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# Application of multilevel linear spline models for analysis of growth trajectories in a cohort with repeat antenatal and postnatal measures of growth: a prospective cohort study

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**Abstract**

**Objectives:** To apply linear spline multilevel models to model trajectories of antenatal and postnatal growth in a randomised controlled trial with repeat prospective assessments of growth.

**Methods:** Prospective follow-up data from 720-759 mother-child pairs from the ROLO study (initially a randomized controlled trial of a low glycemic index diet in pregnancy to prevent recurrence of macrosomia [birthweight > 4K]) were analysed. Fetal measurements were obtained at 20-and 34-weeks gestation, including abdominal circumference (AC) and head circumference (HC). An estimated fetal weight was obtained at 20-and 34-weeks gestation. At delivery, six months, two years and five years AC, HC, weight and length were also recorded. Linear spline multilevel models were used to examine trajectories from 20 weeks gestation (AC, HC and weight) or birth (length/height) to five years.

**Results:** Over 50% of women had 3rd level education and 90% were of White ethnicity. Women were a mean (SD) age of 32 (4.2) at recruitment. The best fitting model for AC, HC and weight included a model with knots at each measurement occasion giving rise to five linear spline periods. The best fitting models for length/height included a model with three linear spline periods from birth to six months, six months to two years and two years to five years. Comparison of observed and predicted values for each model demonstrated good model fit. For all growth measures, fetal growth rates were generally fastest in pregnancy or immediately postpartum (for length/height), with rates of growth slowing after birth and becoming slower still as infancy and childhood progressed.

**Conclusion:** We demonstrate the application of multilevel linear spline models for examining growth trajectories when both antenatal and postnatal measures of growth are available. The approach may be useful for cohort studies or randomised controlled trials with repeat prospective assessments of growth.

## Introduction

Antenatal and childhood growth are important indicators of fetal and child health and development and are associated with health in adult life (1, 2). Consequently, modelling of growth trajectories, identifying causes and predictors of different growth trajectories and relating growth trajectories in the early life course to later life health is important for informing a life course approach to disease prevention (3-5).

A key aspect of understanding growth patterns, their causes, predictors and outcomes includes appropriate modelling of longitudinal growth data (3). Since repeated measures of growth within individuals are not independent of each other and the scale and variance of growth measures often changes over time, traditional approaches to analysis of growth data, such as Z-score based methods analysed using multiple regression, do not take account of the clustering of repeated measures within individuals (3). Moreover, the true shape of growth trajectories cannot be modelled using such approaches. While appropriate methods for the study of longitudinal growth data have been applied to antenatal and childhood growth measures in many cohort studies, most studies to date have examined antenatal growth (6, 7) or postnatal growth as separate processes/trajectories (8-14). Appropriate modelling of growth data as a continuum from antenatal to postnatal life is important to accurately characterise the shape of growth from early gestation into childhood to better understand it's aetiology. In addition, it also allows such trajectories to be examined as outcomes for pre-conception or early pregnancy exposures or to be examined themselves as exposures for later health outcomes (3).

Using data from the prospective follow-up of a randomised controlled trial of a low glycaemic index diet in pregnancy (ROLO study), we demonstrate the application of linear spline

multilevel models for modelling antenatal and postnatal growth trajectories using four measures of anthropometry (abdominal circumference [AC], head circumference [HC], weight and length/height) from 20 weeks' gestation to age five years.

## Methods

### Study population

The ROLO study is a randomised control trial of a low glycaemic index diet in pregnancy that recruited 800 secundigravid women who had previously given birth to a baby weighing over 4kg between 2007-2011 at the National Maternity Hospital, Dublin, Ireland (15). Women were recruited at first antenatal consultation. Women with any underlying medical disorders, including a previous history of gestational diabetes, those on any drugs, those unable to give full informed consent, aged less than 18 years, of gestation greater than 18 weeks, and having multiple pregnancies were excluded. Women were randomised to either the intervention group which received dietary advice on a low glycaemic diet, or the control group who received routine antenatal care. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

### Patient and Public Involvement

None.

### Measurement of anthropometry

*Antenatal measures*

Fetal measurements were obtained from ultrasound scans performed on mothers at medians of 20 + 6 (Interquartile Range [IQR]: 20 + 1 to 21 + 5) and 34 + 1 (IQR: 33 + 5 to 34 + 5) weeks' gestation, including AC and HC. An estimated fetal weight (EFW) at 20- and 34-weeks' gestation was calculated using the Hadlock 4-parameter formula. Ultrasound measurements were taken by two ultrasonographers using a Voluson 730 Expert (GE Medical Systems, Germany) using standard procedures.

*Postnatal measures*

At delivery, infants' AC, HC, weight and length were recorded. Follow-up anthropometry assessments were also obtained in childhood at six months, two years and five years (15-17). All measurements were obtained and calculated by a trained member of the research team. At six months, two years and five years, weight (kg) of the child was measured using a calibrated stand on digital weighing scale (SECA 813) to the nearest 0.1 kg by a trained research team member. Children were measured in light clothing without shoes. Standing height was measured, without shoes, with head aligned in the Frankfort plain, using a free-standing stadiometer (SECA 217) and measurements recorded to the nearest 0.1cm. The child's head and abdominal circumferences were measured using a SECA ergonomic circumference measuring tape, to the nearest 0.1cm. All measurements were recorded three times and the average calculated to improve reliability.

**Statistical analysis**

We used multilevel models to examine trajectories of change in AC, HC, weight and length/height from 20 weeks gestation to age five years (18, 19). Multilevel models estimate mean trajectories of the outcome while accounting for the non-independence (i.e. clustering) of repeated measurements within individuals, change in scale and variance of measures over time and differences in the number and timing of measurements between individuals (using all available data from all eligible participants under a Missing at Random [MAR] assumption) (3, 20).

Change in all four growth measures was estimated here using linear spline multilevel models (two levels: measurement occasion and individual) (3). Linear splines allow knot points to be

fit at different ages to derive periods in which change is approximately linear. The optimal linear spline model for each growth measure was selected by examining observed data for each growth measure and comparing model fit statistics of different models including models that assumed linear change over time to models with knot points at different ages (strategies for selection of knot points are described elsewhere in detail (3)). Model fit statistics examined included Akaike's Information Criterion and observed and predicted values of each growth measure across the age range of the model. Following exploration of a series of models, the best fitting model for AC, HC and weight included a model with knots at each measurement occasion giving rise to five linear spline periods from 20 weeks' to 34 weeks' gestation, 34 weeks' gestation to birth, birth to six months, six months to two years and two years to five years while the best fitting models for length/height included a model with three linear spline periods from birth to six months, six months to two years and two years to five years.

All outcomes were normally distributed at each measurement occasion. Except for length/height which did not include antenatal measures, trajectories were centred on the first available measure (20 weeks gestation) for AC, HC and weight. Length/height trajectories were centred at birth. For all models we placed no restrictions on the variance-covariance matrices of level two (individual level) random effects. Given the substantial change in scale and variance of growth from antenatal to postnatal life, we also aimed to allow occasion level measurement error to vary with age (level one random effects for the slope). Therefore, all models included a level one random effect for the slope while the HC model also included a level one random effect for the intercept. The final models for growth trajectories from 20 weeks gestation took the following form: $AC_{ij}$ / $HC_{ij}$ /$weight_{ij}$= $\beta_0 + u_{0j} + (\beta_1 + u_{1j})s_{ij1} + (\beta_2 + u_{2j})s_{ij2}$

$+ (\beta_3 + u_{3j})s_{ij3} + (\beta_4 + u_{4j})s_{ij4} + (\beta_5 + u_{5j})s_{ij5} + e_{ij}$ where for person j at measurement occasion i; $\beta_0$ represents the fixed effect coefficient for the average intercept, $\beta_1$ to $\beta_5$ represent fixed effect coefficients for the average linear slopes of each linear spline, $u_{0j}$ to $u_{5j}$ indicate person-specific random effects for the intercept and slopes respectively, and $e_{ij}$ represents the occasion-specific residuals or measurement error which were allowed to vary with age. The final model for length took a similar form but with only three linear spline periods due to the absence of measures prior to birth. Code for the application of these models using the "runmlwin" command from MlWin (21) within Stata 16 (22) is included in Supplementary Material.

**Results**

754, 756 and 759 offspring were included in analyses of AC, HC, and weight respectively while 720 offspring were included in analyses of length/height. Table 1 includes the number of measures of each growth measure at each measurement occasion with number of measures available broadly similar across growth measures; for example, weight measures available on each occasion included 655 measures at 20 weeks gestation, 730 at 34 weeks gestation, 756 at birth, 280 at six months, 339 at two years and 387 at five years.

Of participants included in analyses (Table 2), over 50% had completed third level education and a majority (>90%) were of White ethnicity. Among mothers of male babies, mean age (standard deviation (SD)) at delivery was approximately 32.3 (4.2) years, mean (SD) BMI at delivery was 27.1 (5.2) kg/m$^2$, mean (SD) birthweight at delivery was 4.1 (0.5) kg and median (interquartile range (IQR)) gestational age was 40.4 (39.6, 41.1) weeks. Mothers of male babies had relatively low levels of deprivation as indicated by the mean (SD) Pobal HP index of 5.3 (10.8). Characteristics were broadly similar for mothers of female babies though mothers of female babies had somewhat higher levels of third level education (~60%). Model fit as judged by differences between observed growth measures and those predicted by the models for AC, HC, weight and length are shown in Tables 3-6. Overall, our models have good model fit as all reference ranges for the difference between observed and predicted are less than the SD of the observed or less than 10% of the observed value which can be used as a rule of thumb for the assessment of model fit.

Trajectories of AC, HC and weight from 20 weeks' gestation to five years and trajectories of length/height from birth to five years by intervention status and sex are shown in Table 7 and

Figures 1-4. AC and HC had the fastest rates of growth from 20 to 34 weeks' gestation with growth rates continuing to slow thereafter up to age five years. Weight had the fastest growth rate from 34 weeks' gestation to birth with growth rates slowing somewhat from birth to six months and continuing to slow thereafter until five years. Length/height had the fastest growth rates from birth to two years, with the growth rate decreasing thereafter and slowing further from two years to five years.

We found no strong evidence of differences in trajectories of AC, weight and length/height between the intervention and control group, but we found some evidence of slightly greater HC (difference 0.27 cm (95% Confidence Interval [CI] = 0.03, 0.51) emerging among the control group at five years. AC trajectories did not differ between males and females, though we found some evidence of modest differences in HC, weight and length/height trajectories between males and females. Females had lower HC at 20 weeks gestation with this difference widening at birth and persisting at age five years (difference at five years: -0.91cm, 95% CI = -1.14, -0.68). Females had -0.15kg (95% CI = -0.21, -0.08) lower birth weight and slower postnatal growth rates in weight leading to -0.50 kg (95% CI = -0.96, -0.05) lower weight among females at five years. Similarly, females were -0.83 cm (95% CI = -1.17, -0.48) shorter in length at birth and had slower postnatal growth rates in length/height leading to -1.22 cm (95% CI = -2.01, -0.43) shorter height among females at five years.

## Discussion

In this prospective follow-up of a randomised control trial of approximately 750 infants at high risk of macrosomia, we demonstrated the use of linear spline multilevel modelling to examine trajectories of AC, HC, weight and length/height from 20 weeks' gestation to age five years. We showed their applicability to data with repeated measures of growth which span the antenatal and postnatal period, even when as few as four repeat assessments are available (in the case of length/height) and measures are sparse. This work may be of value to other studies including randomised control trials with follow-up data such as ours in demonstrating the application of a multilevel modelling approach to examine growth trajectories which can subsequently be used as exposures or outcomes to better understand determinants and outcomes of growth in early life.

There are several strengths to the approach used here including ability to maximise sample sizes for analyses and reduce selection bias compared with traditional Z-score approaches since multilevel models can include all participants with at least one growth period under a MAR assumption (3). This is particularly advantageous where attrition rates from cohorts are high. Further advantages include more precise standard errors which consider the non-independence of repeated measures and here we have shown that the approach is implementable with as little as four repeated measures and with repeated measures that span antenatal and postnatal life. Limitations of this work include an inability to explore other non-linear growth patterns such as fractional polynomials due to the sparsity of measures which did not allow a range of possible shapes of growth trajectories to be explored (3). In cohorts with greater numbers of repeated measures and density of repeats, linear spline multilevel modelling can be implemented and compared to other possible shapes include

fractional polynomials which have been shown to provide a more biologically intuitive shape of change(3). However, the linear spline approach demonstrated here provides many practical advantages including being more easily interpretable, allowing analysts to split trajectories into distinct periods of change that can then be easily related to exposures and outcomes. It should be noted that this cohort are unlikely to represent the growth rates or trajectories of a general population since their development is above average compared to what would be expected from an age and gender matched general population (the cohort is roughly approximated to the 75$^{th}$ centile based on a crude comparison of means and SDs on the UK-WHO (Ireland) chart) (23).

**Conclusion**

We demonstrate the application of multilevel linear spline models for examining growth trajectories when both antenatal and postnatal measures of growth are available. The approach may be useful for cohort studies or randomised control trials with repeat prospective assessments of fetal growth spanning pregnancy and childhood.

**Table 1 N repeated measures included in analyses for each growth measure**

| | 20 weeks | 34 weeks | Birth | 6 months | 2 years | 5 y ears |
|---|---|---|---|---|---|---|
| **Abdominal circumference** | 656 | 732 | 265 | 280 | 336 | 385 |
| **Head circumference** | 656 | 700 | 634 | 280 | 333 | 386 |
| **Weight** | 655 | 730 | 756 | 280 | 339 | 387 |
| **Length/height** | | | 634 | 280 | 339 | 386 |

**Table 2 Characteristics of ROLO participants included in the analysis of length/height, by sex**

| | Male N=358 | Female N=362 |
|---|---|---|
| | **n (%)** | **n (%)** |
| **Completed 3rd level education** | 151 (50.3) | 187 (60.9) |
| **Non-white ethnicity** | 5 (1.4) | 9 (2.5) |
| | **Mean (SD)** | **Mean (SD)** |
| **Mothers age at delivery (years)** | 32.3 (4.2) | 32.6 (4.2) |
| **HP index (unit)** | 5.3 (10.8) | 5.4 (9.7) |
| **Mothers BMI (kg/m²)** | 27.1 (5.2) | 26.2 (4.4) |
| **Birthweight (kg)** | 4.1 (0.5) | 4.0 (0.4) |
| | **Median (IQR)** | **Median (IQR)** |
| **Gestational age at delivery (weeks)** | 40.4 (39.6, 41.1) | 40.3 (39.6, 41.1) |

SD, standard deviation; IQR, interquartile range.

**Table 3 Model details for abdominal circumference**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 531 | 22.48 (6.92) | 22.59 (6.86) | -0.08 | -1.13 to 0.97 |
| **34 wks to birth** | 517 | 32.22 (2.08) | 32.35 (1.19) | 0.08 | -2.01 to 2.17 |
| **Birth to 6 months** | 315 | 38.21 (5.93) | 36.01 (4.51) | -0.05 | -3.64 to 3.53 |
| **6 months to 2 years** | 272 | 47.91 (5.37) | 47.84 (3.96) | 0.09 | -4.25 to 4.42 |
| **2 years to 5 years** | 681 | 50.25 (8.98) | 54.03 (2.72) | -0.06 | -6.11 to 6.00 |

SD, standard deviation; wks, weeks.

**Table 4 Model details for head circumference**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 292 | 22.90 (4.87) | 22.86 (4.83) | 0.09 | -0.55 to 0.73 |
| **34 wks to birth** | 680 | 32.54 (1.67) | 32.63 (1.53) | -0.01 | -0.61 to 0.59 |
| **Birth to 6 months** | 642 | 37.57 (3.70) | 37.39 (3.46) | 0.00 | -0.48 to 0.47 |
| **6 months to 2 years** | 274 | 47.29 (2.98) | 47.28 (2.87) | 0.01 | -0.47 to 0.49 |
| **2 years to 5 years** | 661 | 48.89 (6.16) | 51.18 (1.64) | -0.01 | -0.75 to 0.73 |

SD, standard deviation; wks, weeks.

**Table 5 Model details for weight**

| | Total number of observations | Mean observed (SD) in kg | Mean predicted (SD) in kg | Mean difference (observed – predicted) in kg | 95% level of agreement between observed and predicted in kg |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 294 | 0.97 (0.81) | 1.04 (0.77) | -0.07 | -0.24 to 0.10 |
| **34 wks to birth** | 708 | 2.93 (0.58) | 2.89 (0.51) | 0.04 | -0.31 to 0.38 |
| **Birth to 6 months** | 735 | 4.87 (1.87) | 4.88 (1.81) | -0.01 | -0.44 to 0.43 |
| **6 months to 2 years** | 276 | 10.92 (2.59) | 10.91 (2.53) | 0.01 | -0.36 to 0.38 |
| **2 years to 5 years** | 695 | 15.54 (6.60) | 18.30 (3.88) | -0.01 | -0.42 to 0.41 |

SD, standard deviation; wks, weeks.

**Table 6 Model details for length**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **Birth to 6 months** | 475 | 57.55 (7.51) | 57.14 (7.25) | 0.0001 | -0.03 to 0.03 |
| **6 months to 2 years** | 304 | 81.03 (10.12) | 81.03 (10.08) | -0.002 | -0.57 to 0.56 |
| **2 years to 5 years** | 574 | 104.07 (12.24) | 106.42 (9.79) | 0.0004 | -2.92 to 2.92 |

SD, standard deviation; wks, weeks.

**Table 7 Mean trajectories of anthropometry and mean difference in trajectories by intervention status and sex in the ROLO cohort**

| | Mean trajectory (95% CI) in intervention | Mean difference in trajectory (95% CI) in controls | Mean trajectory (95% CI) in males | Mean difference in trajectory (95% CI) in females |
|---|---|---|---|---|
| **Abdominal circumference** | | | | |
| 20 weeks (cm) | 15.96 (15.85,16.07) | -0.02 (-0.17,0.14) | 16.05 (15.94,16.16) | -0.20 (-0.35,-0.04) |
| 20 wks to 34 wks (cm/week)* | 1.20 (1.18,1.22) | 0.01 (-0.02,0.03) | 1.20 (1.18,1.22) | 0.002 (-0.02,0.03) |
| 34 wks to birth (cm/week)* | 0.26 (0.19,0.33) | 0.03 (-0.07,0.13) | 0.28 (0.21,0.35) | -0.01 (-0.11,0.09) |
| Birth (cm) | 34.31 (33.94,34.68) | 0.26 (-0.26,0.77) | 34.55 (34.18,34.93) | -0.22 (-0.74,0.29) |
| Birth to 6 months (cm/week)* | 0.40 (0.37,0.43) | -0.01 (-0.05,0.03) | 0.41 (0.38,0.44) | -0.03 (-0.06,0.01) |
| 6 months to 2 years (cm/week)* | 0.08 (0.07,0.09) | -0.001 (-0.01,0.01) | 0.07 (0.06,0.08) | 0.02 (0.004,0.03) |
| 2 years to 5 years (cm/week)* | 0.03 (0.02,0.03) | -0.0002 (-0.01,0.01) | 0.03 (0.02,0.03) | -0.002 (-0.01,0.01) |
| 5 years (cm) | 55.46 (54.91,56.02) | -0.03 (-0.82,0.76) | 55.33 (54.76,55.90) | 0.23 (-0.57,1.02) |
| **Head circumference** | | | | |
| 20 weeks (cm) | 18.60 (18.52,18.68) | -0.11 (-0.22,0.01) | 18.68 (18.60,18.76) | -0.27 (-0.38,-0.16) |
| 20 wks to 34 wks (cm/week)* | 1.01 (1.00,1.02) | -0.002 (-0.02,0.01) | 1.02 (1.01,1.03) | -0.01 (-0.02,0.004) |
| 34 wks to birth (cm/week)* | 0.64 (0.61,0.67) | 0.05 (0.004,0.09) | 0.69 (0.66,0.72) | -0.06 (-0.10,-0.01) |
| Birth (cm) | 36.62 (36.46,36.78) | 0.14 (-0.08,0.37) | 37.07 (36.91,37.22) | -0.75 (-0.97,-0.53) |
| Birth to 6 months (cm/week)* | 0.33 (0.32,0.35) | -0.01 (-0.03,0.003) | 0.34 (0.33,0.35) | -0.03 (-0.04,-0.01) |
| 6 months to 2 years (cm/week)* | 0.06 (0.05,0.06) | 0.004 (-0.001,0.01) | 0.06 (0.05,0.06) | 0.005 (-0.0003,0.009) |
| 2 years to 5 years (cm/week)* | 0.01 (0.01,0.01) | 0.001 (-0.001,0.003) | 0.01 (0.01,0.01) | 0.001 (-0.001,0.003) |
| 5 years (cm) | 51.91 (51.74,52.08) | 0.27 (0.03,0.51) | 52.50 (52.33,52.67) | -0.91 (-1.14,-0.68) |
| **Weight** | | | | |
| 20 weeks (kg) | 0.40 (0.39,0.42) | 0.002 (-0.02,0.02) | 0.41 (0.39,0.42) | 0.002 (-0.02,0.02) |
| 20 wks to 34 wks (kg/week)* | 0.16 (0.16,0.17) | -0.002 (-0.01,0.001) | 0.16 (0.16,0.17) | -0.002 (-0.01,0.002) |
| 34 wks to birth (kg/week)* | 0.24 (0.24,0.25) | 0.01 (-0.003,0.02) | 0.26 (0.25,0.26) | -0.02 (-0.03,-0.01) |
| Birth (kg) | 4.16 (4.11,4.21) | 0.01 (-0.06,0.08) | 4.24 (4.19,4.28) | -0.15 (-0.21,-0.08) |
| Birth to 6 months (kg/week)* | 0.17 (0.17,0.18) | -0.01 (-0.02,-0.001) | 0.18 (0.17,0.19) | -0.02 (-0.04,-0.01) |
| 6 months to 2 years (kg/week)* | 0.05 (0.05,0.06) | 0.005 (0.001,0.01) | 0.05 (0.05,0.06) | 0.004 (-0.0004,0.009) |
| 2 years to 5 years (kg/week)* | 0.04 (0.04,0.05) | 0.0004 (-0.002,0.003) | 0.04 (0.04,0.05) | -0.0003 (-0.002,0.002) |
| 5 years (kg) | 19.75 (19.43,20.08) | 0.15 (-0.31,0.61) | 20.08 (19.75,20.41) | -0.50 (-0.96,-0.05) |
| **Length/height** | | | | |

| | | | | |
|---|---|---|---|---|
| Birth (cm) | 52.81 (52.56,53.06) | -0.13 (-0.48,0.22) | 53.16 (52.91,53.40) | -0.83 (-1.17,-0.48) |
| Birth to 6 months (cm/month)* | 0.66 (0.64,0.68) | -0.02 (-0.04,0.01) | 0.68 (0.66,0.70) | -0.06 (-0.08,-0.03) |
| 6 months to 2 years (cm/month)* | 0.24 (0.24,0.25) | 0.01 (0.001,0.02) | 0.24 (0.23,0.25) | 0.01 (0.01,0.02) |
| 2 years to 5 years (cm/month)* | 0.13 (0.13,0.14) | 0.0003 (-0.004,0.005) | 0.13 (0.13,0.14) | -0.0003 (-0.005,0.004) |
| 5 years (cm) | 109.72 (109.17,110.28) | 0.25 (-0.54,1.04) | 110.46 (109.90,111.03) | -1.22 (-2.01,-0.43) |

*Change in growth per week/per month.

**Ethical Approval Statement**
ROLO received institutional ethical approval and maternal written consent carried out at the National Maternity Hospital, Dublin, Ireland.

**Ethical approval reference/ID**
ROLO received institutional ethical approval and maternal written consent carried out at the National Maternity Hospital, Dublin, Ireland. A reference or ID for this is not available.

**Contributorship Statement**
LMOK designed the study, conducted the analyses and wrote the paper up for publication. All other authors contributed critical revisions to the manuscript.

**Competing Interests**
None of the authors have any conflicts of interest to declare.

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**Data Sharing Agreement**
Data are available upon submission and approval of a research proposal to the PI of ROLO, Professor Fionnuala McAuliffe. Email Fionnuala.McAuliffe@ucd.ie

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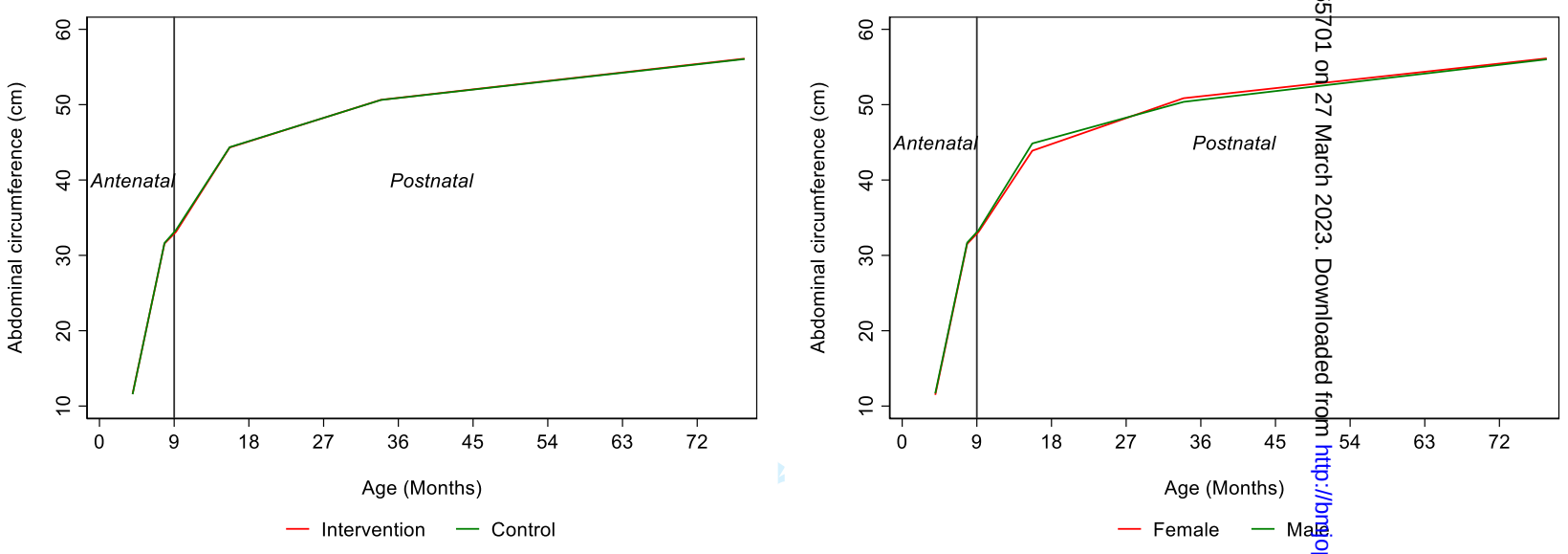
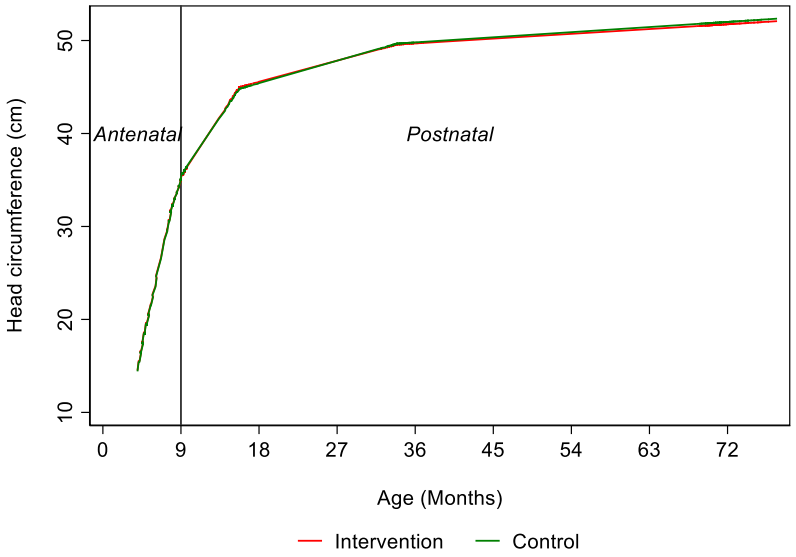



**Figure 1 Trajectories of abdominal circumference from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.



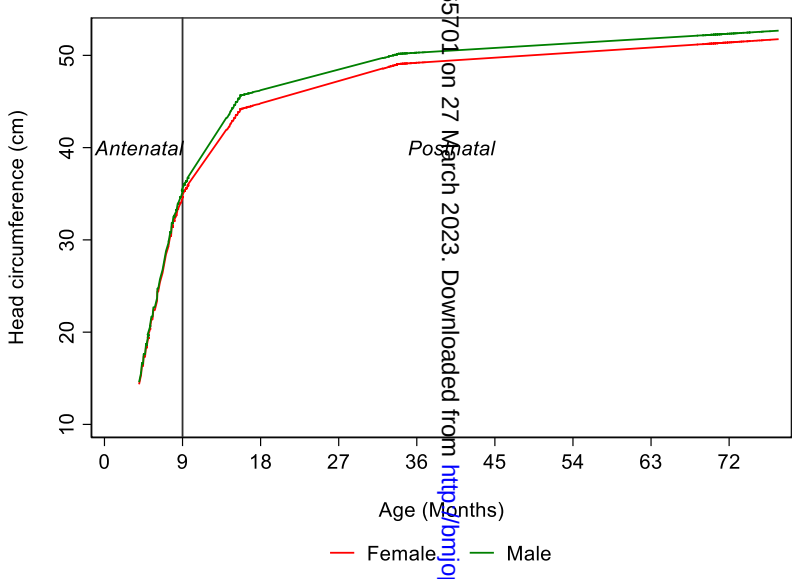




**Figure 2 Trajectories of head circumference from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.

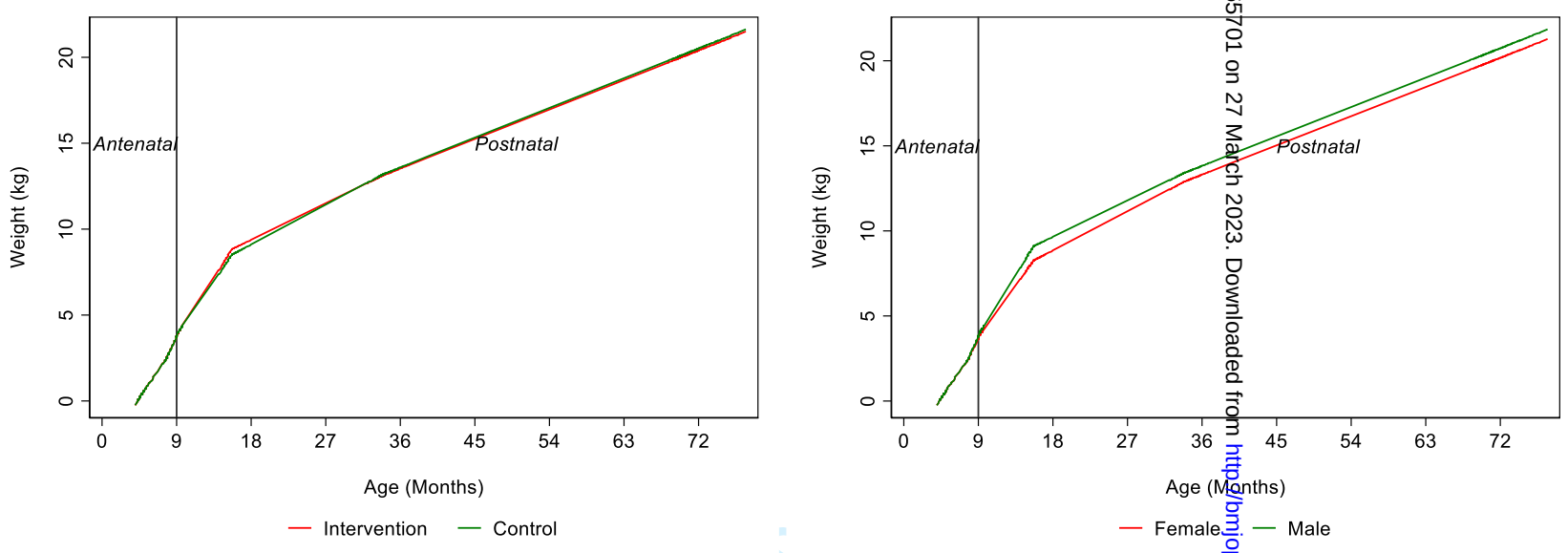



**Figure 3 Trajectories of weight from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.

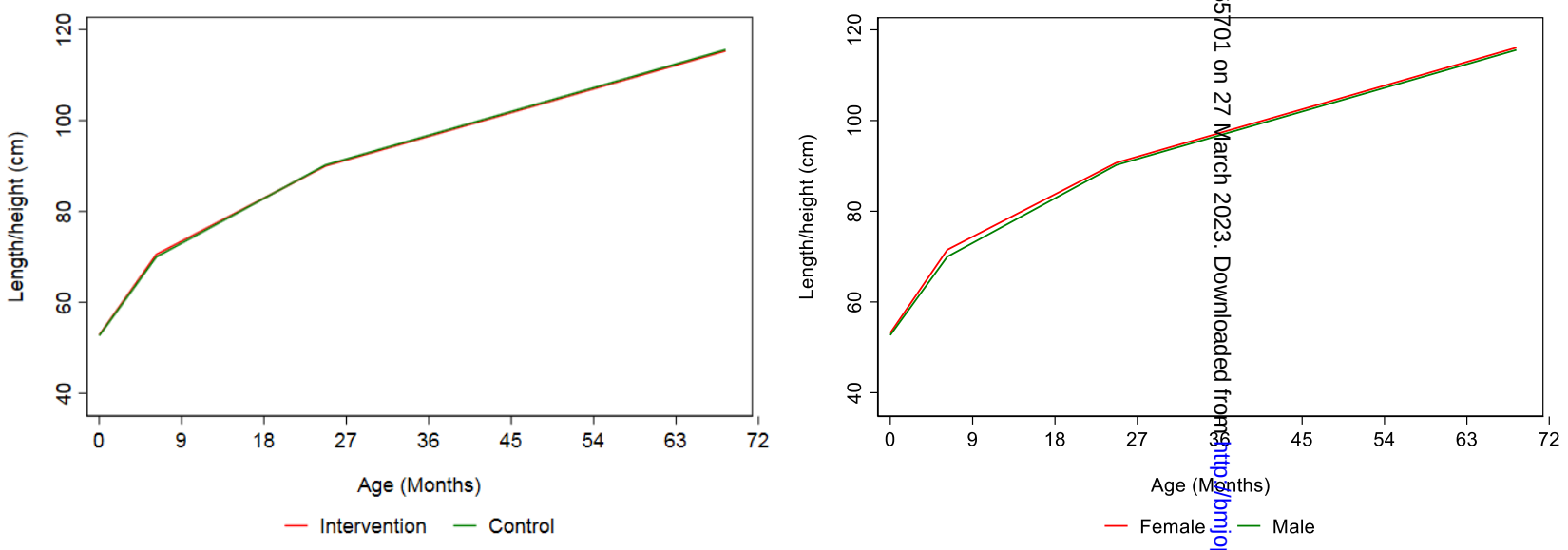



**Figure 4 Trajectories of length/height from birth to age five years by intervention status and sex. Legend:** Note that X axis displays time in months. because trajectory spans the antenatal and postnatal period.

## Supplementary Material

### Sample code for implementing linear spline multilevel models using "runmlwin" command

This syntax utilises the user-written command 'runmlwin' which must be installed prior to use. The most recent version of MLwiN must be installed to be able to use this command and this package is available for use within Stata and R. Below we demonstrate the basic steps involved in implementing linear spline multilevel modelling suing "runmlwin" in Stata. Code below assumes data are in long format and that a variable called "occasion" exists identifying the ordering of observations within individuals. Sample code below applies to length/height from birth to five years.

**Generate the spline variable**

First, three new variables are created: s1 (spline 1 from birth to 6 months), s2 (6 months to 2 year), s3 (2 years to 5 years).

```
mkspline s1_birth_6m 27 s2_6m_2 107 s3_2_max = age_lw
```

**Generate a constant term**

MLwiN does not automatically include a constant term, so this must be generated and included in models.

```
gen cons=1
```

**Identify the location of MLwiN**

```
global MLwiN_path "C:\Program Files\MLwiN v3.05\mlwin.exe"
```

**Run the multilevel model, sorting the data by person and occasion/age first.**

```
sort study_id age
runmlwin length cons s1_birth_6m s2_6m_2 s3_2_max ///
level2 (study_id: cons  s1_birth_6m s2_6m_2 s3_2_max ,  reset(var) residuals (res, var)) ///
level1 (occ: age_lw, reset(var) diag) nopause maxiterations(150)
```

**Adding covariates**

The following assumes covariates are binary and coded 0 and 1 or for covariates with multiple categories, dummy variables have been created. The addition of continuous covariates should be undertaken in the same manner as for categorical covariates but continuous covariates should be centred on the mean so that the baseline trajectory in the model is for the individuals with the mean level of the continuous covariate. Here we demonstrate the steps required for addition of sex as a covariate.

**Multiply covariate by splines**

Once the covariate is coded in the format of 0/1 representing 0 for the baseline category, we multiply the covariate by the splines, creating interaction terms for inclusion in our model.

```
gen s1_birth_6m_fem = s1_birth_6m*female
gen s2_6m_2_fem = s2_6m_2*female
gen s3_2_max_fem = s3_2_max*female
```

**Run model now including covariate terms**

The model is then ran as before but this time including a term for the covariate in question, here “female” and each of the above female*spline interaction terms generated. This allows the mean trajectory to differ for females and males. Because in this example the variable female is coded 0 for male and 1 for female the baseline trajectory is now for males with coefficients for “female”, s1_birth_6m_fem, s2_6m_2_fem, s3_2_max_fem representing the difference in the intercept, spline 1 and spline 2 and spline 3 in females compared with males.

```
sort study_id age
runmlwin length cons s1_birth_6m s2_6m_2 s3_2_max female2*, ///
level2 (study_id: cons  s1_birth_6m s2_6m_2 s3_2_max ,  reset(var) residuals (res, var)) ///
level1 (occ: age_lw, reset(var) diag) nopause maxiterations(150)
```

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 2 |
| | | (*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 | 3 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5-6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 5 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| 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 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7-8 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5-7 |
| 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 | |
| | | (*c*) Explain how missing data were addressed | |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | |
| **Results** | | | |
| 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 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 9 |

| | | | |
|---|---|---|---|
| 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<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 9-10 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | NA |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11 |
| 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 | 12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11-12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12 |
| **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 | 1 |

*Give information separately for exposed and unexposed groups.

**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 http://www.strobe-statement.org.

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# Application of multilevel linear spline models for analysis of growth trajectories in a cohort with repeat antenatal and postnatal measures of growth: a prospective cohort study



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# Application of multilevel linear spline models for analysis of growth trajectories in a cohort with repeat antenatal and postnatal measures of growth: a prospective cohort study

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**Word count:** 3006

**Abstract**

**Objectives:** To apply linear spline multilevel models to model trajectories of antenatal and postnatal growth.

**Design:** Prospective cohort study.

**Setting:** Maternity hospital in Dublin, Ireland.

**Participants:** 720-759 mother-child pairs from the ROLO study (initially a randomised controlled trial of a low glycemic index diet in pregnancy to prevent recurrence of macrosomia [birthweight > 4Kg]).

**Primary outcomes:** Trajectories of growth from 20 weeks gestation [abdominal circumference (AC), head circumference (HC) and weight] or birth (length/height) to five years

**Results:** Over 50% of women had 3rd level education and 90% were of White ethnicity. Women were a mean (SD) age of 32 years (4.2) at recruitment. The best fitting model for AC, HC and weight included a model with five linear spline periods. The best fitting models for length/height included a model with three linear spline periods from birth to six months, six months to two years and two years to five years. Comparison of observed and predicted values for each model demonstrated good model fit. For all growth measures, growth rates were generally fastest in pregnancy or immediately postpartum (for length/height), with rates of growth slowing after birth and becoming slower still as infancy and childhood progressed.

**Conclusion:** We demonstrate the application of multilevel linear spline models for examining growth trajectories when both antenatal and postnatal measures of growth are available. The approach may be useful for cohort studies or randomised controlled trials with repeat prospective assessments of growth.

**Strengths and limitations of this study**

- Using prospective follow up of a randomised control trial of macrocosmic infants, trajectories of antenatal and postnatal growth up to age 5 years were modelled as a single trajectory.
- The linear spline multilevel modelling method used maximises sample sizes for analyses, reduces selection bias and produces more precise standard errors compared with single-level approaches.
- We were not able to explore non-linear growth due to sparsity of repeated measures and this cohort are unlikely to represent the growth rates of a general population since their development is above average compared to what would be expected from an age-and gender-matched general population

## Introduction

Antenatal and childhood growth are important indicators of fetal and child health and development and are associated with health in adult life (1, 2). Consequently, modelling of growth trajectories, identifying causes and predictors of different growth trajectories and relating growth trajectories in the early life course to later life health is important for informing a life course approach to disease prevention (3-5).

A key aspect of understanding growth patterns, their causes, predictors and outcomes includes appropriate modelling of longitudinal growth data (3). Since repeated measures of growth within individuals are not independent of each other and the scale and variance of growth measures often changes over time, traditional approaches to analysis of growth data, such as single-level multiple regression, do not take account of the clustering of repeated measures within individuals (3). Moreover, the true shape of growth trajectories cannot be modelled using such approaches. While appropriate methods for the study of longitudinal growth data have been applied to antenatal and childhood growth measures in many cohort studies, most studies to date have examined antenatal growth (6, 7) or postnatal growth as separate processes/trajectories (8-14). Appropriate modelling of growth data as a continuum from antenatal to postnatal life is important to accurately characterise the shape of growth from early gestation into childhood to better understand it's aetiology. In addition, it also facilitates such trajectories to be examined as outcomes for pre-conception or early pregnancy exposures or to be examined themselves as exposures for later health outcomes (3).

Using data from the prospective follow-up of a randomised controlled trial of a low glycaemic index diet in pregnancy (ROLO study), we demonstrate the application of linear spline

multilevel models for modelling antenatal and postnatal growth trajectories using four measures of anthropometry (abdominal circumference [AC], head circumference [HC], weight and length/height) from 20 weeks' gestation to age five years.

## Methods

### Study population

The ROLO study is a randomised control trial of a low glycaemic index diet in pregnancy that recruited 800 secundigravid women who had previously given birth to a baby weighing over 4kg between 2007-2011 at the National Maternity Hospital, Dublin, Ireland (15). Women were recruited at first antenatal consultation. Women with any underlying medical disorders, including a previous history of gestational diabetes, those on any drugs, those unable to give full informed consent, aged less than 18 years, of gestation greater than 18 weeks, and having multiple pregnancies were excluded. Women were randomised to either the intervention group which received dietary advice on a low glycaemic diet, or the control group who received routine antenatal care. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

### Patient and Public Involvement

None.

### Measurement of anthropometry

*Antenatal measures*

Fetal measurements were obtained from ultrasound scans performed on mothers at medians of 20 + 6 (Interquartile Range [IQR]: 20 + 1 to 21 + 5) and 34 + 1 (IQR: 33 + 5 to 34 + 5) weeks' gestation, including AC and HC. An estimated fetal weight (EFW) at 20- and 34-weeks' gestation was calculated using the Hadlock 4-parameter formula. Ultrasound measurements were taken by two ultrasonographers using a Voluson 730 Expert (GE Medical Systems, Germany) using standard procedures.

*Postnatal measures*

At delivery, infants' AC, HC, weight and length were recorded. Follow-up anthropometry assessments were also obtained in childhood at six months, two years and five years (15-17). All measurements were obtained and calculated by a trained member of the research team. At six months, two years and five years, weight (kg) of the child was measured using a calibrated stand on digital weighing scale (SECA 813) to the nearest 0.1 kg by a trained research team member. Children were measured in light clothing without shoes. Standing height was measured, without shoes, with head aligned in the Frankfort plain, using a free-standing stadiometer (SECA 217) and measurements recorded to the nearest 0.1cm. The child's head and abdominal circumferences were measured using a SECA ergonomic circumference measuring tape, to the nearest 0.1cm. All measurements were recorded three times and the average calculated to improve reliability.

**Statistical analysis**

We used multilevel models to examine trajectories of change in AC, HC, weight and length/height from 20 weeks gestation to age five years (18, 19). Multilevel models estimate mean trajectories of the outcome while accounting for the non-independence (i.e. clustering) of repeated measurements within individuals, change in scale and variance of measures over time and differences in the number and timing of measurements between individuals (using all available data from all eligible participants under a Missing at Random [MAR] assumption) (3, 20). Table 1 shows the measures available at each occasion, demonstrating differences in the number of measurements available between individuals over time. The MLM approach used here therefore was advantageous as it allowed us to include data from all participants, regardless of whether they had one or multiple measures as shown in Table 1.

Change in all four growth measures was estimated here using linear spline multilevel models (two levels: measurement occasion and individual) (3). Linear splines allow knot points to be fit at different ages to derive periods in which change is approximately linear. The optimal linear spline model for each growth measure was selected by examining observed data for each growth measure and comparing model fit statistics of different models. Model fit statistics examined included Akaike's Information Criterion and observed and predicted values of each growth measure across the age range of the model. For AC, HC and weight, we compared the following models; models that assumed linear change over time, models with knots placed at birth and five years only (two spline periods), models with knots placed at birth, two years, and five years (three spline periods), models with knots placed at birth, six months, two years, and five years (four spline periods) and finally a model which knots at 34 weeks, birth, six months, two years and five years.The best fitting model for these included a model with knots at each measurement occasion giving rise to five linear spline periods from 20 weeks' to 34 weeks' gestation, 34 weeks' gestation to birth, birth to six months, six months to two years and two years to five years. For length/height we compared the following models: a model which assumed linear change over time, a model with knots at two and five years only (two spline periods) and a model with knots at six months, two years and five years (three spline periods). The best fitting model for length/height included a model with three linear spline periods from birth to six months, six months to two years and two years to five years.

All outcomes were normally distributed at each measurement occasion. Except for length/height which did not include antenatal measures, trajectories were centred on the first

available measure (20 weeks gestation) for AC, HC and weight. Length/height trajectories were centred at birth. For all models we placed no restrictions on the variance-covariance matrices of level two (individual level) random effects. Given the substantial change in scale and variance of growth from antenatal to postnatal life, we also aimed to allow occasion level measurement error to vary with age (level one random effects for the slope). Therefore, all models included a level one random effect for the slope while the HC model also included a level one random effect for the intercept. The final models for growth trajectories from 20 weeks gestation took the following form: $AC_{ij}$ / $HC_{ij}$ /$weight_{ij}$= $\beta_0 + u_{0j} + (\beta_1 + u_{1j})s_{ij1} + (\beta_2 + u_{2j})s_{ij2} + (\beta_3 + u_{3j})s_{ij3} + (\beta_4 + u_{4j})s_{ij4} + (\beta_5 + u_{5j})s_{ij5} + e_{ij}$ where for person j at measurement occasion i; $\beta_0$ represents the fixed effect coefficient for the average intercept, $\beta_1$ to $\beta_5$ represent fixed effect coefficients for the average linear slopes of each linear spline, $u_{0j}$ to $u_{5j}$ indicate person-specific random effects for the intercept and slopes respectively, and $e_{ij}$ represents the occasion-specific residuals or measurement error which were allowed to vary with age. The final model for length took a similar form but with only three linear spline periods due to the absence of measures prior to birth. Code for the application of these models using the "runmlwin" command from MlWin (21) within Stata 16 (22) is included in Supplementary Material.

**Results**

754, 756 and 759 offspring were included in analyses of AC, HC, and weight respectively while 720 offspring were included in analyses of length/height. Table 1 includes the number of measures of each growth measure at each measurement occasion with number of measures available broadly similar across growth measures; for example, weight measures available on each occasion included 655 measures at 20 weeks gestation, 730 at 34 weeks gestation, 756 at birth, 280 at six months, 339 at two years and 387 at five years.

Of participants included in analyses (Table 2), over 50% had completed third level education and a majority (>90%) were of White ethnicity. Among mothers of male babies, mean age (standard deviation (SD)) at delivery was approximately 32.3 (4.2) years, mean (SD) BMI at delivery was 27.1 (5.2) kg/m$^2$, mean (SD) birthweight at delivery was 4.1 (0.5) kg and median (interquartile range (IQR)) gestational age was 40.4 (39.6, 41.1) weeks. Mothers of male babies had relatively low levels of deprivation as indicated by the mean (SD) Pobal HP (Haase and Pratschke) index of 5.3 (10.8) [note the Pobal HP index is a census-based deprivation index for the Republic of Ireland which has a mean of 0 (SD=10) in the general population and ranges from -39 (most deprived) to 40 (most affluent)] (23) Characteristics were broadly similar for mothers of female babies though mothers of female babies had somewhat higher levels of third level education (~60%). Model fit as judged by differences between observed growth measures and those predicted by the models for AC, HC, weight and length are shown in Tables 3-6. Overall, our models have good model fit as all reference ranges for the difference between observed and predicted are less than the SD of the observed or less than 10% of the observed value which can be used as a rule of thumb for the assessment of model fit.

Trajectories of AC, HC and weight from 20 weeks' gestation to five years and trajectories of length/height from birth to five years by intervention status and sex are shown in Table 7, Table 8 and Figures 1-4. AC and HC had the fastest rates of growth from 20 to 34 weeks' gestation with growth rates continuing to slow thereafter up to age five years. Weight had the fastest growth rate from 34 weeks' gestation to birth with growth rates slowing somewhat from birth to six months and continuing to slow thereafter until five years. Length/height had the fastest growth rates from birth to two years, with the growth rate decreasing thereafter and slowing further from two years to five years.

We found no strong evidence of differences in trajectories of AC, weight and length/height between the intervention and control group, but we found some evidence of slightly greater HC (difference 0.27 cm (95% Confidence Interval [CI] = 0.03, 0.51) emerging among the control group at five years. AC trajectories did not differ between males and females, though we found some evidence of modest differences in HC, weight and length/height trajectories between males and females. Females had lower HC at 20 weeks gestation with this difference widening at birth and persisting at age five years (difference at five years: -0.91cm, 95% CI = -1.14, -0.68). Females had -0.15kg (95% CI = -0.21, -0.08) lower birth weight and slower postnatal growth rates in weight leading to -0.50 kg (95% CI = -0.96, -0.05) lower weight among females at five years. Similarly, females were -0.83 cm (95% CI = -1.17, -0.48) shorter in length at birth and had slower postnatal growth rates in length/height leading to -1.22 cm (95% CI = -2.01, -0.43) shorter height among females at five years.

## Discussion

In this prospective follow-up of a randomised control trial of approximately 750 infants at high risk of macrosomia, we demonstrated the use of linear spline multilevel modelling to examine trajectories of AC, HC, weight and length/height from 20 weeks' gestation to age five years. We showed their applicability to data with repeated measures of growth which span the antenatal and postnatal period, even when as few as four repeat assessments are available (in the case of length/height) and measures are sparse.

All women in this study previously had a macrosomic infant, and over half of infants had a birthweight in the macrosomic range (>4kg). To our knowledge previous analyses have not examined antenatal and postnatal growth trajectories together. Other cohorts have examined the antenatal or postnatal trajectories of infants like ours (24, 25), but differences in methodological approaches such as the use of a group-based approach in the LIFECODES cohort make comparisons challenging (25). Our findings for antenatal growth are however broadly similar to a study examining the growth trajectories of abdominal circumference in macrosomic infants from 20 weeks gestation to birth in 244 singleton pregnancies (26). For example, the macrosomic infants of mothers with gestational diabetes had a fetal abdominal circumference of approximately 1.3cm at 20 weeks, increasing linearly leading to an abdominal circumference of 3.6cm at birth which is broadly comparable with the abdominal circumferences and growth rates found in our study. In comparison with findings from analyses of growth in the Born in Bradford, Generation XXI, Pelotas and PROBIT cohorts (3), our postnatal growth rates are expectedly slower than the growth rates of these general population cohorts. This is consistent with the "catch-down" or slower postnatal growth expected in our high birthweight cohort (27). For example, infants in our cohort were born

52.8cm in length and grew at 2.85cm per month in the first three months after birth; this growth rate is slower than that of infants in the ALSPAC cohort, born 50cm in length and growing at the faster rate of 3.57cm per month in the 3 months after birth. Our growth rates are more comparable to those of the PROBIT cohort which included only infants greater than 2500g birthweight and is the cohort in this analysis likely to be most similar to ours (birth length 51.4cm and growth rate of 2.96cm per month in the three months after birth). However, comparisons with previous studies should be undertaken with caution due to population differences (large birthweight vs general populations) and differences in methodological approaches.

There are several strengths to the general approach of multilevel models taken here; these include the ability to maximise sample sizes for analyses and reduce selection bias compared with single-level approaches since multilevel models can include all participants with at least one growth measure under a MAR assumption (3). This is particularly advantageous where attrition rates from cohorts are high. Further advantages include more precise standard errors which consider the non-independence of repeated measures. There are also additional advantages of the approach of modelling antenatal and postnatal growth together in our cohort. A practical advantage includes the ability to examine this trajectory as a single trajectory outcome for pre-pregnancy or gestational exposures. This allows associations of pre-pregnancy or gestational exposures with antenatal growth rates to be examined, thereby providing insights into the timing of the impact of exposures during pregnancy that analyses of summary birth anthropometry measures alone (such as birth weight) cannot provide. Moreover, within the same model, the associations of such pre-pregnancy or gestational exposures with postnatal growth rates can be examined. This can provide important insights

into whether associations of pre-pregnancy or gestational exposures have intrauterine mechanisms compared with those that have an impact on growth across intrauterine and postnatal life or perhaps have an impact on intrauterine growth that persists in postnatal life; this latter finding would be overlooked entirely in a model which examines postnatal growth trajectories alone because associations in intrauterine life would be missed due to only examining the postnatal trajectory. . In addition, the modelling of antenatal and postnatal growth together allows participants with only one antenatal measure and no postnatal measure or vice versa to be included in analyses, with a full trajectory estimated for that participant under the previously discussed MAR assumption. This can boost sample sizes (28) and reduce selection bias induced by analysing antenatal and postnatal trajectories separately because participants with no antenatal or no postnatal measure would be excluded from their respective trajectory analyses whereas in the joint-modelling approach a full trajectory (from antenatal to postnatal life) can be estimated for these participants. Limitations of this work include an inability to explore other non-linear growth patterns such as fractional polynomials due to the sparsity of measures which did not allow a range of possible shapes of growth trajectories to be explored (3). In cohorts with greater numbers of repeated measures and density of repeats, linear spline multilevel modelling can be implemented and compared to other possible shapes include fractional polynomials which have been shown to provide a more biologically intuitive shape of change(3). However, the linear spline approach demonstrated here provides many practical advantages including being more easily interpretable, allowing analysts to split trajectories into distinct periods of change that can then be easily related to exposures and outcomes. It should be noted that this cohort are unlikely to represent the growth rates or trajectories of a general population since their development is above average compared to what would be expected from an age

and gender matched general population (the cohort is roughly approximated to the 75$^{th}$ centile based on a crude comparison of means and SDs on the UK-WHO (Ireland) chart) (29).

**Conclusion**

We demonstrate the application of multilevel linear spline models for examining growth trajectories when both antenatal and postnatal measures of growth are available. The approach may be useful for cohort studies or randomised control trials with repeat prospective assessments of fetal growth spanning pregnancy and childhood.

**Table 1 N repeated measures included in analyses for each growth measure**

| | 20 weeks | 34 weeks | Birth | 6 months | 2 years | 5 years |
|---|---|---|---|---|---|---|
| **Abdominal circumference** | 656 | 732 | 265 | 280 | 336 | 385 |
| **Head circumference** | 656 | 700 | 634 | 280 | 333 | 386 |
| **Weight** | 655 | 730 | 756 | 280 | 339 | 387 |
| **Length/height** | | | 634 | 280 | 339 | 386 |

**Table 2 Characteristics of ROLO participants included in the analysis of length/height, by sex**

| | Male N=358 | Female N=362 |
|---|---|---|
| | **n (%)** | **n (%)** |
| **Completed 3rd level education** | 151 (50.3) | 187 (60.9) |
| **Non-white ethnicity** | 5 (1.4) | 9 (2.5) |
| | **Mean (SD)** | **Mean (SD)** |
| **Mothers age at delivery (years)** | 32.3 (4.2) | 32.6 (4.2) |
| **Pobal HP index (unit)** | 5.3 (10.8) | 5.4 (9.7) |
| **Mothers BMI (kg/m²)** | 27.1 (5.2) | 26.2 (4.4) |
| **Birthweight (kg)** | 4.1 (0.5) | 4.0 (0.4) |
| | **Median (IQR)** | **Median (IQR)** |
| **Gestational age at delivery (weeks)** | 40.4 (39.6, 41.1) | 40.3 (39.6, 41.1) |

SD, standard deviation; IQR, interquartile range. The Pobal HP index is a census-based deprivation index for the Republic of Ireland which has a mean of 0 (SD=10) in the general population and ranges from -39 (most deprived) to 40 (most affluent).

**Table 3 Model details for abdominal circumference**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 531 | 22.48 (6.92) | 22.59 (6.86) | -0.08 | -1.13 to 0.97 |
| **34 wks to birth** | 517 | 32.22 (2.08) | 32.35 (1.19) | 0.08 | -2.01 to 2.17 |
| **Birth to 6 months** | 315 | 38.21 (5.93) | 36.01 (4.51) | -0.05 | -3.64 to 3.53 |
| **6 months to 2 years** | 272 | 47.91 (5.37) | 47.84 (3.96) | 0.09 | -4.25 to 4.42 |
| **2 years to 5 years** | 681 | 50.25 (8.98) | 54.03 (2.72) | -0.06 | -6.11 to 6.00 |

SD, standard deviation; wks, weeks. Model fit is summarised for age periods rather than specific ages as there may not be enough people at specific ages to provide a meaningful summary.

**Table 4 Model details for head circumference**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 292 | 22.90 (4.87) | 22.86 (4.83) | 0.09 | -0.55 to 0.73 |
| **34 wks to birth** | 680 | 32.54 (1.67) | 32.63 (1.53) | -0.01 | -0.61 to 0.59 |
| **Birth to 6 months** | 642 | 37.57 (3.70) | 37.39 (3.46) | 0.00 | -0.48 to 0.47 |
| **6 months to 2 years** | 274 | 47.29 (2.98) | 47.28 (2.87) | 0.01 | -0.47 to 0.49 |
| **2 years to 5 years** | 661 | 48.89 (6.16) | 51.18 (1.64) | -0.01 | -0.75 to 0.73 |

SD, standard deviation; wks, weeks. Model fit is summarised for age periods rather than specific ages as there may not be enough people at specific ages to provide a meaningful summary

**Table 5 Model details for weight**

| | Total number of observations | Mean observed (SD) in kg | Mean predicted (SD) in kg | Mean difference (observed – predicted) in kg | 95% level of agreement between observed and predicted in kg |
|---|---|---|---|---|---|
| **20 wks to 34 wks** | 294 | 0.97 (0.81) | 1.04 (0.77) | -0.07 | -0.24 to 0.10 |
| **34 wks to birth** | 708 | 2.93 (0.58) | 2.89 (0.51) | 0.04 | -0.31 to 0.38 |
| **Birth to 6 months** | 735 | 4.87 (1.87) | 4.88 (1.81) | -0.01 | -0.44 to 0.43 |
| **6 months to 2 years** | 276 | 10.92 (2.59) | 10.91 (2.53) | 0.01 | -0.36 to 0.38 |
| **2 years to 5 years** | 695 | 15.54 (6.60) | 18.30 (3.88) | -0.01 | -0.42 to 0.41 |

SD, standard deviation; wks, weeks. Model fit is summarised for age periods rather than specific ages as there may not be enough people at specific ages to provide a meaningful summary

**Table 6 Model details for length**

| | Total number of observations | Mean observed (SD) in cm | Mean predicted (SD) in cm | Mean difference (observed – predicted) in cm | 95% level of agreement between observed and predicted in cm |
|---|---|---|---|---|---|
| **Birth to 6 months** | 475 | 57.55 (7.51) | 57.14 (7.25) | 0.0001 | -0.03 to 0.03 |
| **6 months to 2 years** | 304 | 81.03 (10.12) | 81.03 (10.08) | -0.002 | -0.57 to 0.56 |
| **2 years to 5 years** | 574 | 104.07 (12.24) | 106.42 (9.79) | 0.0004 | -2.92 to 2.92 |

SD, standard deviation; wks, weeks. Model fit is summarised for age periods rather than specific ages as there may not be enough people at specific ages to provide a meaningful summary

**Table 7 Mean growth rates of anthropometry and mean difference in growth rates by intervention status and sex in the ROLO cohort from 20 weeks to 5 years**

| | Mean growth rate (95% CI) in intervention | Mean growth rate difference (95% CI) in controls compared with intervention | Mean growth rate (95% CI) in males | Mean difference in growth rate (95% CI) in females compared with males |
|---|---|---|---|---|
| **Abdominal circumference** | | | | |
| 20 wks to 34 wks (cm/week) | 1.20 (1.18,1.22) | 0.01 (-0.02,0.03) | 1.20 (1.19,1.22) | 0.002 (-0.02,0.03) |
| 34 wks to birth (cm/week) | 0.26 (0.19,0.33) | 0.03 (-0.07,0.13) | 0.28 (0.21,0.35) | -0.01 (-0.11,0.09) |
| Birth to 6 months (cm/week) | 0.40 (0.37,0.43) | -0.01 (-0.05,0.03) | 0.41 (0.38,0.44) | -0.03 (-0.06,0.01) |
| 6 months to 2 years (cm/week) | 0.08 (0.07,0.09) | -0.001 (-0.01,0.01) | 0.07 (0.06,0.08) | 0.02 (0.004,0.03) |
| 2 years to 5 years (cm/week) | 0.03 (0.02,0.03) | -0.0002 (-0.01,0.01) | 0.03 (0.02,0.03) | -0.002 (-0.01,0.01) |
| **Head circumference** | | | | |
| 20 wks to 34 wks (cm/week) | 1.01 (1.00,1.02) | -0.002 (-0.02,0.01) | 1.02 (1.01,1.03) | -0.01 (-0.02,0.004) |
| 34 wks to birth (cm/week) | 0.64 (0.61,0.67) | 0.05 (0.004,0.09) | 0.69 (0.66,0.72) | -0.06 (-0.10,-0.01) |
| Birth to 6 months (cm/week) | 0.33 (0.32,0.35) | -0.01 (-0.03,0.003) | 0.34 (0.33,0.35) | -0.03 (-0.04,-0.01) |
| 6 months to 2 years (cm/week) | 0.06 (0.05,0.06) | 0.004 (-0.001,0.01) | 0.06 (0.05,0.06) | 0.005 (-0.0003,0.009) |
| 2 years to 5 years (cm/week) | 0.01 (0.01,0.01) | 0.001 (-0.001,0.003) | 0.01 (0.01,0.01) | 0.001 (-0.001,0.003) |
| **Weight** | | | | |
| 20 wks to 34 wks (kg/week) | 0.16 (0.16,0.17) | -0.002 (-0.01,0.001) | 0.16 (0.16,0.17) | -0.002 (-0.01,0.002) |
| 34 wks to birth (kg/week) | 0.24 (0.24,0.25) | 0.01 (-0.003,0.02) | 0.26 (0.25,0.26) | -0.02 (-0.03,-0.01) |
| Birth to 6 months (kg/week) | 0.17 (0.17,0.18) | -0.01 (-0.02,-0.001) | 0.18 (0.17,0.19) | -0.02 (-0.04,-0.01) |
| 6 months to 2 years (kg/week) | 0.05 (0.05,0.06) | 0.005 (0.001,0.01) | 0.05 (0.05,0.06) | 0.004 (-0.0004,0.009) |
| 2 years to 5 years (kg/week) | 0.04 (0.04,0.05) | 0.0004 (-0.002,0.003) | 0.04 (0.04,0.05) | -0.0003 (-0.002,0.002) |
| **Length/height** | | | | |
| Birth to 6 months (cm/week) | 0.66 (0.64,0.68) | -0.02 (-0.04,0.01) | 0.68 (0.66,0.70) | -0.06 (-0.08,-0.03) |
| 6 months to 2 years (cm/week) | 0.24 (0.24,0.25) | 0.01 (0.001,0.02) | 0.24 (0.23,0.25) | 0.01 (0.01,0.02) |
| 2 years to 5 years (cm/week) | 0.13 (0.13,0.14) | 0.0003 (-0.004,0.005) | 0.13 (0.13,0.14) | -0.0003 (-0.005,0.004) |

*Change in growth per week/per month.

**Table 8 Mean absolute growth measure and difference in absolute growth measure by intervention status and sex in the ROLO cohort at 20 weeks gestation, birth and 5 years**

| | Mean (95% CI) in intervention | Mean difference in controls compared with intervention | Mean (95% CI) in males | Mean difference in females compared with males |
|---|---|---|---|---|
| **Abdominal circumference** | | | | |
| 20 weeks (cm) | 15.96 (15.85,16.07) | -0.02 (-0.17,0.14) | 16.05 (15.94,16.16) | -0.20 (-0.35,-0.04) |
| Birth (cm) | 34.31 (33.94,34.68) | 0.26 (-0.26,0.77) | 34.55 (34.18,34.93) | -0.22 (-0.74,0.29) |
| 5 years (cm) | 55.46 (54.91,56.02) | -0.03 (-0.82,0.76) | 55.33 (54.76,55.90) | 0.23 (-0.57,1.02) |
| **Head circumference** | | | | |
| 20 weeks (cm) | 18.60 (18.52,18.68) | -0.11 (-0.22,0.01) | 18.68 (18.60,18.76) | -0.27 (-0.38,-0.16) |
| Birth (cm) | 36.62 (36.46,36.78) | 0.14 (-0.08,0.37) | 37.07 (36.91,37.22) | -0.75 (-0.97,-0.53) |
| 5 years (cm) | 51.91 (51.74,52.08) | 0.27 (0.03,0.51) | 52.50 (52.33,52.67) | -0.91 (-1.14,-0.68) |
| **Weight** | | | | |
| 20 weeks (kg) | 0.40 (0.39,0.42) | 0.002 (-0.02,0.02) | 0.41 (0.39,0.42) | 0.002 (-0.02,0.02) |
| Birth (kg) | 4.16 (4.11,4.21) | 0.01 (-0.06,0.08) | 4.24 (4.19,4.28) | -0.15 (-0.21,-0.08) |
| 5 years (kg) | 19.75 (19.43,20.08) | 0.15 (-0.31,0.61) | 20.08 (19.75,20.41) | -0.50 (-0.96,-0.05) |
| **Length/height** | | | | |
| Birth (cm) | 52.81 (52.56,53.06) | -0.13 (-0.48,0.22) | 53.16 (52.91,53.40) | -0.83 (-1.17,-0.48) |
| 5 years (cm) | 109.72 (109.17,110.28) | 0.25 (-0.54,1.04) | 110.46 (109.90,111.03) | -1.22 (-2.01,-0.43) |

**Ethical Approval Statement**
ROLO received institutional ethical approval and maternal written consent carried out at the National Maternity Hospital, Dublin, Ireland.

**Ethical approval reference/ID**
ROLO received institutional ethical approval and maternal written consent carried out at the National Maternity Hospital, Dublin, Ireland. A reference or ID for this is not available.

**Contributorship Statement**
LMOK and FMM designed and planned the study, conducted the analyses, and wrote the paper up for publication. CAY, HCB, KNON, CMc provided input on the modelling strategy, contributed to interpretation of data and provided critical revisions to the manuscript.

**Data Sharing Agreement**
Data are available upon submission and approval of a research proposal to the PI of ROLO, Professor Fionnuala McAuliffe. Email Fionnuala.McAuliffe@ucd.ie

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**Conflict of Interest Statement**
None of the authors have any conflicts of interest to declare.

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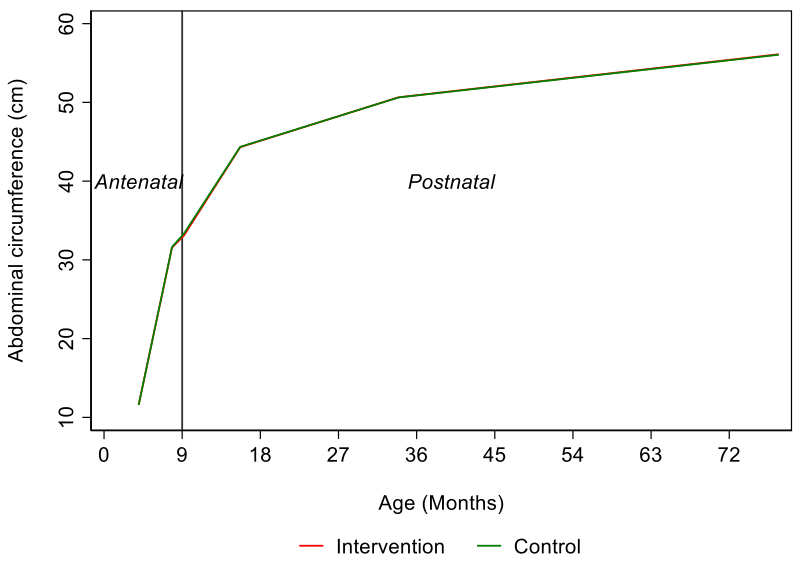


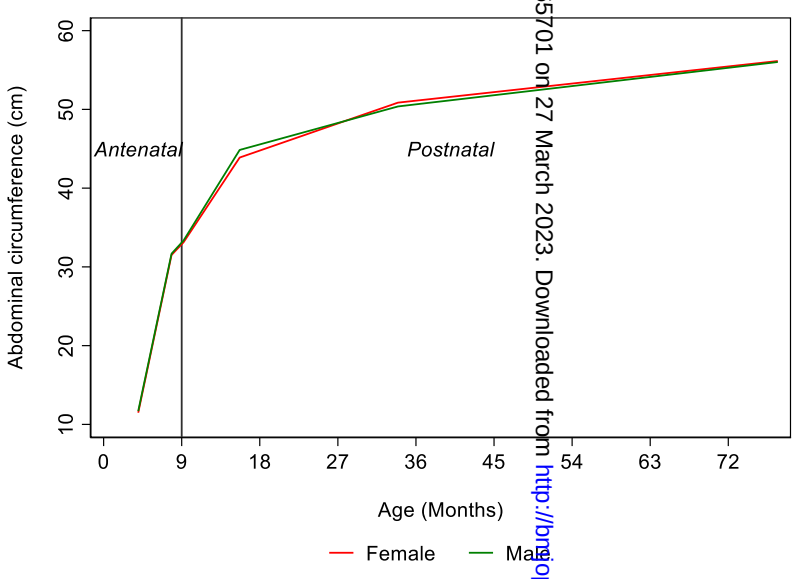


**Figure 1 Trajectories of abdominal circumference from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.

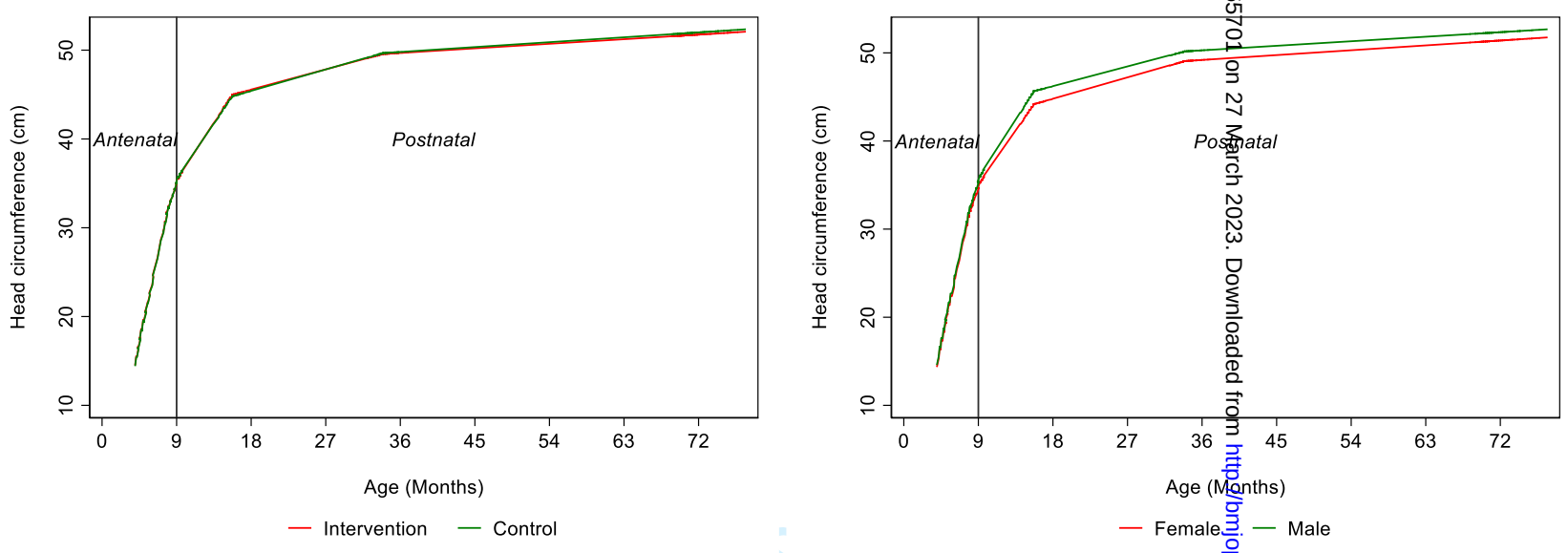


**Figure 2 Trajectories of head circumference from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.

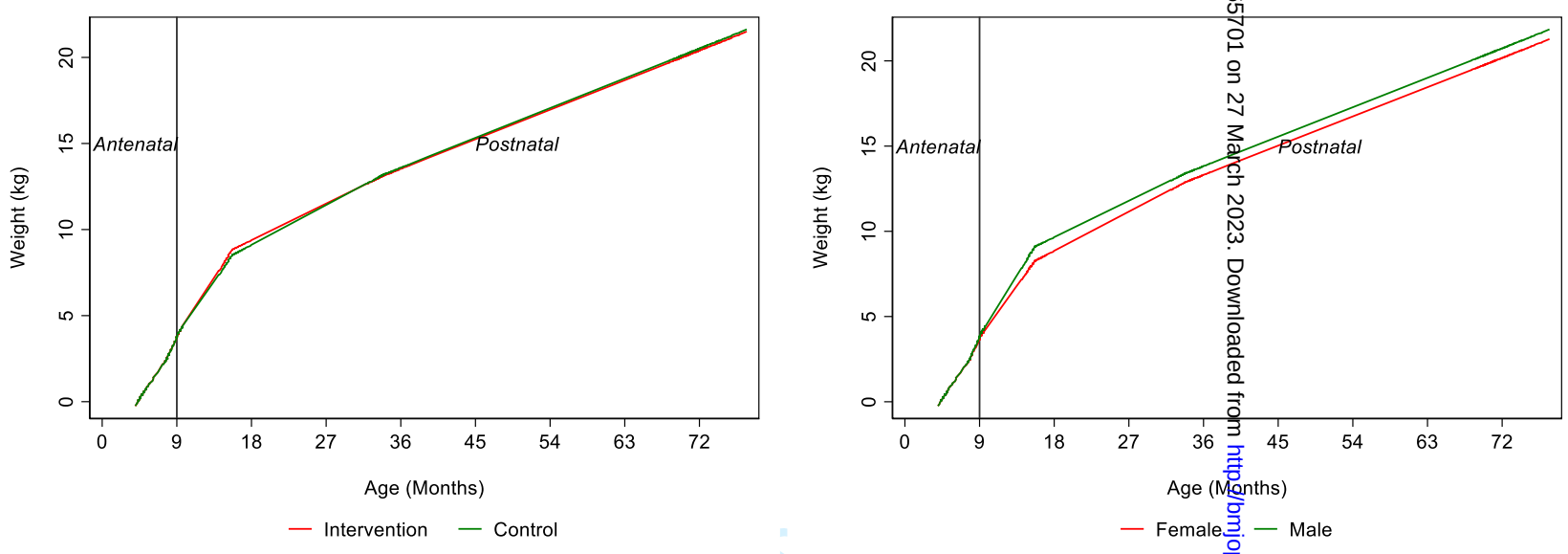


**Figure 3 Trajectories of weight from 20 weeks gestation to age five years by intervention status and sex. Legend:** Note that X axis displays time in months because trajectory spans the antenatal and postnatal period.

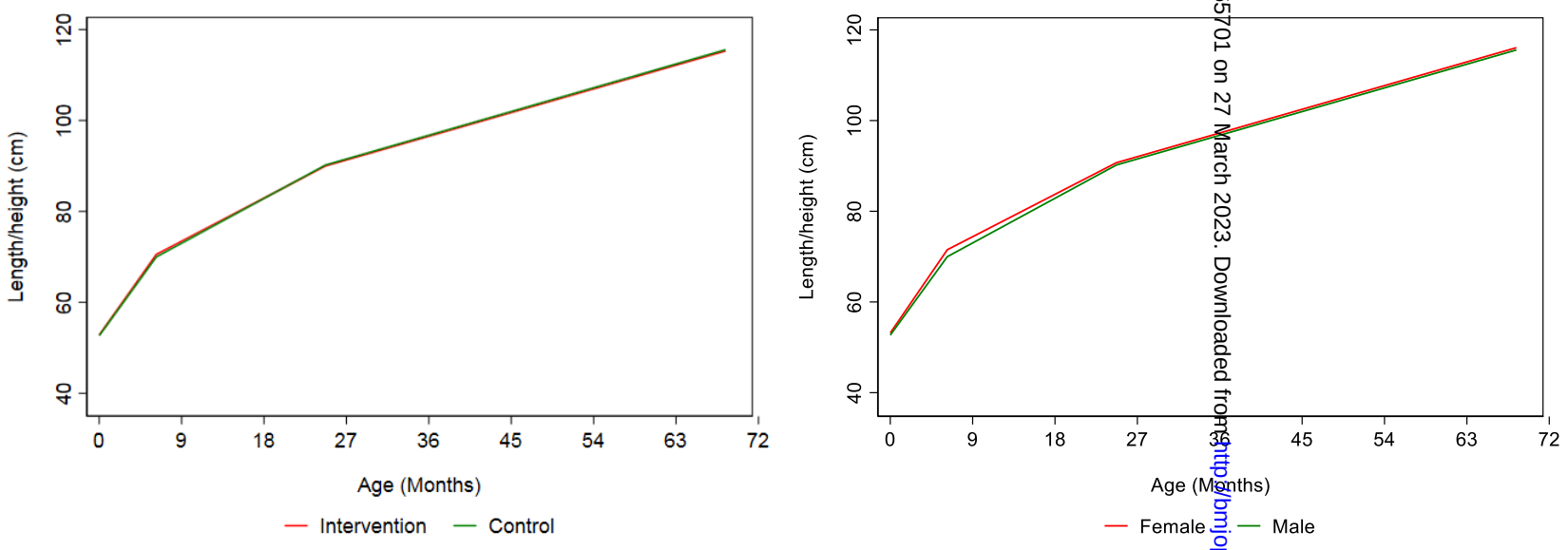


**Figure 4 Trajectories of length/height from birth to age five years by intervention status and sex. Legend:** Note that X axis displays time in months. because trajectory spans the antenatal and postnatal period.

# Supplementary Material

## Sample code for implementing linear spline multilevel models using "runmlwin" command

This syntax utilises the user-written command 'runmlwin' which must be installed prior to use. The most recent version of MLwiN must be installed to be able to use this command and this package is available for use within Stata and R. Below we demonstrate the basic steps involved in implementing linear spline multilevel modelling suing "runmlwin" in Stata. Code below assumes data are in long format and that a variable called "occasion" exists identifying the ordering of observations within individuals. Sample code below applies to length/height from birth to five years.

### Generate the spline variable

First, three new variables are created: s1 (spline 1 from birth to 6 months), s2 (6 months to 2 year), s3 (2 years to 5 years).

```
mkspline s1_birth_6m 27 s2_6m_2 107 s3_2_max = age_lw
```

### Generate a constant term

MLwiN does not automatically include a constant term, so this must be generated and included in models.

```
gen cons=1
```

### Identify the location of MLwiN

```
global MLwiN_path "C:\Program Files\MLwiN v3.05\mlwin.exe"
```

### Run the multilevel model, sorting the data by person and occasion/age first.

```
sort study_id age
runmlwin length cons s1_birth_6m s2_6m_2 s3_2_max ///
level2 (study_id: cons  s1_birth_6m s2_6m_2 s3_2_max ,  reset(var) residuals (res, var)) ///
level1 (occ: age_lw, reset(var) diag) nopause maxiterations(150)
```

### Adding covariates

The following assumes covariates are binary and coded 0 and 1 or for covariates with multiple categories, dummy variables have been created. The addition of continuous covariates should be undertaken in the same manner as for categorical covariates but continuous covariates should be centred on the mean so that the baseline trajectory in the model is for the individuals with the mean level of the continuous covariate. Here we demonstrate the steps required for addition of sex as a covariate.

**Multiply covariate by splines**

Once the covariate is coded in the format of 0/1 representing 0 for the baseline category, we multiply the covariate by the splines, creating interaction terms for inclusion in our model.

```
gen s1_birth_6m_fem = s1_birth_6m*female
gen s2_6m_2_fem = s2_6m_2*female
gen s3_2_max_fem = s3_2_max*female
```

**Run model now including covariate terms**

The model is then ran as before but this time including a term for the covariate in question, here "female" and each of the above female*spline interaction terms generated. This allows the mean trajectory to differ for females and males. Because in this example the variable female is coded 0 for male and 1 for female the baseline trajectory is now for males with coefficients for "female", s1_birth_6m_fem, s2_6m_2_fem, s3_2_max_fem representing the difference in the intercept, spline 1 and spline 2 and spline 3 in females compared with males.

```
sort study_id age
runmlwin length cons s1_birth_6m s2_6m_2 s3_2_max female2*, ///
level2 (study_id: cons  s1_birth_6m s2_6m_2 s3_2_max ,  reset(var) residuals (res, var)) ///
level1 (occ: age_lw, reset(var) diag) nopause maxiterations(150)
```

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 2 |
| | | (*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 | 3 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5-6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 5 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| 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 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7-8 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5-7 |
| 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 | |
| | | (*c*) Explain how missing data were addressed | |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | |
| **Results** | | | |
| 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 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 9 |

| | | | |
|---|---|---|---|
| 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<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 9-10 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | NA |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11 |
| 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 | 12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11-12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12 |
| **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 | 1 |

*Give information separately for exposed and unexposed groups.

**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 http://www.strobe-statement.org.