abstract:

Objective: To investigate whether changing levels of exercise during pregnancy are related to altered neonatal adiposity. **Design**: Secondary analysis of data from a prospective cohort study. **Setting**: Cork, Ireland. **Participants**: 1200 mother-infant pairs recruited as part of a prospective birth cohort, Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE). **Main outcome measures**: Neonatal adiposity was assessed within several days of birth using air displacement plethysmography (PEAPOD). BF% as a continuous outcome and a pair of dichotomous variables; high or low adiposity, representing BF% >90th or <10th centile, respectively. **Results**: Crude analysis revealed no effect of a changing level of exercise (since becoming pregnant) at 15 weeks' gestation. At 20 weeks' gestation, analyses revealed that relative to women who do not change their exercise level up to 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46). This association was maintained after adjustment for putative confounders (OR: 1.62; 95% CI: 1.06; 2.47). **Conclusions**: We observed a possible sensitive period for the effect of changing exercise levels, with no effect observed with exercise recall for the first 15 weeks of gestation, but an effect of a decreasing level of exercise between 15 and 20 weeks. These results should be interpreted in line with the limitations of the study and further studies utilising objectively measured estimates of exercise are required in order to replicate these findings.

Article Summary:

Strength and limitations of this study

- Air displacement plethysmography (PEAPOD) was used to measure neonatal body composition
- Directed acyclic graphs (DAGs), based on an understanding of the causal network linking the variables in the analysis, were used to identify putative confounding variables
- Exercise variables were based on maternal self-report and therefore subject to recall bias
- Pre-pregnancy exercise data were not available, meaning we were unable to ascertain what pre-pregnancy exercise level women had changed from

Introduction:

In their 2006 guideline, the Royal College of Obstetricians and Gynaecologists (RCOG) concluded that pregnant women should be 'encouraged to initiate or continue exercise to derive the health benefits associated with such activities'. [1]

The benefits of physical activity during pregnancy are likely to operate through an increased blood flow and oxygenation to the fetus. [2,3] It has also been proposed that the impact of exercise on fetal growth is mediated by its effect on maternal insulin sensitivity, although a recent study has cast doubt on this. [4] Another mechanism by which exercise could exert its effect is via the functioning of the uteroplacental unit, for example by affecting placental function, volume and growth rates. [5-7] However, the apparent beneficial effects of exercise appear to be dependent upon the timing of when exercise is undertaken. For example, Clapp et al (2002) demonstrated that women who performed a high quantity of moderate exercise in early pregnancy and then cut back in late pregnancy (hi-lo) delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume (lo-hi). The hi-lo exercise regimen was also associated with a greater placental volume at delivery, relative to the other two groups, [6] presumably as a result of faster placental growth in early gestation. Those who either maintained moderate exercise or increased to a high volume of exercise in late gestation (relative to the hi-lo group) did not exhibit this increased placental volume at birth, suggesting that early gestation is a critical period for any exercise effects on placental development to be enacted, with a potentially suppressive effect in late gestation. [2] Furthermore, it has been reported that the transient changes in glucose regulation observed after bouts of exercise differ depending on when in pregnancy the exercise load is occurring, with increases in blood glucose observed after exercise early in pregnancy, but decreases in later pregnancy. [8] These fluctuations in nutrient supply, depending on the timing of exercise, could also contribute to differential effects on fetal growth.

The data surrounding the effects of physical activity on neonatal body composition (as opposed to size) from large scale observational studies is limited. Data from a limited number of relatively small randomised controlled trials report either a null or reducing effect of physical activity on neonatal adiposity, [4 6 7] with potentially greater effects if the exercise intervention is administered at later gestations. Findings from a recent observational study, the Healthy Start cohort (n=826), also suggested that increasing physical activity levels in later pregnancy could result in a reduction in neonatal adiposity, even after adjusting for putative confounders (e.g. maternal age, race or ethnicity, educational status, household income, pre-pregnancy BMI, and prenatal smoking status).[9]

It is now well established that the *in utero* milieu experienced by the developing fetus could influence long-term risk for the development of obesity and obesity-related non-communicable diseases (OR-NCDs). [10-12] Maternal behaviour during this critical period of developmental plasticity has the potential to permanently alter susceptibility to later chronic disease via alterations in the offspring's metabolic and endocrinological phenotype. [13-15] Consequently, we hypothesise that maternal exercise in pregnancy is associated with neonatal adiposity. Any changes in neonatal adiposity could be indicative of an altered phenotypic profile in the offspring, which may increase susceptibility to later chronic disease.

The objective of the current study was to investigate whether changes in maternal exercise during pregnancy were associated with offspring adiposity in the neonatal period, measured using air displacement plethysmography in a large homogeneous population.

Methods:

Neonatal participants were recruited as part of the Cork BASELINE birth cohort study (ClinicalTrials.gov NCT: 01498965 www.birthcohorts.net) [16] between August 2008 and August 2011 from women who had participated in SCOPE (Screening for Pregnancy Endpoints) Ireland. SCOPE was a multicentre prospective cohort study with the aim of developing screening tests to predict various complications of pregnancy (e.g. pre-eclampsia, small-for-gestational-age (SGA) infants, and spontaneous preterm birth) (ACTRN12607000551493). [17] Methods are described in detail elsewhere. [17 18] In brief, participants were healthy nulliparous women with singleton pregnancies recruited antenatally between February 2007 and February 2011 in Cork, Ireland. Women were recruited, interviewed and all measurements obtained at 15±1 and 20±1 weeks' gestation. [17 19] Exclusion criteria included: a high risk for pre-eclampsia/delivery of a SGA neonate/spontaneous preterm birth because of underlying medical conditions; three or more previous miscarriages; three or more terminations of pregnancy; or having received interventions such as aspirin that might modify pregnancy outcome. At the time of interview, data were entered onto an internet-accessed central database with a complete audit trail designed and hosted by MedSciNet, Sweden. Participants were followed up prospectively, with pregnancy outcome data collected by trained research midwives.

Neonatal adiposity was assessed in the majority of neonates within 72 hours of birth by calculating neonatal body fat percentage (BF%) using the PEAPOD air displacement plethysmography. The mean time of measuring BF% in those infants born over 37 weeks' gestation was 1.8 days (standard deviation 0.97 days). Of those infants born <37 weeks' gestation, the mean time of testing was 2.4 days (standard deviation 1.2 days). Measurement of neonatal BF% involves direct measurement of body mass using precise scale and body volume in an airtight, enclosed chamber. Body composition assessment by densitometry involves the measurement of the density of the whole body. Body density is then used in a two-compartment model to calculate the percentage of fat, fat mass, and

fat-free mass.[20] The PEAPOD has excellent test-to-test reproducibility and is safe, non-invasive and fast.[21 22]

Exercise data were collected at both the 15 and 20 week visits in a standardised manner. At both time points, women were asked how many times per week they engaged in vigorous activity (which made the woman breathe harder or puff or pant),[23] moderate activity (did not breathe harder or puff or pant) or walking for recreation or exercise. At 15 weeks, women were asked: *'Has your level of exercise (physical activity) changed since you've been pregnant?*', to which they could respond *'decreased'*, *'unchanged'* or *'increased'*. At 20 weeks, women were then asked: *'Has your level of exercise changed since last SCOPE visit?'*, with the same possible response options.

Statistical analysis:

We used linear regression models to investigate the effect of changing levels of self-reported maternal exercise during pregnancy on birthweight (g) and %BF measured as continuous variables. For descriptive results, we generated a 'no exercise' binary variable with a value of 1 indicating women who reported doing no vigorous nor moderate nor recreational walking activity per week. Change in exercise levels was coded as a categorical variable: no change (reference group) versus decreased versus increased. Regression diagnostics did not reveal any violations to linear regression assumptions (i.e. normally distributed residuals and homogeneity of variance). We subsequently generated separate binary variables (0= no; 1=yes) exercise on these dichotomous variables using logistic regression models. Low and high adiposity was defined as below/above the gestational age- and sex-specific 10^{th}/90^{th} adiposity centiles respectively, according to the centiles produced by Hawkes et al (2011).[24] We performed sensitivity analyses limiting the sample to only those born at term (n=1180) and separately, to those born non-low birthweight (>2500g) (n=1180) but effect estimates did not markedly change and thus these infants were retained in the analysis. Furthermore, as the analysis sample was based on those that had complete data for the exposure, outcome and covariates, we also performed sensitivity analyses to identify whether we had introduced a selection bias by only including those with complete data (supplementary tables 1 and 2).

In order to identify less biased associations between our exposures and outcome, we produced a directed acyclic graph (DAG) using Daggity. [25] DAGs provide a method for formalising and clarifying the causal hypothesised assumptions a researcher may make regarding the variables they wish to analyse [26] and thus justify modelling choices. [27 28] These graphs are especially useful for identifying variables which potentially confound the relationship between two variables, thus providing researchers with sets of variables for which adjustment (and importantly non-adjustment) is necessary, in order to obtain unbiased estimates of the relationship between a set of variables. For a more detailed discussion on the use of these graphs in epidemiology, see Greenland, Pearl and Robins. [29] Daggity is a web-based interface which allows researchers to construct and edit a directed acyclic graph, with the ultimate aim of identifying sufficient sets of variables for adjustment which will minimise bias when estimating the effect of an exposure on the outcome. The set of variables identified by Daggity as necessary for adjustment were socioeconomic status, maternal employment, smoking status, alcohol intake, body mass index (BMI) and level of education. These variables were then incorporated into multivariable regression models. All analyses were conducted in Stata/IC v14.1.

Results:

1258 neonates had PEAPOD measurements taken, of which 1200 had complete exposure, outcome and covariate data. Of these 1200, 612 (51.00%) were male and 98.25% (n=1 179) were of White European ethnic origin. The mean birthweight was 3510g (95% CI: 3484 - 3537) and the median gestational age was 40 weeks (interquartile range: 39 - 41).

Change in exercise level reported in the first 15 weeks of pregnancy

It is shown in Table 1 that at 15 weeks' gestation, more than a quarter (n=327, 27.25%) of women reported engaging in vigorous exercise at least once per week, with approximately three quarters reporting doing some form of moderate exercise per week (n=892, 74.33%). 104 (8.67%) women reported not engaging in any form of exercise per week.

Table 2 provides descriptive statistics for various maternal characteristics and neonatal outcomes, stratified by type of change of exercise in pregnancy. Compared to women who reported no change in exercise level, those who decreased their level of exercise were older (30.51 years (4.17) vs. 28.89 years (4.74)), with a higher level socioeconomic status (44.33 (16.15) vs. 39.10 (15.40)), less likely to have a household income below €21 000 (5.80% vs. 13.16%) and less likely to have smoked during the first trimester (6.03% vs. 15.90%). Compared to women who increased their exercise levels, no differences were observed in women who decreased their exercise levels, apart from a much lower likelihood of having a household income below €21 000 (5.80% vs. 17.07%).

The effect of changing exercise levels on birthweight and neonatal adiposity is shown in Table 3. Relative to women who did not change their exercise level in pregnancy up to 15 weeks, there was no difference in any of the outcomes in those women who either increased or decreased their level of exercise, in both crude and adjusted analyses. Changing the reference group in order to compare those who decreased relative to those who increased also revealed no differences in neonatal outcomes.

Table 1: Descriptive statistics of self-reported activity levels at 15 and 20 weeks

	Exercise level at 15 weeks (n=1200)	Exercise level at 20 weeks (n=1200)
Vigorous at least once per week (yes) (n; % of 1200)	327 (27.25)	377 (31.42)
Moderate at least once per week (yes) (n; % of 1200)	892 (74.33)	908 (75.67)
Recreational at least once per week (yes) (n; % of 1200)	1040 (86.67)	1057 (88.08)
No exercise per week (n; % of 1200)	104 (8.67)	100 (8.33)

	Change in exercise level between 15-20 weeks		
	Decreased (n=263)	Unchanged (n=665)	Increased (n=272)
Any exercise per week at 15 weeks			
No (n; % of column total)	7 (2.66)	72 (10.83)	25 (9.19)
Yes (n; % of column total)	256 (97.34)	593 (89.17)	247 (90.81)

Table 2: Descriptive statistics in those with changing levels of physical activity during pregnancy

	Change in exercise level in pregnancy to 15 weeks (n=1200)		
	Decreased (n=813)	Unchanged (n=346)	Increased (n=41)
Maternal characteristics:			
Maternal age (mean;SD)	30.51 (4.17)†	28.89 (4.74)	28.88 (5.19)
Maternal BMI (mean;SD)	25.02 (4.12)	24.49 (4.21)	24.18 (3.85)
Maternal years schooling (mean;SD)	13.27 (0.83)	13.18 (0.81)	13.15 (0.73)
Maternal socioeconomic status (mean;SD)	44.33 (16.15)†	39.10 (15.40)	43.51 (16.35)
Maternal household income <€21 000 (n:%)	47 (5.80)†‡	45 (13.16)	7 (17.07)
Maternal smoking in 1st trimester (n;%)	49 (6.03)†	55 (15.90)	5 (12.20)
Maternal alcohol intake in 1st trimester (units/week)	4.61 (5.76)	5.39 (6.97)	5.99 (8.10)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.16)	40 (1.24)	40 (1.00)
Birth weight (g) (mean;SD)	3525 (460)	3471 (478)	3541 (449)
Neonatal adiposity (%)(mean;SD)	11.06 (4.15)	11.03 (4.06)	11.22 (4.13)
Adiposity<10th centile (yes) (n;%)	68 (8.36)	26 (7.51)	3 (7.32)
Adiposity>90th centile (yes) (n;%)	86 (10.58)	39 (11.27)	7 (17.07)
	Change in exercise level in pregnancy: 15 to 20 weeks (n=1200)		
	Decreased (n=263)	Unchanged (n=665)	Increased (n=272)
Maternal characteristics:			
Maternal age (mean;SD)	30.74 (4.13)†	29.52 (4.58)‡	30.39 (4.25)
Maternal BMI (mean;SD)	25.07 (4.06)	24.60 (4.01)	25.20 (4.52)
Maternal years schooling	13.28 (0.72)	13.23 (0.86)	13.22 (0.82)
Maternal socioeconomic status	44.33 (15.49)†	40.96 (16.08)‡	45.79 (16.21)
Maternal household income <€21 000 (n:%)	15 (5.70)†	66 (10.03)	18 (6.62)
Maternal smoking in 1st trimester (n;%)	23 (8.75)	73 (10.98)‡	13 (4.78)
Maternal alcohol intake in 1st trimester (units/week)	4.91 (5.79)	5.24 (6.82)‡	3.98 (5.01)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.20)	40 (1.19)	40 (1.14)
Birth weight (g) (mean;SD)	3541 (498)	3487 (458)	3537 (448)
Neonatal adiposity (%)(mean;SD)	11.44 (4.66)	10.90 (4.02)	11.08 (3.79)
Adiposity<10th centile (yes) (n;%)	22 (8.37)	58 (8.87)	16 (5.88)
Adiposity>90th centile (yes) (n;%)	41 (15.59)	68 (10.23)	23 (8.46)

†different to 'unchanged' ‡different to 'increased'

Change in exercise level between 15 and 20 weeks

At 20 weeks, similar levels of exercise were reported, with approximately 30% of women reporting doing vigorous exercise at least once per week, and three quarters of the sample engaging in some form of moderate exercise. Just over 8% of women reported taking part in no form of exercise at 20 weeks (Table 1). Table 1 also reveals that of the 665 women who reported no change in their exercise levels between 15-20 weeks, approximately 10% of these (n=72) had engaged in no exercise at 15 weeks. Similarly, of those who increased their exercise levels between 15-20 weeks, just under 10% (n=25) had reported no exercise at 15 weeks (Table 1).

Compared to women who reported no change in exercise level between 15 and 20 weeks, those who decreased their level of exercise were older (30.74 years (4.13) vs. 29.52 years (4.58)), with a higher level socioeconomic status (44.33 (15.49) vs. 40.96 (16.08)) and less likely to have a household income below €21 000 (n=15 (5.70%) vs. n=66 (10.03%)). Women who increased their exercise levels between 15 and 20 weeks, relative to those who reported no change, were also older and with a higher SES, with a reduced alcohol intake (3.98 (5.01) units/week vs. 5.24 (6.82) units/week) and lower likelihood of smoking during the 1st trimester (n=13 (4.78%) vs. n=73 (10.98%) (Table 2).

Crude analysis shows that relative to women who do not change their exercise level between 15 and 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46) (Table 3). This association was maintained after adjustment for the putative confounders (OR: 1.62; 95% CI: 1.06; 2.47). When changing the reference group in order to compare women who decreased exercise levels relative to those who increased exercise, it was observed that those who decreased were twice as likely to give birth to a neonate with an adiposity above the 90th centile (OR: 2.00; 95% CI: 1.16 - 3.44), which again was also maintained on adjustment (OR: 2.05; 95% CI: 1.19 - 3.55). Birthweight was not associated with differences in exercise (Table 3).

Compared to all of those enrolled without a PEADOD measurement (n=513) cohort, those enrolled in Cork with a PEAPOD measurement taken (n=1258) were approximately 130g (95% CI: 80-190) heavier and born approximately 2 days later (95% CI: 0.15-0.43), but with no differences in any maternal biological or demographic data (Supplementary table 1). Although 1258 had PEAPOD measurements taken, 58 infants were not included in the final analysis due to: all PEAPOD data being lost/mis-entered (n=16), being born too early or late for adiposity centiles to be generated (n=23) and having incomplete exposure and covariate data (19), leaving a final analysis sample of 1200. Compared to those with PEAPOD measurements but not in the final analysis sample, those who were in the final analysis had higher birthweight (187.81; 95% CI: 64.45-311.17), but with no differences in gestational age or any maternal biological or demographic data (Supplementary table 2).

Table 3: Effect of changing exercise levels during pregnancy on neonatal adiposity

	Change in exercise level in pregnancy to 15 weeks (coefficient; 95%CI)					
	Crude			***Multivariable******		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.39 (-4.16; 112.93)	*(reference)*	70.24 (-80.40; 220.89)	22.10 (-37.27; 81.48)	*(reference)*	74.10 (-37.27; 81.48)
Neonatal adiposity (%)*	0.03 (-0.49; 0.55)	*(reference)*	0.19 (-1.15; 1.53)	-0.01 (-0.54; 0.52)	*(reference)*	0.33 (-1.01; 1.67)
Adiposity<10th centile**	1.12 (0.70; 1.80)	*(reference)*	0.97 (0.28; 3.36)	1.21 (0.74; 1.97)	*(reference)*	0.81 (0.23; 2.89)
Adiposity>90th centile**	0.93 (0.62; 1.39)	*(reference)*	1.62 (0.67; 3.90)	0.93 (0.62; 1.41)	*(reference)*	1.75 (0.71; 4.30)
	Change in exercise level in pregnancy: 15 to 20 weeks (coefficient; 95%CI)					
	Crude			***Multivariable******		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.47 (-11.96; 120.90)	*(reference)*	50.11 (-15.53; 115.75)	42.59 (-23.45; 108.62)	*(reference)*	22.29 (-43.44; 88.01)
Neonatal adiposity (%)*	0.54 (-0.05; 1.13)	*(reference)*	0.18 (-0.40; 0.76)	0.53 (-0.06; 1.12)	*(reference)*	0.12 (-0.46; 0.71)
Adiposity<10th centile**	0.94 (0.56; 1.56)	*(reference)*	0.64 (0.36; 1.14)	0.96 (0.57; 1.61)	*(reference)*	0.70 (0.39; 1.25)
Adiposity>90th centile**	1.62 (1.07; 2.46)	*(reference)*	0.81 (0.49; 1.33)	1.62 (1.06; 2.47)	*(reference)*	0.79 (0.48; 1.31)

*Linear regression for continuous outcomes (β); **logistic regression for dichotomous outcomes (OR) ***adjusted for: socioeconomic status, years of schooling, employment status, maternal BMI, smoking in 1st trimester, alcohol intake in 1st trimester

Discussion

In this cohort of white European mother-offspring pairs, we report the effect of changing levels of exercise during pregnancy and neonatal adiposity measured using air displacement plethysmography (PEAPOD). We observed that pregnant women who reported a decrease in exercise levels between 15 and 20 weeks, had a 60% higher risk of having a baby with adiposity above the 90th centile when compared with women who reported no change. This risk was approximately double (OR: 2.00; 95% CI: 1.16 - 3.44) when women who reported a decrease in exercise levels between 15 and 20 weeks were compared to women who reported an increase in exercise levels. This association was maintained after adjustment for a set of putative confounders including maternal education, employment status, smoking, alcohol intake, BMI and socioeconomic status. The exercise effect was only apparent between 15 and 20 weeks and not for changing exercise levels prior to 15 weeks, raising the possibility that there is a potential sensitive period with regard to the effect of a change on exercise level on the development of offspring adiposity.

A major strength of the study is the use of air-displacement plethysmography for the estimates of body composition. This method is a quick, safe and non-invasive technique, which has shown to be a reliable and accurate instrument for determining body fat percentage in infants. [21 30 31] As such, it has been deemed the primary method for measuring body density in paediatric populations. [32] Inter-observer variability was reduced by having one trained midwife perform almost all of the measurements. However, repeated measurements were not performed and thus we were unable to assess intra-observer variability. The prospective design of the cohort, allowing us to comprehend the temporal relationship between variables and the rich collection of covariates available for adjustment further strengthens the study. Another strength of this study is the use of a directed acyclic graph (DAG) which is based on an understanding of the causal network linking the variables in the analysis. As such, the DAG allows for the appropriate adjustment for a set of putative confounders in order to obtain a less biased estimate of the effect of changing levels of exercise on neonatal adiposity. We are, however, cautious not to refer to any effect as 'causal' as we cannot exclude the possibility of the presence of both residual confounding and, in particular with this subjective measurement of exercise, measurement error.

Arguably the greatest limitation is the subjective nature of the exercise variables, which were based on maternal report. Whilst the recall period was relatively short (5-15 weeks), and reduced the potential effect of any recall bias, an objectively measured assessment of physical activity (e.g. an accelerometer), which is not subject to any recall bias, would have more optimally identified whether changes in exercise had occurred. Nonetheless, in large-scale cohort studies a compromise is often sought, with participant burden and cost-effectiveness on the one side and a more precisely measured variable on the other. Furthermore, it has been reported that pregnant women may wear monitors placed at the hip incorrectly due to changes in their girth. [33 34] Accordingly, a recent systematic review found that in epidemiological studies amongst pregnant women, self-reported physical activity measures were the most common assessment method.[35] Research on agreement between subjective estimates of physical activity and objectives measures has generated mixed results, [36 37] with the same systematic review concluding that the agreement between questionnaires and objective measures of physical activity assessment, ranged from 'poor to substantial'.[35]

A related limitation is that, as recruitment commenced during pregnancy, pre-pregnancy exercise data was not available and as a result we were unable to determine what pre-pregnancy exercise level women had changed from. It could be speculated that women who reported no change in activity level at 15 weeks did not do any exercise to start with. We have shown that those women whose activity remained unchanged at 15 weeks (compared to those who decreased) were more likely to smoke during the first trimester, be of lower socioeconomic status and more likely to have a lower household income, all of which are associated with reduced levels of exercise and fetal growth. Whilst we adjusted for these confounding factors, the lack of baseline activity limits the interpretability of our findings. For example, it would have been interesting to determine whether the effect of a decreasing exercise level (vs. unchanged level) was the same across differing categories of baseline activity.

A final limitation is the potential lack of generalisability of our results to other groups. For example, study recruitment was limited to primiparous women with singleton pregnancies and notably, a majority of White European gravidas (approximately 98.25%) were recruited into the study. This predominance of White European gravidas does, however, reflect the demographic profile of females aged 15 to 44 in Ireland as a whole (95%).[38] Unfortunately, a number of infants (513/1771) were unable to have a body composition assessment. Possible reasons for this include a lag period between the start of the study and the arrival of the PEAPOD, and admittance of the infant to the neonatal intensive care unit (NICU). Whilst this this will have reduced the statistical power of the study, we have shown that although these infants differed slightly in terms of birthweight (median difference: 130g; 95% CI: 80-190g) and gestational age (median difference: 0.29 weeks; 95% CI: 0.15 - 0.43 weeks), there were no differences in the maternal characteristics of those with and without a PEAPOD measurement (supplementary table 1), and thus we are confident we have not introduced a substantial selection bias into the analysis. The employment of a complete-case analysis could also have introduced a degree of selection bias into the analysis, however, supplementary table 2 shows that, apart from birthweight, there no differences in the offspring or maternal characteristics of those with complete vs. incomplete data. A complete case analysis would, however, reduce the statistical power of the analysis.

To the authors' knowledge, this is the first study looking at the effect of changing exercise levels in pregnancy on neonatal adiposity using air displacement plethysmography. Previous studies have either used different measurement techniques (sum of skinfolds [6 7] or dual-energy x-ray absorptiometry (DXA)[4]) or were not looking at changing levels of exercise. [9] A recent large observational study observed that the lowest quartile of late-pregnancy energy expenditure was associated with a substantially higher neonatal fat mass (290.5g vs 249.4g, p=0.03) within the first

72-hours, which was not mirrored in neonatal fat-free mass [9]. Unlike our study, however, no differences were observed in either mid- or early pregnancy. However, the study of Harrod et al [9] was not investigating intra-pregnancy change and also relied on a statistically driven method to identify potential confounders, ignoring the causal framework underpinning any possible associations. We observed a possible sensitive period for the effect of changing exercise levels, with no effect observed with exercise recall for the first 15 weeks of gestation, but an effect of a decreasing level of exercise between 15 and 20 weeks. This provides support for the findings of Clapp et al,[6] who found that women who performed a high volume of moderate exercise in early pregnancy and then cut back in late pregnancy delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume. [6] Indeed in our study we observed a markedly increased risk of delivering an infant with neonatal adiposity above the 90th centile in pregnant women who reported having increased their exercise levels up to 15 weeks, but then reported a decrease between 15 and 20 weeks, relative to those who reported no change at both time points (OR: 5.87; 95% CI: 1.74-19.80, data not shown), though the uncertainty of this estimate can be observed in the wide confidence interval, reflecting the small number of women on which this finding was based.

Further studies utilising objectively measured estimates of physical activity in a range of different population groups are required in order to replicate this finding. For example, the cohort of women in this analysis exhibited relatively low levels of activity, with almost 75% of women never doing any vigorous activity at 15 weeks and only approximately 50% of the women doing moderate activity more than once a week. If results appear consistent and robust to these differences in methodology and population, then these findings have significant implications, which extend beyond the short-term. However, the lack of follow-up studies with body composition assessment at birth limits our ability to explicitly link increased adiposity and later risk. Nonetheless, if the effects of a reduced level of exercise are able to manifest in the offspring as an altered adiposity at birth, the wider implication is that, during this critical period of developmental plasticity, some sort of programing has occurred, potentially permanently altering the offspring's metabolic and endocrinological phenotype (13-15),[13-15] and altering its long-term susceptibility to a variety of non-communicable diseases (NCDs). It is hoped that with the increasing incorporation of body composition assessment methods in infancy, particularly air-displacement plethysmography, these questions will be able to be investigated.

Conclusion:

A decreasing level of maternal reported exercise between 15 and 20 weeks' gestation was associated with an increased risk of delivering an infant with a high adiposity. This effect was maintained after appropriate adjustment for confounding variables as identified using knowledge of the causal network. However, these findings need interpreting in line with the limitations of the study. Accordingly, further research utilising objective measures of physical activity and in different populations needs to be conducted in order to validate results.

Acknowledgements:

We thank the pregnant women who participated in the SCOPE study and the mothers who allowed their newborn infants to participate in the BASELINE study.

Funding:

SCOPE Ireland was funded by the Health Research Board, Ireland (CSA 2007/2). The BASELINE cohort was supported by the National Children's Research Centre, Dublin, Ireland, and the Food Standards Agency of the United Kingdom (grant no. TO7060). SCOPE and BASELINE are supported by INFANT, an SFI funded Research Centre (grant no 12/RC/2272). The funders had no involvement in the study design, data collection, analysis and interpretation, as well as in the writing of the manuscript.

Competing interests:

None declared.

Contribution to authorship:

LCK is guarantor. TN designed the study, analysed and interpreted the data, alongside LCK and PNB. TN, AK, FMC, DMM, MK, J O'B H, LCK and PNB took part in drafting the article or revising it for critically important intellectual content and all gave final approval of the version to be published.

Ethics approval:

Ethical approval was obtained from the local ethics committees (Cork ECM5(10)05/02/08; approved 5 February 2008) and all women provided written informed consent.

Data sharing statement: There are no additional data available.

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Supplementary table 1: Descriptive results for those vs those without PEAPOD measurements

	With PEAPOD (n=1258)	Without PEAPOD (n=513)	Difference (Cork_with – Cork_without) (95% CI)
Sex of infant (% (n) female)	48.57 (611)	50.68 (260)	-2.11 (-7.25; 3.02)*
Birth weight (g) (median;IQR)	3500 (3180;3800)	3380 (3020;3730)	130 (80;190)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41)	40 (38.71;40.86)	0.29 (0.15;0.43)**
Maternal age (years) (mean:SD)	29.95 (4.44)	29.88 (4.61)	0.07 (-0.39;0.53)**
Maternal BMI (kg/m^2) (median:IQR)	23.9 (22;26.9)	24 (22;26.9)	-0.1 (-0.30;0.50)**
Maternal Socioeconomic status (median;IQR)	45 (29;51)	45 (29;50)	0 (-1;0)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13;14)	0 (0;0)**
Household income (<€21 000) (yes)(% (n))	8.47 (106)	8.22 (42)	0.25 (-2.58;3.10)*
Maternal unemployment (yes) (% (n))	5.56 (70)	3.51 (18)	2.06 (0.02;4.09)*
1st trimester smoking (yes) (% (n))	9.30 (117)	11.70 (60)	-2.40 (-5.60;0.82)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	2.8 (0.62;5)	0 (-0.5;0)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)

Supplementary table 2: Descriptive results for those with vs those without complete data

	Complete data group (n=1200)	Missing data group (n=58)	Difference (Complete – incomplete) (95% CI)
Sex of infant (% (n) female)	49.00 (588)	39.66 (23)	9.34 -(3.56;22.25)*
Birth weight (g) (mean;SD)	3510 (465)	3322 (518)	188 (64;311)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41.00)	40.57 (39.29;42.00)	-0.43 (-0.86;0.15)**
Maternal age (years) (mean:SD)	29.98 (4.44)	29.13 (4.54)	0.85 (-0.33;2.02)**
Maternal BMI (kg/m^2) (median;IQR)	23.9 (22.00;26.8)	24.7 (22.00;29.00)	-0.70 (-1.90;0.40)**
Maternal Socioeconomic status (median;IQR)	45 (29;51.00)	44.5 (29;50.00)	0 (-3.00;4.00)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13:14)	0 (0;0)**
Household income <€21 000 (yes) (% (n))	8.30 (99)	12.07 (7)	-3.77 (-12.30; 4.76)*
Maternal unemployment (yes) (% (n))	5.50 (66)	6.90 (4)	-1.40 (-8.04;5.25)*
1st trimester smoking (yes) (% (n))	9.08 (109)	13.79 (8)	-4.71 (-13.73;4.31)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	3 (0.6;10)	-0.10 (-1.5;0.67)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	

(*e*) Describe any sensitivity analyses

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,11
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,12-14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Do changing levels of maternal exercise during pregnancy affect neonatal adiposity? Secondary analysis of the Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) birth cohort (Cork, Ireland)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017987.R1
Article Type:	Research
Date Submitted by the Author:	27-Jul-2017
Complete List of Authors:	Norris, Tom; University of Leicester College of Medicine Biological Sciences and Psychology, Health Sciences McCarthy, Fergus; The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Department of Obstetrics and Gynecology Khashan, Ali; University College Cork, Department of Epidemiology and Public Health Murray, Deidre; University College Cork, Paediatrics and Child Health Kiely, Mairead; University College Cork, Food and Nutritional Sciences Hourihane, Jonathan; University College, Cork, Ireland, Paediatrics and Child Health Baker, Philip ; University of Leicester, College of Medicine Kenny, Louise; University College Cork, Obstetrics and Gynaecology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	adiposity, exercise, pregnancy, PEAPOD

Do changing levels of maternal exercise during pregnancy affect neonatal adiposity? Secondary analysis of the Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) birth cohort (Cork, Ireland).

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Abstract:

Objective: To investigate whether changing levels of exercise during pregnancy are related to altered neonatal adiposity. **Design**: Secondary analysis of data from a prospective cohort study. **Setting**: Cork, Ireland. **Participants**: 1200 mother-infant pairs recruited as part of a prospective birth cohort, Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE). **Main outcome measures**: Neonatal adiposity was assessed within several days of birth using air displacement plethysmography (PEAPOD). Percent body fat (BF%) as a continuous outcome and a pair of dichotomous variables; high or low adiposity, representing BF% >90th or <10th centile, respectively. Multivariable linear and logistic regression models were used to investigate the relationship between exercise and the respective outcomes. **Results**: Crude analysis revealed no association between a changing level of exercise (since becoming pregnant) at 15 weeks' gestation and any of the outcomes (%BF, low adiposity, high adiposity). At 20 weeks' gestation, analyses revealed that relative to women who do not change their exercise level up to 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46). This association was maintained after adjustment for putative confounders (OR: 1.62; 95% CI: 1.06; 2.47). **Conclusions**: We observed a possible critical period for the association between changing exercise levels and neonatal adiposity, with no association observed with exercise recall for the first 15 weeks of gestation, but an association with a decreasing level of exercise between 15 and 20 weeks. These results should be interpreted in line with the limitations of the study and further studies utilising objectively measured estimates of exercise are required in order to replicate these findings.

Article Summary:

Strength and limitations of this study

- Air displacement plethysmography (PEAPOD) was used to measure neonatal body composition
- Directed acyclic graphs (DAGs), based on an understanding of the causal network linking the variables in the analysis, were used to identify putative confounding variables
- Exercise variables were based on maternal self-report and therefore subject to error
- Pre-pregnancy exercise data were not available, meaning we were unable to ascertain what pre-pregnancy exercise level women had changed from

Introduction:

In their 2006 guideline, the Royal College of Obstetricians and Gynaecologists (RCOG) concluded that pregnant women should be 'encouraged to initiate or continue exercise to derive the health benefits associated with such activities'. [1]

The benefits of physical activity during pregnancy are likely to operate through an increased blood flow and oxygenation to the fetus. [2 3] It has also been proposed that the impact of exercise on fetal growth is mediated by its effect on maternal insulin sensitivity, glucose metabolism and gestational weight gain.[4 5] Another mechanism by which exercise could exert its effect is via the functioning of the uteroplacental unit, for example by affecting placental function, volume and growth rates. [6-8] However, the apparent beneficial effects of exercise appear to be dependent upon the timing of when exercise is undertaken. For example, Clapp et al (2002) demonstrated that women who performed a high quantity of moderate exercise in early pregnancy and then cut back in late pregnancy (hi-lo) delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume (lo-hi). The hi-lo exercise regimen was also associated with a greater placental volume at delivery, relative to the other two groups, [7] presumably as a result of faster placental growth in early gestation. Those who either maintained moderate exercise or increased to a high volume of exercise in late gestation (relative to the hi-lo group) did not exhibit this increased placental volume at birth, suggesting that early gestation is a critical period for any exercise effects on placental development to be enacted, with a potentially suppressive effect in late gestation. [2] Furthermore, it has been reported that the transient changes in glucose regulation observed after bouts of exercise differ depending on when in pregnancy the exercise load is occurring, with increases in blood glucose observed after exercise early in pregnancy, but decreases in later pregnancy. [9] These fluctuations in nutrient supply, depending on the timing of exercise, could also contribute to differential effects on fetal growth.

The data surrounding the effects of physical activity on neonatal body composition (as opposed to size) from large scale observational studies is limited. Data from a limited number of relatively small randomised controlled trials report either a null or reducing effect of physical activity on neonatal adiposity, [7 8 10] with potentially greater effects if the exercise intervention is administered at later gestations. Findings from a recent observational study, the Healthy Start cohort (n=826), also suggested that increasing physical activity levels in later pregnancy could result in a reduction in neonatal adiposity, even after adjusting for putative confounders (e.g. maternal age, race or ethnicity, educational status, household income, pre-pregnancy body mass index (BMI), and prenatal smoking status).[11]

It is now well established that the *in utero* milieu experienced by the developing fetus could influence long-term risk for the development of obesity and obesity-related non-communicable diseases (OR-NCDs). [12-14] Maternal behaviour during this critical period of developmental plasticity has the potential to permanently alter susceptibility to later chronic disease via alterations in the offspring's metabolic and endocrinological phenotype. [15-17] Consequently, we hypothesise that maternal exercise in pregnancy will be associated with altered neonatal adiposity, such that an increasing/decreasing exercise level in pregnancy will be associated with a reduction/increase in adiposity, respectively. Any changes in neonatal adiposity could be indicative of an altered phenotypic profile in the offspring, which may increase susceptibility to later chronic disease.

The objective of the current study was to investigate whether changes in maternal exercise during pregnancy were associated with offspring adiposity in the neonatal period, measured using air displacement plethysmography in a large homogeneous population.

Methods:

Neonatal participants were recruited as part of the Cork BASELINE birth cohort study (ClinicalTrials.gov NCT: 01498965 www.birthcohorts.net) [18] between August 2008 and August 2011 from women who had participated in SCOPE (Screening for Pregnancy Endpoints) Ireland. SCOPE was a multicentre prospective cohort study with the aim of developing screening tests to predict various complications of pregnancy (e.g. pre-eclampsia, small-for-gestational-age (SGA) infants, and spontaneous preterm birth) (ACTRN12607000551493). [19] Methods are described in detail elsewhere. [19 20] In brief, participants were healthy nulliparous women with singleton pregnancies recruited antenatally between February 2007 and February 2011 in Cork, Ireland. Women were recruited, interviewed and all measurements obtained at 15±1 and 20±1 weeks' gestation. [19 21] Exclusion criteria included: a high risk for pre-eclampsia/delivery of a SGA neonate/spontaneous preterm birth because of underlying medical conditions; three or more previous miscarriages; three or more terminations of pregnancy; or having received interventions such as aspirin that might modify pregnancy outcome. At the time of interview, data were entered onto an internet-accessed central database with a complete audit trail designed and hosted by MedSciNet, Sweden. Participants were followed up prospectively, with pregnancy outcome data collected by trained research midwives.

Neonatal adiposity was assessed in the majority of neonates within 72 hours of birth by calculating neonatal body fat percentage (BF%) using the PEAPOD air displacement plethysmography. The mean time of measuring BF% in those infants born over 37 weeks' gestation was 1.8 days (standard deviation 0.97 days). Of those infants born <37 weeks' gestation, the mean time of testing was 2.4 days (standard deviation 1.2 days). Measurement of neonatal BF% involves direct measurement of body mass using precise scale and body volume in an airtight, enclosed chamber. Body composition assessment by densitometry involves the measurement of the density of the whole body. Body density is then used in a two-compartment model to calculate the percentage of fat, fat mass, and

fat-free mass.[22] The PEAPOD has excellent test-to-test reproducibility and is safe, non-invasive and fast.[23 24]

Exercise data were collected at both the 15 and 20 week visits in a standardised manner. At both time points, women were asked how many times per week they engaged in vigorous activity (which made the woman breathe harder or puff or pant),[25] moderate activity (did not breathe harder or puff or pant) or walking for recreation or exercise. At 15 weeks, women were asked: *'Has your level of exercise (physical activity) changed since you've been pregnant?*', to which they could respond *'decreased'*, *'unchanged'* or *'increased'*. At 20 weeks, women were then asked: *'Has your level of exercise changed since last SCOPE visit?'*, with the same possible response options.

Statistical analysis:

Differences in maternal characteristics and birth outcomes, stratified by change in exercise level, were explored using one way analysis of variance for continuous variables (with scheffe test for post-hoc pairwise comparisons) and chi^2 test for categorical variables (table 1). Descriptive statistics (frequencies and percentages) of the different levels of exercise were summarised and are shown in table 2. We generated a 'no exercise' binary variable with a value of 1 indicating women who reported doing no vigorous nor moderate nor recreational walking activity per week.

We used linear regression models to investigate the effect of changing levels of self-reported maternal exercise during pregnancy on birthweight (g) and %BF measured as continuous variables. Change in exercise levels was coded as a categorical variable: no change (reference group) versus decreased versus increased. Regression diagnostics did not reveal any violations to linear regression assumptions (i.e. normally distributed residuals and homogeneity of variance). We subsequently generated separate binary variables (0= no; 1=yes) indicating the presence of either low or high adiposity. Low and high adiposity was defined as below/above the gestational age- and sex-specific 10^{th}/90^{th} adiposity centiles respectively, according to the centiles produced by Hawkes et al (2011).[26]

The effect of changes in physical activity on these dichotomous variables was investigated using logistic regression models.

We performed sensitivity analyses limiting the sample to only those born at term (n=1180) and separately, to those born non-low birthweight (>2500g) (n=1180) but effect estimates did not markedly change and thus these infants were retained in the analysis. Furthermore, as the analysis sample was based on those that had complete data for the exposure, outcome and covariates, we also investigated whether we had introduced a selection bias by only including those with complete data (supplementary tables 1 and 2).

In order to identify less biased associations between our exposures and outcome, we produced a directed acyclic graph (DAG) using Daggity. [27] DAGs provide a method for formalising and clarifying the causal hypothesised assumptions a researcher may make regarding the variables they wish to analyse [28] and thus justify modelling choices. [29 30] These graphs are especially useful for identifying variables which potentially confound the relationship between two variables, thus providing researchers with sets of variables for which adjustment (and importantly non-adjustment) is necessary, in order to obtain unbiased estimates of the relationship between a set of variables. For a more detailed discussion on the use of these graphs in epidemiology, see Greenland, Pearl and Robins. [31] Daggity is a web-based interface which allows researchers to construct and edit a directed acyclic graph, with the ultimate aim of identifying sufficient sets of variables for adjustment which will minimise bias when estimating the effect of an exposure on the outcome. The set of variables identified by Daggity as necessary for adjustment were socioeconomic status, maternal employment, smoking status, alcohol intake, BMI, level of education, maternal age and whether the mother's job was physically active (see supplementary figure 1 for analysis DAG). These variables were then incorporated into multivariable regression models. All analyses were conducted in Stata/IC v14.1.

Results:

Descriptive statistics of the sample (and those omitted)

Compared to all of those enrolled without a PEADOD measurement (n=513) cohort, those enrolled in Cork with a PEAPOD measurement taken (n=1258) were approximately 130g (95% CI: 80-190) heavier and born approximately 2 days later (95% CI: 1.05-3.01), but with no differences in any maternal biological or demographic data (Supplementary table 1). Although 1258 had PEAPOD measurements taken, 58 infants were not included in the final analysis due to: all PEAPOD data being lost/mis-entered (n=16), being born too early or late for adiposity centiles to be generated (n=23) and having incomplete exposure and covariate data (n=19), leaving a final analysis sample of 1200. Compared to those with PEAPOD measurements but not in the final analysis sample, those who were in the final analysis had higher birthweight (187.81; 95% CI: 64.45-311.17), but with no differences in gestational age or any maternal biological or demographic data (Supplementary table 2).

Of the 1200 neonates with complete exposure, outcome and covariate data, 612 (51.00%) were male and 98.25% (n=1 179) were of White European ethnic origin. The mean birthweight was 3510g (95% CI: 3484 - 3537) and the median gestational age was 40 weeks (interquartile range: 39 - 41).

Change in exercise level reported in the first 15 weeks of pregnancy

Table 1 provides descriptive statistics for various maternal characteristics and neonatal outcomes, stratified by type of change of exercise in pregnancy. Compared to women who reported no change in exercise level, those who decreased their level of exercise were older (30.51 years (4.17) vs. 28.89 years (4.74)), with a higher level socioeconomic status (44.33 (16.15) vs. 39.10 (15.40)), less likely to have a household income below €21 000 (5.80% vs. 13.16%) and less likely to have smoked during the first trimester (6.03% vs. 15.90%). The small proportion of women who reported increasing their exercise levels from the time they became pregnant to 15 weeks gestation (<4%) did not differ substantially from the cohort, with the exception of having a higher likelihood of a lower household income (Table 1).

It is shown in Table 2 that at 15 weeks' gestation, more than a quarter (n=327, 27.25%) of women reported engaging in vigorous exercise at least once per week, with approximately three quarters reporting doing some form of moderate exercise per week (n=892, 74.33%). 104 (8.67%) women reported not engaging in any form of exercise per week.

The effect of changing exercise levels on birthweight and neonatal adiposity is shown in Table 3. Relative to women who did not change their exercise level in pregnancy up to 15 weeks, there was no difference in any of the outcomes in those women who either increased or decreased their level of exercise, in both crude and adjusted analyses. Changing the reference group in order to compare those who decreased relative to those who increased also revealed no differences in neonatal outcomes.

Table 1: Descriptive statistics in those with changing levels of physical activity during pregnancy

	Change in exercise level in pregnancy to 15 weeks (n=1200)		
	Decreased (n=813 (67.8%))	Unchanged (n=346(28.8%))	Increased (n=41 (3.4%))
Maternal characteristics:			
Maternal age (mean;SD)	30.51 (4.17)†	28.89 (4.74)	28.88 (5.19)
Maternal BMI at 15 weeks (mean;SD)	25.02 (4.12)	24.49 (4.21)	24.18 (3.85)
Maternal years schooling (mean;SD)	13.27 (0.83)	13.18 (0.81)	13.15 (0.73)
Maternal socioeconomic status (mean;SD)	44.33 (16.15)†	39.10 (15.40)	43.51 (16.35)
Maternal household income <€21 000 (n:%)	47 (5.80)†‡	45 (13.16)	7 (17.07)
Maternal smoking in 1st trimester (n;%)	49 (6.03)†	55 (15.90)	5 (12.20)
Maternal alcohol intake in 1st trimester (units/week)	4.61 (5.76)	5.39 (6.97)	5.99 (8.10)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.16)	40 (1.24)	40 (1.00)
Birth weight (g) (mean;SD)	3525 (460)	3471 (478)	3541 (449)
Neonatal adiposity (%)(mean;SD)	11.06 (4.15)	11.03 (4.06)	11.22 (4.13)
Adiposity<10th centile (yes) (n;%)	68 (8.36)	26 (7.51)	3 (7.32)
Adiposity>90th centile (yes) (n;%)	86 (10.58)	39 (11.27)	7 (17.07)
	Change in exercise level in pregnancy: 15 to 20 weeks (n=1200)		
	Decreased (n=263 (21.9%))	Unchanged (n=665 (55.4%))	Increased (n=272 (22.7%))
Maternal characteristics:			
Maternal age (mean;SD)	30.74 (4.13)†	29.52 (4.58)‡	30.39 (4.25)
Maternal BMI at 15 weeks (mean;SD)	25.07 (4.06)	24.60 (4.01)	25.20 (4.52)
Maternal years schooling	13.28 (0.72)	13.23 (0.86)	13.22 (0.82)
Maternal socioeconomic status	44.33 (15.49)†	40.96 (16.08)‡	45.79 (16.21)
Maternal household income <€21 000 (n:%)	15 (5.70)†	66 (10.03)	18 (6.62)
Maternal smoking in 1st trimester (n;%)	23 (8.75)	73 (10.98)‡	13 (4.78)
Maternal alcohol intake in 1st trimester (units/week)	4.91 (5.79)	5.24 (6.82)‡	3.98 (5.01)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.20)	40 (1.19)	40 (1.14)
Birth weight (g) (mean;SD)	3541 (498)	3487 (458)	3537 (448)
Neonatal adiposity (%)(mean;SD)	11.44 (4.66)	10.90 (4.02)	11.08 (3.79)
Adiposity<10th centile (yes) (n;%)	22 (8.37)	58 (8.87)	16 (5.88)
Adiposity>90th centile (yes) (n;%)	41 (15.59)	68 (10.23)	23 (8.46)

†different to 'unchanged' ‡different to 'increased'

Table 2: Descriptive statistics of self-reported activity levels at 15 and 20 weeks

	Exercise level at 15 weeks (n=1200)	Exercise level at 20 weeks (n=1200)
Vigorous at least once per week (yes) (n; % of 1200)	327 (27.25)	377 (31.42)
Moderate at least once per week (yes) (n; % of 1200)	892 (74.33)	908 (75.67)
Recreational at least once per week (yes) (n; % of 1200)	1040 (86.67)	1057 (88.08)
No exercise per week (n; % of 1200)	104 (8.67)	100 (8.33)

	Change in exercise level between 15-20 weeks		
	Decreased (n=263)	Unchanged (n=665)	Increased (n=272)
Any exercise per week at 15 weeks			
No (n; % of column total)	7 (2.66)	72 (10.83)	25 (9.19)
Yes (n; % of column total)	256 (97.34)	593 (89.17)	247 (90.81)

Change in exercise level between 15 and 20 weeks

Compared to women who reported no change in exercise level between 15 and 20 weeks, those who decreased their level of exercise were older (30.74 years (4.13) vs. 29.52 years (4.58)), with a higher level socioeconomic status (44.33 (15.49) vs. 40.96 (16.08)) and less likely to have a household income below €21 000 (n=15 (5.70%) vs. n=66 (10.03%)). Women who increased their exercise levels between 15 and 20 weeks, relative to those who reported no change, were also older and with a higher SES, with a reduced alcohol intake (3.98 (5.01) units/week vs. 5.24 (6.82) units/week) and lower likelihood of smoking during the 1st trimester (n=13 (4.78%) vs. n=73 (10.98%) (Table 1).

At 20 weeks, similar levels of exercise were reported, with approximately 30% of women reporting doing vigorous exercise at least once per week, and three quarters of the sample engaging in some form of moderate exercise. Just over 8% of women reported taking part in no form of exercise at 20 weeks (Table 2). Table 2 also reveals that of the 665 women who reported no change in their exercise levels between 15-20 weeks, approximately 10% of these (n=72) had engaged in no exercise at 15 weeks. Similarly, of those who increased their exercise levels between 15-20 weeks, just under 10% (n=25) had reported no exercise at 15 weeks (Table 2).

Crude analysis shows that relative to women who do not change their exercise level between 15 and 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46) (Table 3). This association was maintained after adjustment for the putative confounders (OR: 1.66; 95% CI: 1.09; 2.54). When changing the reference group in order to compare women who decreased exercise levels relative to those who increased exercise, it was observed that those who decreased were twice as likely to give birth to a neonate with an adiposity above the 90th centile (OR: 2.00; 95% CI: 1.16 - 3.44), which again was also maintained on adjustment (OR: 2.09; 95% CI: 1.20 - 3.61). Birthweight was not associated with differences in exercise (Table 3).

Table 3: Effect of changing exercise levels during pregnancy on neonatal adiposity

	Change in exercise level in pregnancy to 15 weeks (coefficient; 95%CI)					
	Crude			***Multivariable****		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.39 (-4.16; 112.93)	*(reference)*	70.24 (-80.40; 220.89)	22.03 (-37.61; 81.67)	*(reference)*	74.40 (-75.84;224.64)
Neonatal adiposity (%)*	0.03 (-0.49; 0.55)	*(reference)*	0.19 (-1.15; 1.53)	0.01 (-0.52; 0.55)	*(reference)*	0.33 (-1.01; 1.67)
Adiposity<10th centile**	1.12 (0.70; 1.80)	*(reference)*	0.97 (0.28; 3.36)	1.20 (0.73; 1.95)	*(reference)*	0.82 (0.23; 2.94)
Adiposity>90th centile**	0.93 (0.62; 1.39)	*(reference)*	1.62 (0.67; 3.90)	0.96 (0.62; 1.41)	*(reference)*	1.75 (0.71; 4.31)
	Change in exercise level in pregnancy: 15 to 20 weeks (coefficient; 95%CI)					
	Crude			***Multivariable****		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.47 (-11.96; 120.90)	*(reference)*	50.11 (-15.53; 115.75)	42.68 (-23.62; 108.98)	*(reference)*	22.30 (-43.49; 88.09)
Neonatal adiposity (%)*	0.54 (-0.05; 1.13)	*(reference)*	0.18 (-0.40; 0.76)	0.56 (-0.03; 1.15)	*(reference)*	0.13 (-0.46; 0.72)
Adiposity<10th centile**	0.94 (0.56; 1.56)	*(reference)*	0.64 (0.36; 1.14)	0.94 (0.56; 1.59)	*(reference)*	0.69 (0.39; 1.24)
Adiposity>90th centile**	1.62 (1.07; 2.46)	*(reference)*	0.81 (0.49; 1.33)	1.66 (1.09; 2.54)	*(reference)*	0.80 (0.48; 1.32)

*Linear regression for continuous outcomes (β); **logistic regression for dichotomous outcomes (OR) ***adjusted for: socioeconomic status, years of schooling, employment status, maternal BMI, smoking in 1st trimester, alcohol intake in 1st trimester, maternal age, exercise as part of job

Discussion

In this cohort of white European mother-offspring pairs, we report the effect of changing levels of exercise during pregnancy and neonatal adiposity measured using air displacement plethysmography (PEAPOD). We observed that pregnant women who reported a decrease in exercise levels between 15 and 20 weeks, had a 60% higher risk of having a baby with adiposity above the 90th centile when compared with women who reported no change. This risk was approximately double when women who reported a decrease in exercise levels between 15 and 20 weeks were compared to women who reported an increase in exercise levels. This association was maintained after adjustment for a set of putative confounders including maternal education, employment status, smoking, alcohol intake, BMI socioeconomic status, maternal age and whether her occupation was physically active. The exercise effect was only apparent between 15 and 20 weeks and not for changing exercise levels prior to 15 weeks, raising the possibility that there is a potential critical period with regard to the effect of a change on exercise level on the development of offspring adiposity.

A major strength of the study is the use of air-displacement plethysmography for the estimates of body composition. This method is a quick, safe and non-invasive technique, which has shown to be a reliable and accurate instrument for determining body fat percentage in infants. [23 32 33] As such, it has been deemed the primary method for measuring body density in paediatric populations. [34] Inter-observer variability was reduced by having a small, highly trained team of midwives and researchers who conducted all of the assessments to strict protocols. However, repeated measurements were not performed and thus we were unable to assess intra-observer variability. The prospective design of the cohort, allowing us to comprehend the temporal relationship between variables and the rich collection of covariates available for adjustment further strengthens the study. Another strength of this study is the use of a directed acyclic graph (DAG) which is based on an understanding of the causal network linking the variables in the analysis. As such, the DAG allows for the appropriate adjustment for a set of putative confounders in order to obtain a less biased estimate of the effect of changing levels of exercise on neonatal adiposity. We are, however, cautious not to refer to any effect as 'causal' as we cannot exclude the possibility of the presence of both residual confounding and, in particular with this subjective measurement of exercise, measurement error.

Arguably the greatest limitation is the subjective nature of the exercise data. Whilst the questionnaire regarding physical exercise was not validated for any population, the definition of vigorous exercise (daily exercise leading to heavy breathing or being out of breath) has previously been used in other studies.[25] As the exercise variables were based on maternal report, this introduced a potential error due to women not accurately remembering their exercise levels (e.g. due to social desirability of reporting higher levels or age). The recall period was relatively short, considering only the very recent past, and focussed on habitual activity, thus reducing the extent of the error introduced. An objectively measured assessment of physical activity (e.g. an accelerometer), would have been of benefit to estimate actual activity. Nonetheless, in large-scale cohort studies a compromise is often sought, with participant burden and cost-effectiveness on the one side and a more precisely measured variable on the other. Furthermore, it has been reported that pregnant women may wear monitors placed at the hip incorrectly due to changes in their girth. [35 36] Accordingly, a recent systematic review found that in epidemiological studies amongst pregnant women, self-reported physical activity measures were the most common assessment method.[37] Research on agreement between subjective estimates of physical activity and objectives measures has generated mixed results, [38 39] with the same systematic review concluding that the agreement between questionnaires and objective measures of physical activity assessment, ranged from 'poor to substantial'.[37]

A related limitation is that, as recruitment commenced during pregnancy, pre-pregnancy exercise data was not available and as a result we were unable to determine what pre-pregnancy exercise level women had changed from. It could be speculated that women who reported no change in activity level at 15 weeks did not do any exercise to start with. We have shown that those women whose activity remained unchanged at 15 weeks (compared to those who decreased) were more likely to smoke during the first trimester, be of lower socioeconomic status and more likely to have a lower household income, all of which are associated with reduced levels of exercise and fetal growth. Whilst we adjusted for these confounding factors, the lack of baseline activity limits the interpretability of our findings. For example, it would have been interesting to determine whether the effect of a decreasing exercise level (vs. unchanged level) was the same across differing categories of baseline activity.

We were unable to adjust our estimates for the likely mediating role of gestational hyperglycaemia as these data were not available. Similarly, we did not adjust our estimates for the effect of gestational weight gain. In line with the published literature[4 5 40 41], these variables are likely to operate along the causal pathway between maternal exercise and neonatal adiposity. While adjusting for them may mask part of the association between exercise and adiposity, it would have been of benefit to conduct *a priori* analysis to examine whether a change exercise was associated with neonatal adiposity independently of pre-pregnancy obesity, gestational weight gain or impaired glycaemic control. Acknowledging these data gaps, the current paper did not aim to elucidate possible mechanisms by which the association between exercise and adiposity is enacted, rather, we aimed to identify whether an association existed at all.

A final limitation is the potential lack of generalisability of our results to other groups. For example, study recruitment was limited to primiparous women with singleton pregnancies and notably, a majority of White European gravidas (approximately 98.25%) were recruited into the study. This predominance of White European gravidas does, however, reflect the demographic profile of females aged 15 to 44 in Ireland as a whole (95%).[42] Unfortunately, a number of infants (513/1771) were unable to have a body composition assessment. Possible reasons for this include a lag period between the start of the study and the arrival of the PEAPOD, and admittance of the infant to the neonatal intensive care unit (NICU). We have shown that although these infants differed slightly in terms of birthweight (median difference: 130g; 95% CI: 80-190g) and gestational age (median difference: 0.29 weeks; 95% CI: 0.15 - 0.43 weeks), there were no differences in the maternal characteristics of those with and without a PEAPOD measurement (supplementary table 1), and thus we are confident we have not introduced a substantial selection bias into the analysis. The employment of a complete-case analysis could also have introduced a degree of selection bias into the analysis, however, supplementary table 2 shows that, apart from birthweight, there no differences in the offspring or maternal characteristics of those with complete vs. incomplete data.

To the authors' knowledge, this is the first study looking at the effect of changing exercise levels in pregnancy on neonatal adiposity using air displacement plethysmography. Previous studies have either used different measurement techniques (sum of skinfolds [7,8] or dual-energy x-ray absorptiometry (DXA)[10]) or were not looking at changing levels of exercise. [11] A recent large observational study observed that the lowest quartile of late-pregnancy energy expenditure was associated with a substantially higher neonatal fat mass (290.5g vs 249.4g, p=0.03) within the first 72-hours, which was not mirrored in neonatal fat-free mass [11]. Unlike our study, however, no differences were observed in either mid- or early pregnancy. However, the aforementioned study was not investigating intra-pregnancy change and also relied on a statistically driven method to identify potential confounders, ignoring the causal framework underpinning any possible associations.

We observed a possible critical period for the effect of changing exercise levels, with no effect observed with exercise recall for the first 15 weeks of gestation, but an effect of a decreasing level of exercise between 15 and 20 weeks. This provides support for the findings of Clapp et al,[7] who found that women who performed a high volume of moderate exercise in early pregnancy and then cut back in late pregnancy delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume. [7] Indeed in our study we observed a markedly increased risk of delivering an infant with neonatal adiposity above the 90th centile in pregnant women who reported having increased their exercise levels up to 15 weeks, but then reported a decrease between 15 and 20 weeks, relative to those who reported no change at both time points (OR: 5.87; 95% CI: 1.74-19.80, data not shown), though the uncertainty of this estimate can be observed in the wide confidence interval, reflecting the small number of women on which this finding was based.

The data presented here suggest that a reduction in exercise levels may lead to less favourable outcomes in terms of neonatal adiposity. As such, and given the evidence of maintaining pre-pregnancy exercise levels[43 44], we advocate the continuation of pre- and early pregnancy exercise levels into later pregnancy. Further studies utilising objectively measured estimates of physical activity in a range of different population groups are required in order to replicate this finding. For example, the cohort of women in this analysis exhibited relatively low levels of activity, with almost 75% of women never doing any vigorous activity at 15 weeks and only approximately 50% of the women doing moderate activity more than once a week. If results appear consistent and robust to these differences in methodology and population, then these findings have significant implications, which extend beyond the short-term. For example, it has been shown that the associations between maternal pregnancy exercise levels and offspring adiposity present at birth extend into childhood, with children of women who exercised during pregnancy observed to have a reduced fat mass at age 5 years (37mm ± 1 vs. 44mm ± 4) compared children whose mothers were inactive[45]. However, the overall lack of follow-up studies with body composition assessment at birth limits our ability to explicitly link increased adiposity in early-life and later risk. Nonetheless, if the effects of a reduced level of exercise are able to manifest in the offspring as an altered adiposity at birth, the wider implication is that, during this critical period of developmental plasticity, some sort of programing has occurred, potentially permanently altering the offspring's metabolic and endocrinological phenotype (13-15),[15-17] and altering its long-term susceptibility to a variety of non-communicable diseases (NCDs). It is hoped that with the increasing incorporation of body composition assessment methods in infancy, particularly air-displacement plethysmography, these questions will be able to be investigated.

Conclusion:

A decreasing level of maternal reported exercise between 15 and 20 weeks' gestation was associated with an increased risk of delivering an infant with a high adiposity. This effect was maintained after appropriate adjustment for confounding variables as identified using knowledge of the causal network. However, these findings need interpreting in line with the limitations of the study. Accordingly, further research utilising objective measures of physical activity and in different populations needs to be conducted in order to validate results.

Acknowledgements:

We thank the pregnant women who participated in the SCOPE study and the mothers who allowed their newborn infants to participate in the BASELINE study.

Funding:

SCOPE Ireland was funded by the Health Research Board, Ireland (CSA 2007/2). The BASELINE cohort was supported by the National Children's Research Centre, Dublin, Ireland, and the Food Standards Agency of the United Kingdom (grant no. TO7060). SCOPE and BASELINE are supported by INFANT, an SFI funded Research Centre (grant no 12/RC/2272). The funders had no involvement in the study design, data collection, analysis and interpretation, as well as in the writing of the manuscript.

Competing interests:

None declared.

Contribution to authorship:

LCK is guarantor. TN designed the study, analysed and interpreted the data, alongside LCK and PNB. TN, AK, FMC, DMM, MK, J O'B H, LCK and PNB took part in drafting the article or revising it for critically important intellectual content and all gave final approval of the version to be published.

Ethics approval:

Ethical approval was obtained from the local ethics committees (Cork ECM5(10)05/02/08; approved 5 February 2008) and all women provided written informed consent.

Data sharing statement: There are no additional data available.

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Supplementary table 1: Descriptive results for those vs those without PEAPOD measurements

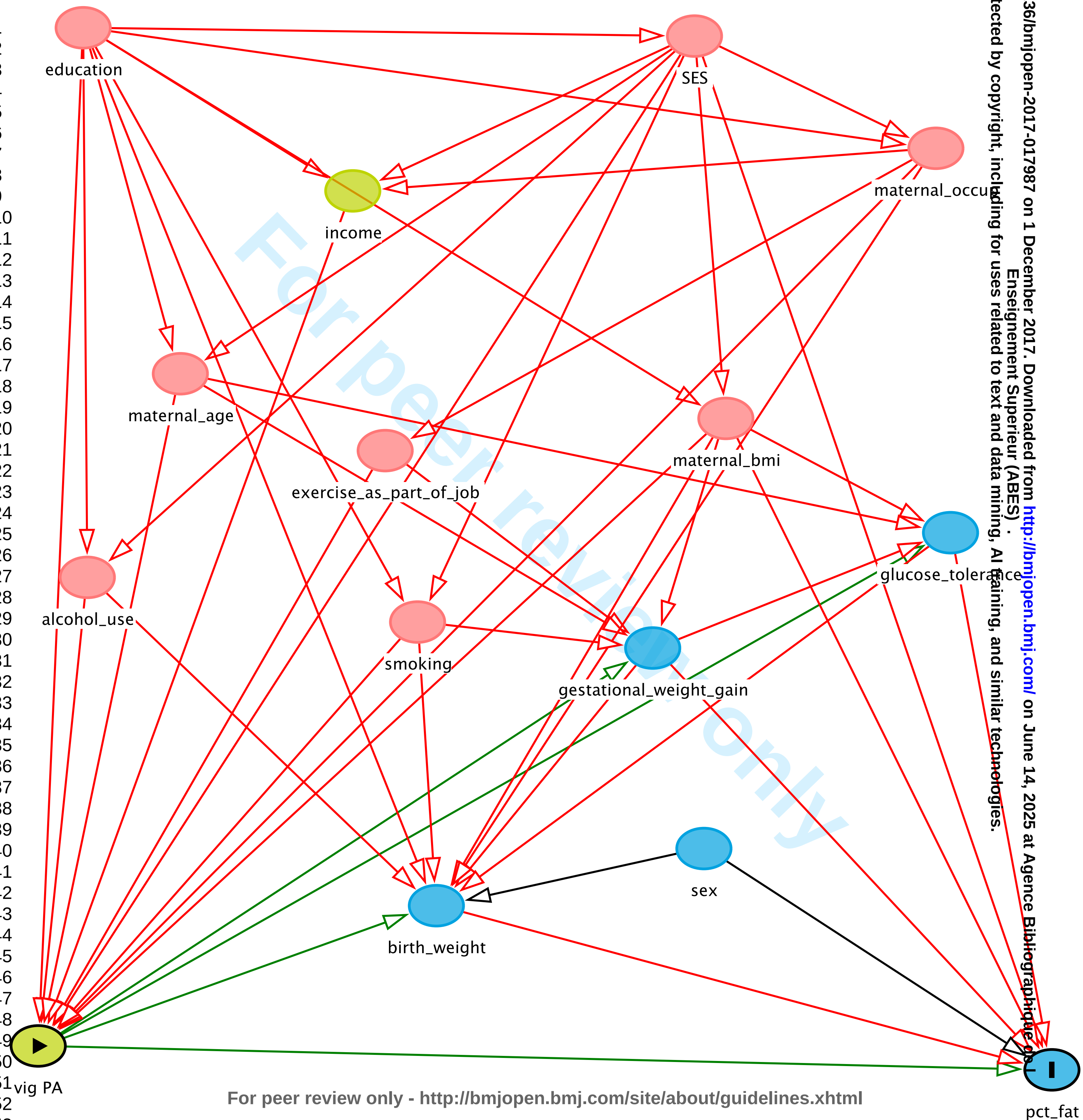
	With PEAPOD (n=1258)	Without PEAPOD (n=513)	Difference (Cork_with – Cork_without) (95% CI)
Sex of infant (% (n) female)	48.57 (611)	50.68 (260)	-2.11 (-7.25; 3.02)*
Birth weight (g) (median;IQR)	3500 (3180;3800)	3380 (3020;3730)	130 (80;190)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41)	40 (38.71;40.86)	0.29 (0.15;0.43)**
Maternal age (years) (mean:SD)	29.95 (4.44)	29.88 (4.61)	0.[illegible]7 (0.39;0.53)**
Maternal BMI (kg/m^2) (median:IQR)	23.9 (22;26.9)	24 (22;26.9)	-0.1 (-0.30;0.50)**
Maternal Socioeconomic status (median;IQR)	45 (29;51)	45 (29;50)	0 (-1;0)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13;14)	0 (0;0)**
Household income (<€21 000) (yes)(% (n))	8.47 (106)	8.22 (42)	0.25 (-2.58;3.10)*
Maternal unemployment (yes) (% (n))	5.56 (70)	3.51 (18)	2.06 (0.02;4.09)*
1st trimester smoking (yes) (% (n))	9.30 (117)	11.70 (60)	-2.40 (-5.60;0.82)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	2.8 (0.62;5)	0 (-0.5;0)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)

Supplementary table 2: Descriptive results for those with vs those without complete data

	Complete data group (n=1200)	Missing data group (n=58)	Difference (Complete – incomplete) (95% CI)
Sex of infant (% (n) female)	49.00 (588)	39.66 (23)	9.34 (-3.56;22.25)*
Birth weight (g) (mean;SD)	3510 (465)	3322 (518)	188 (64;311)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41.00)	40.57 (39.29;42.00)	-0.43 (-0.86;0.15)**
Maternal age (years) (mean:SD)	29.98 (4.44)	29.13 (4.54)	0.85 (-0.33;2.02)**
Maternal BMI (kg/m^2) (median;IQR)	23.9 (22.00;26.8)	24.7 (22.00;29.00)	-0.70 (-1.90;0.40)**
Maternal Socioeconomic status (median;IQR)	45 (29;51.00)	44.5 (29;50.00)	0 (-3.00;4.00)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13:14)	0 (0;0)**
Household income <€21 000 (yes) (% (n))	8.30 (99)	12.07 (7)	-3.77 (-12.30; 4.76)*
Maternal unemployment (yes) (% (n))	5.50 (66)	6.90 (4)	-1.40 (-8.04;5.25)*
1st trimester smoking (yes) (% (n))	9.08 (109)	13.79 (8)	-4.71 (-13.73;4.31)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	3 (0.6;10)	-0.10 (-1.5;0.67)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)


education
SES
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exercise_as_part_of_job
alcohol_use
smoking
gestational_weight_gain
glucose_tolerance
sex
birth_weight
vig PA
pct_fat

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	

(*e*) Describe any sensitivity analyses

Continued on next page

Results			Page
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,11
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,12-14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Do changing levels of maternal exercise during pregnancy affect neonatal adiposity? Secondary analysis of the Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) birth cohort (Cork, Ireland)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017987.R2
Article Type:	Research
Date Submitted by the Author:	04-Sep-2017
Complete List of Authors:	Norris, Tom; University of Leicester College of Medicine Biological Sciences and Psychology, Health Sciences McCarthy, Fergus; The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Department of Obstetrics and Gynecology Khashan, Ali; University College Cork, Department of Epidemiology and Public Health Murray, Deidre; University College Cork, Paediatrics and Child Health Kiely, Mairead; University College Cork, Food and Nutritional Sciences Hourihane, Jonathan; University College, Cork, Ireland, Paediatrics and Child Health Baker, Philip ; University of Leicester, College of Medicine Kenny, Louise; University College Cork, Obstetrics and Gynaecology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	adiposity, exercise, pregnancy, PEAPOD

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Do changing levels of maternal exercise during pregnancy affect neonatal adiposity? Secondary analysis of the Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) birth cohort (Cork, Ireland).

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on behalf of the SCOPE Ireland Cohort study and the Cork BASELINE Birth Cohort Study

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Abstract:

Objective: To investigate whether changing levels of exercise during pregnancy are related to altered neonatal adiposity. **Design**: Secondary analysis of data from a prospective cohort study. **Setting**: Cork, Ireland. **Participants**: 1200 mother-infant pairs recruited as part of a prospective birth cohort, Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE). **Main outcome measures**: Neonatal adiposity was assessed within several days of birth using air displacement plethysmography (PEAPOD). Percent body fat (BF%) as a continuous outcome and a pair of dichotomous variables; high or low adiposity, representing BF% >90th or <10th centile, respectively. Multivariable linear and logistic regression models were used to investigate the relationship between exercise and the respective outcomes. **Results**: Crude analysis revealed no association between a changing level of exercise (since becoming pregnant) at 15 weeks' gestation and any of the outcomes (%BF, low adiposity, high adiposity). At 20 weeks' gestation, analyses revealed that relative to women who do not change their exercise level up to 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46). This association was maintained after adjustment for putative confounders (OR: 1.62; 95% CI: 1.06; 2.47). **Conclusions**: We observed a possible critical period for the association between changing exercise levels and neonatal adiposity, with no association observed with exercise recall for the first 15 weeks of gestation, but an association with a decreasing level of exercise between 15 and 20 weeks. These results should be interpreted in line with the limitations of the study and further studies utilising objectively measured estimates of exercise are required in order to replicate these findings.

Article Summary:

Strength and limitations of this study

- Air displacement plethysmography (PEAPOD) was used to measure neonatal body composition
- Directed acyclic graphs (DAGs), based on an understanding of the causal network linking the variables in the analysis, were used to identify putative confounding variables
- Exercise variables were based on maternal self-report and therefore subject to error
- Pre-pregnancy exercise data were not available, meaning we were unable to ascertain what pre-pregnancy exercise level women had changed from

Introduction:

In their 2006 guideline, the Royal College of Obstetricians and Gynaecologists (RCOG) concluded that pregnant women should be 'encouraged to initiate or continue exercise to derive the health benefits associated with such activities'. [1]

The benefits of physical activity during pregnancy are likely to operate through an increased blood flow and oxygenation to the fetus. [2 3] It has also been proposed that the impact of exercise on fetal growth is mediated by its association with maternal insulin sensitivity, glucose metabolism and gestational weight gain.[4 5] Another mechanism by which exercise may be implicated is via the functioning of the uteroplacental unit, for example by affecting placental function, volume and growth rates. [6-8] However, the apparent beneficial associations of exercise appear to be dependent upon the timing of when exercise is undertaken. For example, Clapp et al (2002) demonstrated that women who performed a high quantity of moderate exercise in early pregnancy and then cut back in late pregnancy (hi-lo) delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume (lo-hi). The hi-lo exercise regimen was also associated with a greater placental volume at delivery, relative to the other two groups, [7] presumably as a result of faster placental growth in early gestation. Those who either maintained moderate exercise or increased to a high volume of exercise in late gestation (relative to the hi-lo group) did not exhibit this increased placental volume at birth, suggesting that early gestation is a critical period for any influence of exercise on placental development to be enacted, with a potentially suppressive association in late gestation. [2] Furthermore, it has been reported that the transient changes in glucose regulation observed after bouts of exercise differ depending on when in pregnancy the exercise load is occurring, with increases in blood glucose observed after exercise early in pregnancy, but decreases in later pregnancy. [9] These fluctuations in nutrient supply, depending on the timing of exercise, could also contribute to differential associations with fetal growth.

The data surrounding the associations between physical activity and neonatal body composition (as opposed to size) from large scale observational studies is limited. Data from a limited number of relatively small randomised controlled trials report either a null or reducing association between physical activity and neonatal adiposity, [7 8 10] with potentially stronger associations if the exercise intervention is administered at later gestations. Findings from a recent observational study, the Healthy Start cohort (n=826), also suggested that increasing physical activity levels in later pregnancy could be associated with a reduction in neonatal adiposity, even after adjusting for putative confounders (e.g. maternal age, race or ethnicity, educational status, household income, pre-pregnancy body mass index (BMI), and prenatal smoking status).[11]

It is now well established that the *in utero* milieu experienced by the developing fetus could influence long-term risk for the development of obesity and obesity-related non-communicable diseases (OR-NCDs). [12-14] Maternal behaviour during this critical period of developmental plasticity has the potential to permanently alter susceptibility to later chronic disease via alterations in the offspring's metabolic and endocrinological phenotype. [15-17] Consequently, we hypothesise that maternal exercise in pregnancy will be associated with altered neonatal adiposity, such that an increasing/decreasing exercise level in pregnancy will be associated with a reduction/increase in adiposity, respectively. Any changes in neonatal adiposity could be indicative of an altered phenotypic profile in the offspring, which may increase susceptibility to later chronic disease.

The objective of the current study was to investigate whether changes in maternal exercise during pregnancy were associated with offspring adiposity in the neonatal period, measured using air displacement plethysmography in a large homogeneous population.

Methods:

Neonatal participants were recruited as part of the Cork BASELINE birth cohort study (ClinicalTrials.gov NCT: 01498965 www.birthcohorts.net) [18] between August 2008 and August 2011 from women who had participated in SCOPE (Screening for Pregnancy Endpoints) Ireland. SCOPE was a multicentre prospective cohort study with the aim of developing screening tests to predict various complications of pregnancy (e.g. pre-eclampsia, small-for-gestational-age (SGA) infants, and spontaneous preterm birth) (ACTRN12607000551493). [19] Methods are described in detail elsewhere. [19 20] In brief, participants were healthy nulliparous women with singleton pregnancies recruited antenatally between February 2007 and February 2011 in Cork, Ireland. Women were recruited, interviewed and all measurements obtained at 15±1 and 20±1 weeks' gestation. [19 21] Exclusion criteria included: a high risk for pre-eclampsia/delivery of a SGA neonate/spontaneous preterm birth because of underlying medical conditions; three or more previous miscarriages; three or more terminations of pregnancy; or having received interventions such as aspirin that might modify pregnancy outcome. At the time of interview, data were entered onto an internet-accessed central database with a complete audit trail designed and hosted by MedSciNet, Sweden. Participants were followed up prospectively, with pregnancy outcome data collected by trained research midwives.

Neonatal adiposity was assessed in the majority of neonates within 72 hours of birth by calculating neonatal body fat percentage (BF%) using the PEAPOD air displacement plethysmography. The mean time of measuring BF% in those infants born over 37 weeks' gestation was 1.8 days (standard deviation 0.97 days). Of those infants born <37 weeks' gestation, the mean time of testing was 2.4 days (standard deviation 1.2 days). Measurement of neonatal BF% involves direct measurement of body mass using precise scale and body volume in an airtight, enclosed chamber. Body composition assessment by densitometry involves the measurement of the density of the whole body. Body density is then used in a two-compartment model to calculate the percentage of fat, fat mass, and

fat-free mass.[22] The PEAPOD has excellent test-to-test reproducibility and is safe, non-invasive and fast.[23 24]

Exercise data were collected at both the 15 and 20 week visits in a standardised manner. At both time points, women were asked how many times per week they engaged in vigorous activity (which made the woman breathe harder or puff or pant),[25] moderate activity (did not breathe harder or puff or pant) or walking for recreation or exercise. At 15 weeks, women were asked: *'Has your level of exercise (physical activity) changed since you've been pregnant?*', to which they could respond *'decreased'*, *'unchanged'* or *'increased'*. At 20 weeks, women were then asked: *'Has your level of exercise changed since last SCOPE visit?'*, with the same possible response options.

Statistical analysis:

Differences in maternal characteristics and birth outcomes, stratified by change in exercise level, were explored using one way analysis of variance for continuous variables (with scheffe test for post-hoc pairwise comparisons) and chi^2 test for categorical variables (table 1). Descriptive statistics (frequencies and percentages) of the different levels of exercise were summarised and are shown in table 2. We generated a 'no exercise' binary variable with a value of 1 indicating women who reported doing no vigorous nor moderate nor recreational walking activity per week.

We used linear regression models to investigate the associations between changing levels of self-reported maternal exercise during pregnancy and birthweight (g) and %BF measured as continuous variables. Change in exercise levels was coded as a categorical variable: no change (reference group) versus decreased versus increased. Regression diagnostics did not reveal any violations to linear regression assumptions (i.e. normally distributed residuals and homogeneity of variance). We subsequently generated separate binary variables (0= no; 1=yes) indicating the presence of either low or high adiposity. Low and high adiposity was defined as below/above the gestational age- and sex-specific 10th/90th adiposity centiles respectively, according to the centiles produced by Hawkes et al (2011).[26] The associations between changes in physical activity and these dichotomous variables were investigated using logistic regression models.

We performed sensitivity analyses limiting the sample to only those born at term (n=1180) and separately, to those born non-low birthweight (>2500g) (n=1180) but estimates did not markedly change and thus these infants were retained in the analysis. Furthermore, as the analysis sample was based on those that had complete data for the exposure, outcome and covariates, we also investigated whether we had introduced a selection bias by only including those with complete data (supplementary tables 1 and 2).

In order to identify less biased associations between our exposures and outcome, we produced a directed acyclic graph (DAG) using Daggity. [27] DAGs provide a method for formalising and clarifying the causal hypothesised assumptions a researcher may make regarding the variables they wish to analyse [28] and thus justify modelling choices. [29 30] These graphs are especially useful for identifying variables which potentially confound the relationship between two variables, thus providing researchers with sets of variables for which adjustment (and importantly non-adjustment) is necessary, in order to obtain unbiased estimates of the relationship between a set of variables. For a more detailed discussion on the use of these graphs in epidemiology, see Greenland, Pearl and Robins. [31] Daggity is a web-based interface which allows researchers to construct and edit a directed acyclic graph, with the ultimate aim of identifying sufficient sets of variables for adjustment which will minimise bias when estimating the association between an exposure and outcome. The set of variables identified by Daggity as necessary for adjustment were socioeconomic status, maternal employment, smoking status, alcohol intake, BMI, level of education, maternal age and whether the mother's job was physically active (see supplementary figure 1 for analysis DAG). These variables were then incorporated into multivariable regression models. All analyses were conducted in Stata/IC v14.1.

Results:

Descriptive statistics of the sample (and those omitted)

Compared to all of those enrolled without a PEADOD measurement (n=513) cohort, those enrolled in Cork with a PEAPOD measurement taken (n=1258) were approximately 130g (95% CI: 80-190) heavier and born approximately 2 days later (95% CI: 1.05-3.01), but with no differences in any maternal biological or demographic data (Supplementary table 1). Although 1258 had PEAPOD measurements taken, 58 infants were not included in the final analysis due to: all PEAPOD data being lost/mis-entered (n=16), being born too early or late for adiposity centiles to be generated (n=23) and having incomplete exposure and covariate data (n=19), leaving a final analysis sample of 1200. Compared to those with PEAPOD measurements but not in the final analysis sample, those who were in the final analysis had higher birthweight (187.81; 95% CI: 64.45-311.17), but with no differences in gestational age or any maternal biological or demographic data (Supplementary table 2).

Of the 1200 neonates with complete exposure, outcome and covariate data, 612 (51.00%) were male and 98.25% (n=1 179) were of White European ethnic origin. The mean birthweight was 3510g (95% CI: 3484 - 3537) and the median gestational age was 40 weeks (interquartile range: 39 - 41).

Change in exercise level reported in the first 15 weeks of pregnancy

Table 1 provides descriptive statistics for various maternal characteristics and neonatal outcomes, stratified by type of change of exercise in pregnancy. Compared to women who reported no change in exercise level, those who decreased their level of exercise were older (30.51 years (4.17) vs. 28.89 years (4.74)), with a higher level socioeconomic status (44.33 (16.15) vs. 39.10 (15.40)), less likely to have a household income below €21 000 (5.80% vs. 13.16%) and less likely to have smoked during the first trimester (6.03% vs. 15.90%). The small proportion of women who reported increasing their exercise levels from the time they became pregnant to 15 weeks gestation (<4%) did not differ substantially from the cohort, with the exception of having a higher likelihood of a lower household income (Table 1).

It is shown in Table 2 that at 15 weeks' gestation, more than a quarter (n=327, 27.25%) of women reported engaging in vigorous exercise at least once per week, with approximately three quarters reporting doing some form of moderate exercise per week (n=892, 74.33%). 104 (8.67%) women reported not engaging in any form of exercise per week.

The associations between changing exercise levels and birthweight and neonatal adiposity are shown in Table 3. Relative to women who did not change their exercise level in pregnancy up to 15 weeks, there was no difference in any of the outcomes in those women who either increased or decreased their level of exercise, in both crude and adjusted analyses. Changing the reference group in order to compare those who decreased relative to those who increased also revealed no differences in neonatal outcomes.

Table 1: Descriptive statistics in those with changing levels of physical activity during pregnancy

	Change in exercise level in pregnancy to 15 weeks (n=1200)		
	Decreased (n=813 (67.8%))	Unchanged (n=346(28.8%))	Increased (n=41 (3.4%))
Maternal characteristics:			
Maternal age (mean;SD)	30.51 (4.17)†	28.89 (4.74)	28.88 (5.19)
Maternal BMI at 15 weeks (mean;SD)	25.02 (4.12)	24.49 (4.21)	24.18 (3.85)
Maternal years schooling (mean;SD)	13.27 (0.83)	13.18 (0.81)	13.15 (0.73)
Maternal socioeconomic status (mean;SD)	44.33 (16.15)†	39.10 (15.40)	43.51 (16.35)
Maternal household income <€21 000 (n:%)	47 (5.80)†‡	45 (13.16)	7 (17.07)
Maternal smoking in 1st trimester (n;%)	49 (6.03)†	55 (15.90)	5 (12.20)
Maternal alcohol intake in 1st trimester (units/week)	4.61 (5.76)	5.39 (6.97)	5.99 (8.10)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.16)	40 (1.24)	40 (1.00)
Birth weight (g) (mean;SD)	3525 (460)	3471 (478)	3541 (449)
Neonatal adiposity (%)(mean;SD)	11.06 (4.15)	11.03 (4.06)	11.22 (4.13)
Adiposity<10th centile (yes) (n;%)	68 (8.36)	26 (7.51)	3 (7.32)
Adiposity>90th centile (yes) (n;%)	86 (10.58)	39 (11.27)	7 (17.07)
	Change in exercise level in pregnancy: 15 to 20 weeks (n=1200)		
	Decreased (n=263 (21.9%))	Unchanged (n=665 (55.4%))	Increased (n=272 (22.7%))
Maternal characteristics:			
Maternal age (mean;SD)	30.74 (4.13)†	29.52 (4.58)‡	30.39 (4.25)
Maternal BMI at 15 weeks (mean;SD)	25.07 (4.06)	24.60 (4.01)	25.20 (4.52)
Maternal years schooling	13.28 (0.72)	13.23 (0.86)	13.22 (0.82)
Maternal socioeconomic status	44.33 (15.49)†	40.96 (16.08)‡	45.79 (16.21)
Maternal household income <€21 000 (n:%)	15 (5.70)†	66 (10.03)	18 (6.62)
Maternal smoking in 1st trimester (n;%)	23 (8.75)	73 (10.98)‡	13 (4.78)
Maternal alcohol intake in 1st trimester (units/week)	4.91 (5.79)	5.24 (6.82)‡	3.98 (5.01)
Birth outcomes:			
Gestational age (weeks) (mean;SD)	40 (1.20)	40 (1.19)	40 (1.14)
Birth weight (g) (mean;SD)	3541 (498)	3487 (458)	3537 (448)
Neonatal adiposity (%)(mean;SD)	11.44 (4.66)	10.90 (4.02)	11.08 (3.79)
Adiposity<10th centile (yes) (n;%)	22 (8.37)	58 (8.87)	16 (5.88)
Adiposity>90th centile (yes) (n;%)	41 (15.59)	68 (10.23)	23 (8.46)

†different to 'unchanged' ‡different to 'increased'

Table 2: Descriptive statistics of self-reported activity levels at 15 and 20 weeks

	Exercise level at 15 weeks (n=1200)	Exercise level at 20 weeks (n=1200)	
Vigorous at least once per week (yes) (n; % of 1200)	327 (27.25)	377 (31.42)	
Moderate at least once per week (yes) (n; % of 1200)	892 (74.33)	908 (75.67)	
Recreational at least once per week (yes) (n; % of 1200)	1040 (86.67)	1057 (88.08)	
No exercise per week (n; % of 1200)	104 (8.67)	100 (8.33)	
	Change in exercise level between 15-20 weeks		
	Decreased (n=263)	Unchanged (n=665)	Increased (n=272)
Any exercise per week at 15 weeks			
No (n; % of column total)	7 (2.66)	72 (10.83)	25 (9.19)
Yes (n; % of column total)	256 (97.34)	593 (89.17)	247 (90.81)

Change in exercise level between 15 and 20 weeks

Compared to women who reported no change in exercise level between 15 and 20 weeks, those who decreased their level of exercise were older (30.74 years (4.13) vs. 29.52 years (4.58)), with a higher level socioeconomic status (44.33 (15.49) vs. 40.96 (16.08)) and less likely to have a household income below €21 000 (n=15 (5.70%) vs. n=66 (10.03%)). Women who increased their exercise levels between 15 and 20 weeks, relative to those who reported no change, were also older and with a higher SES, with a reduced alcohol intake (3.98 (5.01) units/week vs. 5.24 (6.82) units/week) and lower likelihood of smoking during the 1st trimester (n=13 (4.78%) vs. n=73 (10.98%) (Table 1).

At 20 weeks, similar levels of exercise were reported, with approximately 30% of women reporting doing vigorous exercise at least once per week, and three quarters of the sample engaging in some form of moderate exercise. Just over 8% of women reported taking part in no form of exercise at 20 weeks (Table 2). Table 2 also reveals that of the 665 women who reported no change in their exercise levels between 15-20 weeks, approximately 10% of these (n=72) had engaged in no exercise at 15 weeks. Similarly, of those who increased their exercise levels between 15-20 weeks, just under 10% (n=25) had reported no exercise at 15 weeks (Table 2).

Crude analysis shows that relative to women who do not change their exercise level between 15 and 20 weeks, those women who decreased their exercise level were more likely to give birth to a neonate with adiposity above the 90th centile (OR: 1.62; 95% CI:1.07; 2.46) (Table 3). This association was maintained after adjustment for the putative confounders (OR: 1.66; 95% CI: 1.09; 2.54). When changing the reference group in order to compare women who decreased exercise levels relative to those who increased exercise, it was observed that those who decreased were twice as likely to give birth to a neonate with an adiposity above the 90th centile (OR: 2.00; 95% CI: 1.16 - 3.44), which again was also maintained on adjustment (OR: 2.09; 95% CI: 1.20 - 3.61). Birthweight was not associated with differences in exercise (Table 3).

Table 3: Associations between changing exercise levels during pregnancy and birthweight and neonatal adiposity

	Change in exercise level in pregnancy to 15 weeks (coefficient; 95%CI)					
	Crude			***Multivariable****		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.39 (-4.16; 112.93)	*(reference)*	70.24 (-80.40; 220.89)	22.03 (-37.61; 81.67)	*(reference)*	74.40 (-75.84;224.64)
Neonatal adiposity (%)*	0.03 (-0.49; 0.55)	*(reference)*	0.19 (-1.15; 1.53)	0.01 (-0.52; 0.55)	*(reference)*	0.33 (-1.01; 1.67)
Adiposity<10th centile**	1.12 (0.70; 1.80)	*(reference)*	0.97 (0.28; 3.36)	1.20 (0.73; 1.95)	*(reference)*	0.82 (0.23; 2.94)
Adiposity>90th centile**	0.93 (0.62; 1.39)	*(reference)*	1.62 (0.67; 3.90)	0.96 (0.62; 1.41)	*(reference)*	1.75 (0.71; 4.31)
	Change in exercise level in pregnancy: 15 to 20 weeks (coefficient; 95%CI)					
	Crude			***Multivariable****		
	Decreased	*Unchanged*	Increased	Decreased	*Unchanged*	Increased
Birthweight (g)*	54.47 (-11.96; 120.90)	*(reference)*	50.11 (-15.53; 115.75)	42.68 (-23.62; 108.98)	*(reference)*	22.30 (-43.49; 88.09)
Neonatal adiposity (%)*	0.54 (-0.05; 1.13)	*(reference)*	0.18 (-0.40; 0.76)	0.56 (-0.03; 1.15)	*(reference)*	0.13 (-0.46; 0.72)
Adiposity<10th centile**	0.94 (0.56; 1.56)	*(reference)*	0.64 (0.36; 1.14)	0.94 (0.56; 1.59)	*(reference)*	0.69 (0.39; 1.24)
Adiposity>90th centile**	1.62 (1.07; 2.46)	*(reference)*	0.81 (0.49; 1.33)	1.66 (1.09; 2.54)	*(reference)*	0.80 (0.48; 1.32)

*Linear regression for continuous outcomes (β); **logistic regression for dichotomous outcomes (OR) ***adjusted for: socioeconomic status, years of schooling, employment status, maternal BMI, smoking in 1st trimester, alcohol intake in 1st trimester, maternal age, exercise as part of job

Discussion

In this cohort of white European mother-offspring pairs, we report the association between changing levels of exercise during pregnancy and neonatal adiposity measured using air displacement plethysmography (PEAPOD). We observed that pregnant women who reported a decrease in exercise levels between 15 and 20 weeks, had a 60% higher risk of having a baby with adiposity above the 90th centile when compared with women who reported no change. This risk was approximately double when women who reported a decrease in exercise levels between 15 and 20 weeks were compared to women who reported an increase in exercise levels. This association was maintained after adjustment for a set of putative confounders including maternal education, employment status, smoking, alcohol intake, BMI socioeconomic status, maternal age and whether her occupation was physically active. This positive association between decreased exercise level and adiposity was also observed when adiposity was assessed as a continuous variable, though the 95% confidence interval did include the null. The exercise-adiposity association was only apparent between 15 and 20 weeks and not for changing exercise levels prior to 15 weeks, raising the possibility that there is a potential critical period with regard to the association between changes in exercise level and the development of offspring adiposity.

A major strength of the study is the use of air-displacement plethysmography for the estimates of body composition. This method is a quick, safe and non-invasive technique, which has shown to be a reliable and accurate instrument for determining body fat percentage in infants. [23 32 33] As such, it has been deemed the primary method for measuring body density in paediatric populations. [34] Inter-observer variability was reduced by having a small, highly trained team of midwives and researchers who conducted all of the assessments to strict protocols. However, repeated measurements were not performed and thus we were unable to assess intra-observer variability. The prospective design of the cohort, allowing us to comprehend the temporal relationship between variables and the rich collection of covariates available for adjustment further strengthens the study. Another strength of this study is the use of a directed acyclic graph (DAG) which is based on an understanding of the causal network linking the variables in the analysis. As such, the DAG allows for the appropriate adjustment for a set of putative confounders in order to obtain a less biased estimate of the association between changing levels of exercise and neonatal adiposity. We are, however, cautious not to refer to any association as 'causal' as we cannot exclude the possibility of the presence of both residual confounding and, in particular with this subjective measurement of exercise, measurement error.

Arguably the greatest limitation is the subjective nature of the exercise data. Whilst the questionnaire regarding physical exercise was not validated for any population, the definition of vigorous exercise (daily exercise leading to heavy breathing or being out of breath) has previously been used in other studies.[25] As the exercise variables were based on maternal report, this introduced a potential error due to women not accurately remembering their exercise levels (e.g. due to social desirability of reporting higher levels or age). The recall period was relatively short, considering only the very recent past, and focussed on habitual activity, thus reducing the extent of the error introduced. An objectively measured assessment of physical activity (e.g. an accelerometer), would have been of benefit to estimate actual activity. Nonetheless, in large-scale cohort studies a compromise is often sought, with participant burden and cost-effectiveness on the one side and a more precisely measured variable on the other. Furthermore, it has been reported that pregnant women may wear monitors placed at the hip incorrectly due to changes in their girth.[35 36] Accordingly, a recent systematic review found that in epidemiological studies amongst pregnant women, self-reported physical activity measures were the most common assessment method.[37] Research on agreement between subjective estimates of physical activity and objectives measures has generated mixed results, [38 39] with the same systematic review concluding that the agreement between questionnaires and objective measures of physical activity assessment, ranged from 'poor to substantial'.[37]

A related limitation is that, as recruitment commenced during pregnancy, pre-pregnancy exercise data was not available and as a result we were unable to determine what pre-pregnancy exercise level women had changed from. It could be speculated that women who reported no change in activity level at 15 weeks did not do any exercise to start with. We have shown that those women whose activity remained unchanged at 15 weeks (compared to those who decreased) were more likely to smoke during the first trimester, be of lower socioeconomic status and more likely to have a lower household income, all of which are associated with reduced levels of exercise and fetal growth. Whilst we adjusted for these confounding factors, the lack of baseline activity limits the interpretability of our findings. For example, it would have been interesting to determine whether the observed association between a decreasing exercise level (vs. unchanged level) and neonatal adiposity was the same across differing categories of baseline activity.

We were unable to adjust our estimates for the likely mediating role of gestational hyperglycaemia as these data were not available. Similarly, we did not adjust our estimates for gestational weight gain. In line with the published literature[4 5 40 41], these variables are likely to operate along the causal pathway between maternal exercise and neonatal adiposity. While adjusting for them may mask part of the association between exercise and adiposity, it would have been of benefit to conduct *a priori* analysis to examine whether a change exercise was associated with neonatal adiposity independently of pre-pregnancy obesity, gestational weight gain or impaired glycaemic control. Acknowledging these data gaps, the current paper did not aim to elucidate possible mechanisms by which the association between exercise and adiposity is enacted, rather, we aimed to identify whether an association existed at all.

A final limitation is the potential lack of generalisability of our results to other groups. For example, study recruitment was limited to primiparous women with singleton pregnancies and notably, a majority of White European gravidas (approximately 98.25%) were recruited into the study. This predominance of White European gravidas does, however, reflect the demographic profile of females aged 15 to 44 in Ireland as a whole (95%).[42] Unfortunately, a number of infants (513/1771) were unable to have a body composition assessment. Possible reasons for this include a lag period between the start of the study and the arrival of the PEAPOD, and admittance of the infant to the neonatal intensive care unit (NICU). We have shown that although these infants differed slightly in terms of birthweight (median difference: 130g; 95% CI: 80-190g) and gestational age (median difference: 0.29 weeks; 95% CI: 0.15 - 0.43 weeks), there were no differences in the maternal characteristics of those with and without a PEAPOD measurement (supplementary table 1), and thus we are confident we have not introduced a substantial selection bias into the analysis. The employment of a complete-case analysis could also have introduced a degree of selection bias into the analysis, however, supplementary table 2 shows that, apart from birthweight, there no differences in the offspring or maternal characteristics of those with complete vs. incomplete data.

To the authors' knowledge, this is the first study looking at the association between changing exercise levels in pregnancy and neonatal adiposity using air displacement plethysmography. Previous studies have either used different measurement techniques (sum of skinfolds [7 8] or dual-energy x-ray absorptiometry (DXA)[10]) or were not looking at changing levels of exercise. [11] A recent large observational study observed that the lowest quartile of late-pregnancy energy expenditure was associated with a substantially higher neonatal fat mass (290.5g vs 249.4g, p=0.03) within the first 72-hours, which was not mirrored in neonatal fat-free mass [11]. Unlike our study, however, no differences were observed in either mid- or early pregnancy. However, the aforementioned study was not investigating intra-pregnancy change and also relied on a statistically driven method to identify potential confounders, ignoring the causal framework underpinning any possible associations.

We observed a possible critical period for the association between changing exercise levels and neonatal adiposity, with no association observed with exercise recall for the first 15 weeks of gestation, but an association between a decreasing level of exercise between 15 and 20 weeks. This provides support for the findings of Clapp et al,[7] who found that women who performed a high volume of moderate exercise in early pregnancy and then cut back in late pregnancy delivered offspring who were heavier and longer at birth, compared to offspring of women who either did moderate volumes in both early and late pregnancy or a low volume followed by a high volume.[7] Indeed in our study we observed a markedly increased risk of delivering an infant with neonatal adiposity above the 90th centile in pregnant women who reported having increased their exercise levels up to 15 weeks, but then reported a decrease between 15 and 20 weeks, relative to those who reported no change at both time points (OR: 5.87; 95% CI: 1.74-19.80, data not shown), though the uncertainty of this estimate can be observed in the wide confidence interval, reflecting the small number of women on which this finding was based.

The data presented here suggest that a reduction in exercise levels may lead to less favourable outcomes in terms of neonatal adiposity. As such, and given the evidence of maintaining pre-pregnancy exercise levels[43 44], we advocate the continuation of pre- and early pregnancy exercise levels into later pregnancy. Further studies utilising objectively measured estimates of physical activity in a range of different population groups are required in order to replicate this finding. For example, the cohort of women in this analysis exhibited relatively low levels of activity, with almost 75% of women never doing any vigorous activity at 15 weeks and only approximately 50% of the women doing moderate activity more than once a week. If results appear consistent and robust to these differences in methodology and population, then these findings have significant implications, which extend beyond the short-term. For example, it has been shown that the associations between maternal pregnancy exercise levels and offspring adiposity present at birth extend into childhood, with children of women who exercised during pregnancy observed to have a reduced fat mass at age 5 years (37mm ± 1 vs. 44mm ± 4) compared children whose mothers were inactive[45]. However, the overall lack of follow-up studies with body composition assessment at birth limits our ability to explicitly link increased adiposity in early-life and later risk. Nonetheless, if the effects of a reduced level of exercise are able to manifest in the offspring as an altered adiposity at birth, the wider implication is that, during this critical period of developmental plasticity, some sort of programing has occurred, potentially permanently altering the offspring's metabolic and endocrinological phenotype (13-15),[15-17] and altering its long-term susceptibility to a variety of non-communicable diseases (NCDs). It is hoped that with the increasing incorporation of body composition assessment methods in infancy, particularly air-displacement plethysmography, these questions will be able to be investigated.

Conclusion:

A decreasing level of maternal reported exercise between 15 and 20 weeks' gestation was associated with an increased risk of delivering an infant with a high adiposity. This association was maintained after appropriate adjustment for confounding variables as identified using knowledge of the causal network. However, these findings need interpreting in line with the limitations of the study. Accordingly, further research utilising objective measures of physical activity and in different populations needs to be conducted in order to validate results.

Acknowledgements:

We thank the pregnant women who participated in the SCOPE study and the mothers who allowed their newborn infants to participate in the BASELINE study.

Funding:

SCOPE Ireland was funded by the Health Research Board, Ireland (CSA 2007/2). The BASELINE cohort was supported by the National Children's Research Centre, Dublin, Ireland, and the Food Standards Agency of the United Kingdom (grant no. TO7060). SCOPE and BASELINE are supported by INFANT, an SFI funded Research Centre (grant no 12/RC/2272). The funders had no involvement in the study design, data collection, analysis and interpretation, as well as in the writing of the manuscript.

Competing interests:

None declared.

Contribution to authorship:

LCK is guarantor. TN designed the study, analysed and interpreted the data, alongside LCK and PNB. TN, AK, FMC, DMM, MK, J O'B H, LCK and PNB took part in drafting the article or revising it for critically important intellectual content and all gave final approval of the version to be published.

Ethics approval:

Ethical approval was obtained from the local ethics committees (Cork ECM5(10)05/02/08; approved 5 February 2008) and all women provided written informed consent.

Data sharing statement: There are no additional data available.

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Supplementary table 1: Descriptive results for those vs those without PEAPOD measurements

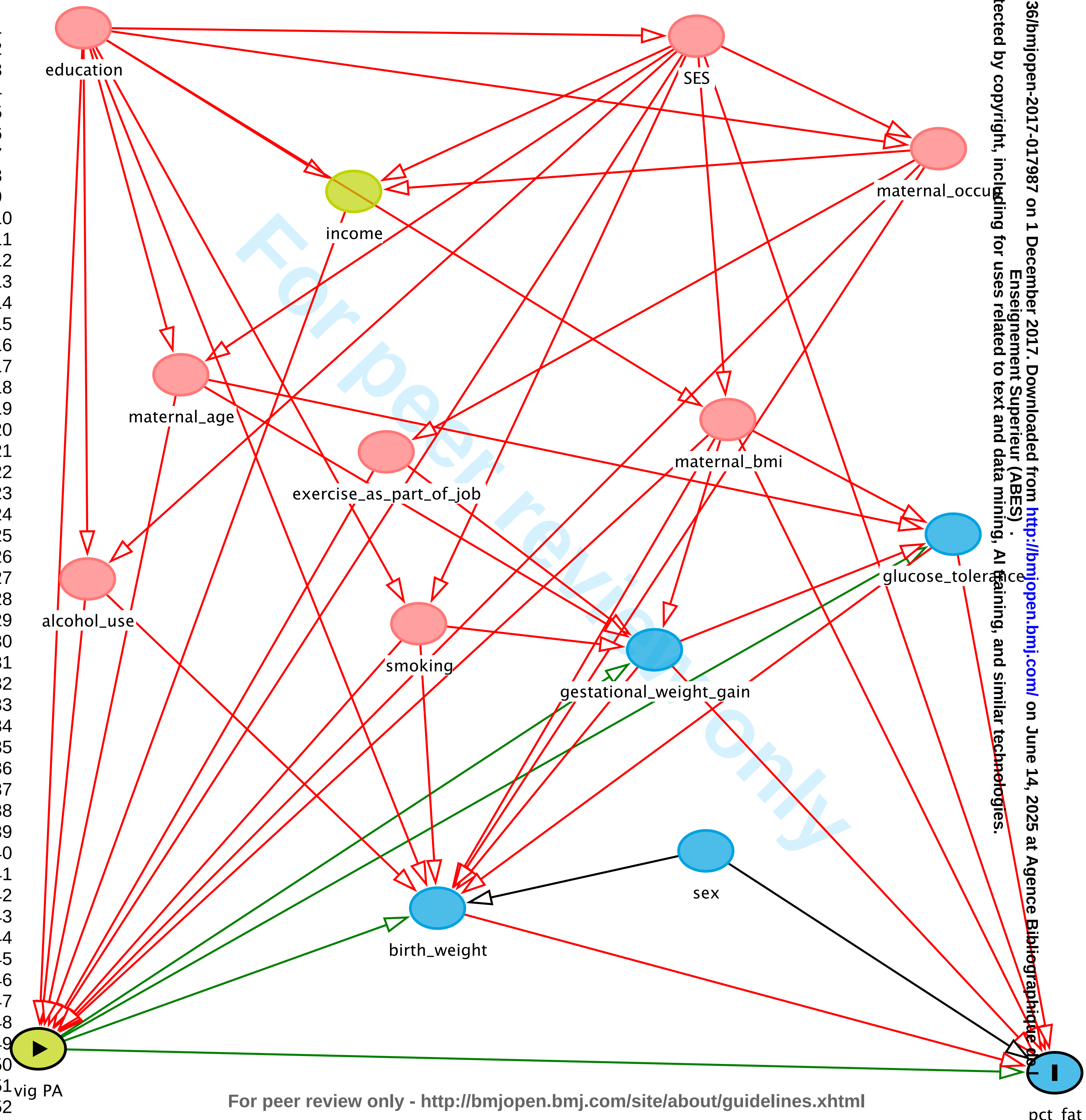
	With PEAPOD (n=1258)	Without PEAPOD (n=513)	Difference (Cork_with – Cork_without) (95% CI)
Sex of infant (% (n) female)	48.57 (611)	50.68 (260)	-2.11 (-7.25; 3.02)*
Birth weight (g) (median;IQR)	3500 (3180;3800)	3380 (3020;3730)	130 (80;190)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41)	40 (38.71;40.86)	0.29 (0.15;0.43)**
Maternal age (years) (mean:SD)	29.95 (4.44)	29.88 (4.61)	0.07 (-0.39;0.53)**
Maternal BMI (kg/m^2) (median:IQR)	23.9 (22;26.9)	24 (22;26.9)	-0.1 (-0.30;0.50)**
Maternal Socioeconomic status (median;IQR)	45 (29;51)	45 (29;50)	0 (-1;0)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13;14)	0 (0;0)**
Household income (<€21 000) (yes)(% (n))	8.47 (106)	8.22 (42)	0.25 (-2.58;3.10)*
Maternal unemployment (yes) (% (n))	5.56 (70)	3.51 (18)	2.06 (0.02;4.09)*
1st trimester smoking (yes) (% (n))	9.30 (117)	11.70 (60)	-2.40 (-5.60;0.82)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	2.8 (0.62;5)	0 (-0.5;0)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)

Supplementary table 2: Descriptive results for those with vs those without complete data

	Complete data group (n=1200)	Missing data group (n=58)	Difference (Complete – incomplete) (95% CI)
Sex of infant (% (n) female)	49.00 (588)	39.66 (23)	9.34 (-3.56;22.25)*
Birth weight (g) (mean;SD)	3510 (465)	3322 (518)	188 (64;311)**
Gestational age (weeks) (median;IQR)	40.29 (39.43;41.00)	40.57 (39.29;42.00)	-0.43 (-0.86;0.15)**
Maternal age (years) (mean:SD)	29.98 (4.44)	29.13 (4.54)	0.85 (-0.33;2.02)**
Maternal BMI (kg/m^2) (median;IQR)	23.9 (22.00;26.8)	24.7 (22.00;29.00)	-0.70 (-1.90;0.40)**
Maternal Socioeconomic status (median;IQR)	45 (29;51.00)	44.5 (29;50.00)	0 (-3.00;4.00)**
Maternal schooling (years) (median;IQR)	13 (13;14)	13 (13:14)	0 (0;0)**
Household income <€21 000 (yes) (% (n))	8.30 (99)	12.07 (7)	-3.77 (-12.30; 4.76)*
Maternal unemployment (yes) (% (n))	5.50 (66)	6.90 (4)	-1.40 (-8.04;5.25)*
1st trimester smoking (yes) (% (n))	9.08 (109)	13.79 (8)	-4.71 (-13.73;4.31)*
1st trimester alcohol intake (units/week) (median;IQR)	3 (0.6;7)	3 (0.6;10)	-0.10 (-1.5;0.67)**

*tests for proportions for categorical variables (% difference); **t-tests/Wilcoxon rank-sum for continuous variables (difference in means/medians)


education
SES
maternal_occup
income
maternal_age
maternal_bmi
exercise_as_part_of_job
glucose_tolerance
alcohol_use
smoking
gestational_weight_gain
sex
birth_weight
vig PA
pct_fat

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	

(*e*) Describe any sensitivity analyses

Continued on next page

Results			Page
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9,11
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,12-14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.