

Report on approaches for longitudinal data/health trajectories in the context of life course analyses

Work package 7 - Task 7.2 - Deliverable 7.2

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Table of contents

1. Introduction	3
2. Work performed	3
2.1. Searchable database of method materials of importance for causal inference in life-course epidemiology	3
2.2. Reviews of causal inference methods of relevance for LifeCycle	4
2.2.1 Methods papers published and in progress with preliminary results	4
2.2.2 Reviews for the preparation of the tutorials	14
2.3. Applied examples in LifeCycle	14
3. Conclusions	24

1. Introduction

The WP7 of LifeCycle focuses on specific methodological aspects of importance for the LifeCycle applied work packages (i.e. WP4 to 6 and 8) and life course research in general. It aims at developing an integrated analysis strategy to apply causal inference methods, model longitudinal data and health trajectories; assessing approaches to analyse multiple exposure and potential mediating data in the context of longitudinal modelling; and, at the same time, enhancing, inside and outside LifeCycle, the knowledge and use of causal inference approaches and methods to model longitudinal (repeatedly assessed data).

Task 7.2 focuses on different methods for analysing repeatedly assessed data from a theoretical and applied approach. Four areas of interest and need were identified early in LifeCycle: (1) understanding different methods from a theoretical and applied perspective; (2) understanding the importance of the research question, together with the data available data in choosing a method/methods; (3) identifying methods for joining repeatedly assessed data from different cohorts together (e.g. doing this across the cohorts in EU Child Cohort Network) so that we can establish life course trajectories that go from birth to adult life and can assess the relationship of exposures with those (full) life-course trajectories and (4) sharing code and practical support so that colleagues within the LifeCycle partnership and beyond can apply these analyses, including in DataShield. This deliverable covers these four aspects. Its results will also contribute to future WP7 deliverables, specifically to the analyses of the exposome on longitudinal (life-course) trajectories (Deliverable 7.3), demonstration of the analytical strategies with tutorials (Deliverable 7.4), and the development of courses on causal inference methods and longitudinal modelling in the context of life course analyses (Deliverable 7.5).

2. Work performed

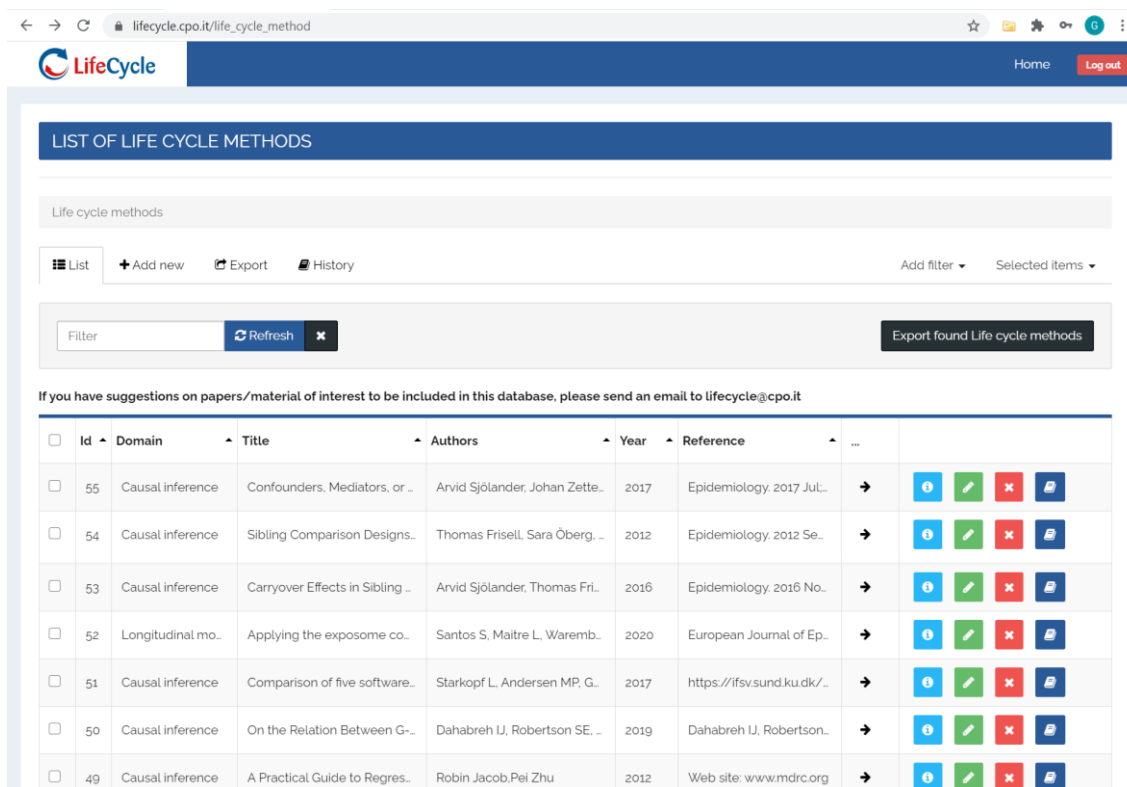
2.1. Searchable database of method materials of importance for causal inference in life-course epidemiology

One of the objectives of WP7 is to enhance the knowledge and use of causal inference approaches, as well as methods to model longitudinal data, within LifeCycle. We intend to provide access to all members of LifeCycle to a variety of methodology materials on the topic of causal inference and longitudinal modelling. For this purpose, we created a searchable database filled in by LifeCycle (mostly WP7) experts as they come along with materials of interest for the broad audience of LifeCycle members. The materials are selected based on their relevance to the specific research projects conducted within LifeCycle and their readability by non-specialists. By accessing the database, any member who wants to get familiar with the field can have a rapid overview of the methods and challenges. This database can also be the basis for deeper study of the field.





























The web application, hosted at Telecom Italia SPC Cloud infrastructure and available at <https://lifecycle.cpo.it>, was built with Ruby on Rails and MySQL. The interface is user-friendly and intuitive with filters and export functionalities (**Figure 1**). It has been filled by ten contributors since 23 April 2018 and contains 70 items as of 15 November 2020. Each item is described through 17 features: unique id, title of the work, source online, type of work (educational, tutorial, review, or research paper), year of publication, topic (causal inference, longitudinal data, or both), authors, reference, key words, short summary, comments, open access (yes or no), contains codes for reuse in statistical

packages (yes or no), date of entry in the database, date of update, contributing member, and full text link (if open access). A total of 87% of the records are open access publications, and 29% of the publications contain programming codes.

Figure 1: Screen shot of the searchable database.



The screenshot shows a web browser window with the URL `lifecycle.cpo.it/life_cycle_method`. The page title is "LIST OF LIFE CYCLE METHODS". Below the title, there are navigation options: "List", "Add new", "Export", and "History". A search bar with a "Filter" input, a "Refresh" button, and an "Export found Life cycle methods" button is present. Below the search bar, there is a message: "If you have suggestions on papers/material of interest to be included in this database, please send an email to lifecycle@cpo.it".

<input type="checkbox"/>	Id	Domain	Title	Authors	Year	Reference	...	
<input type="checkbox"/>	55	Causal inference	Confounders, Mediators, or ...	Arvid Sjölander, Johan Zette...	2017	Epidemiology, 2017 Jul...	→	   
<input type="checkbox"/>	54	Causal inference	Sibling Comparison Designs...	Thomas Frisell, Sara Öberg, ...	2012	Epidemiology, 2012 Se...	→	   
<input type="checkbox"/>	53	Causal inference	Carryover Effects in Sibling ...	Arvid Sjölander, Thomas Fri...	2016	Epidemiology, 2016 No...	→	   
<input type="checkbox"/>	52	Longitudinal mo...	Applying the exposome co...	Santos S, Maitre L, Waremb...	2020	European Journal of Ep...	→	   
<input type="checkbox"/>	51	Causal inference	Comparison of five software...	Starkopf L, Andersen MP, G...	2017	https://ifsv.sund.ku.dk/...	→	   
<input type="checkbox"/>	50	Causal inference	On the Relation Between G...	Dahabreh IJ, Robertson SE, ...	2019	Dahabreh IJ, Robertson...	→	   
<input type="checkbox"/>	49	Causal inference	A Practical Guide to Regres...	Robin Jacob,Pei Zhu	2012	Web site: www.mdrc.org	→	   

Among the 70 items, 49 have a focus on causal inference, nine on longitudinal modelling, six pertain to both topics, and the remain six to the recently added field of exposome and omics. The majority (35 items) are educational papers or books, while 16 are tutorials, reviews, or textbooks, 11 are applied methods papers, and eight are original methods papers.

Please Note We provide a more detailed description of this databse in the Deliverable 7.1 report, which we have submitted alongside this report for deliverable 7.2, and have not repeated all of that detail here. The database is relevant to Tasks 7.1, 7.2 and 7.3.

2.2. Reviews of repeat measures analysis methods of relevance to LifeCycle

2.2.1 Methods papers published and in progress with preliminary results

We have two methods papers at an advanced stage. One of these is published on a preprint sever and submitted to the American Journal of Epidemiology (AJE) and one is at an advanced stage. Both are described here.

Paper 1: Hughes RA, Tilling K, Lawlor DA. Combining longitudinal data from different cohorts to examine the life-course trajectory. MedRxiv 2020 MedRxiv 2020;

<https://medrxiv.org/cgi/content/short/2020.11.24.20237669v1> (under consideration with AJE).

Overview

This paper focuses on methods for combining repeatedly assessed data from several cohorts to generate trajectories that cover a larger age span than any of the single cohorts. The key challenges and how to approach these are described. These are illustrated in a real data example, which used repeat measures of weight from birth to early adulthood from 5 cohorts, and explored the associations of sex, ethnicity and parental (family) socioeconomic position with the weight trajectories. Developing these methods is a key part of task 7.2 and central to the overall aims of LifeCycle.

Extended Summary

Longitudinal data analysis is necessary to reveal changes within the same individual as they age. However, few studies are able to capture multiple decades across the life-course. We investigate the challenges in combining data from cohorts with repeated measurements that cover different and overlapping periods of life and demonstrate methods to overcome these challenges. Our illustrative example examines the effects of parental education, sex, and ethnicity on weight trajectories. Data were from five prospective cohorts (one in Belarus, 4 from distinct regions of the UK) with data spanning from birth to early adulthood during differing calendar periods (1936-1964, 1972-1974, 1991-2015, 2007-2010). We combined all cohorts into a single dataset and fitted a multilevel model with a nonlinear growth trajectory. Three of the key challenges were: (1) Some variables were measured differently

Box 1. Key challenges

Data harmonisation

- Information may be lost during the data harmonisation process in order to include cohorts with a relatively crude measure of a variable.
- Some variables may not be measured by all cohorts. Subject-matter knowledge may provide information about their most likely values.

Accounting for the data's dependence structure

- The data's dependence structure must be appropriately modelled in order to obtain appropriate standard errors.
- Reliable estimation of the random effects' variance components at a particular level depends on the number of units at that level and not the total number of level-1 observations (e.g., repeated measurements).

Model selection across multiple cohorts

- Model selection on the combined data from all studies may be impractical due to the large volume of data.
- Two alternatives are: (1) fit the same model separately to each cohort and perform model selection on the summed likelihoods across the cohorts. (2) Perform model selection on a random sample of individuals, keeping the proportion of individuals from each cohort as per the combined data.
- Model selection based on summed likelihoods may not be feasible when the feature under comparison (e.g., nonlinear trajectory) requires each cohort to cover the same age range (e.g., restricted cubic splines).

Missing data in the outcome (repeated measurements) and the covariates

- Determining the amount of missing outcome data is difficult for cohorts without a prescribed measurement schedule (such as opportunistic health visits).
- Likelihood estimation of a multilevel model can utilise all available observed outcome data and is valid under the missing at random assumption (i.e., differences between the observed and missing data are explained by associations with the observed outcome and covariate data).
- When outcome data are suspected missing not at random, multiple imputation (MI) can use information from auxiliary variables (not included in the multilevel model) to explain the reasons for the missing data and provide valid inference.
- Restricting the analysis to those with complete covariate data can result in a substantial loss of information especially when covariates are systematically missing in some cohorts (missing for all individuals) and/or when there are many covariates each with small amounts of missing data.
- The imputation model of MI should account for the main features of the multilevel model (e.g., its multilevel structure, any interactions).

across the studies. We used data harmonisation to derive new 'harmonised' variables by identifying common elements across all studies. (2) Ethnicity and maternal education were not measured at all by some studies. We used subject-matter knowledge to derive ethnicity and developed a novel method to multiply impute individual-level covariates of a multilevel model with a nonlinear growth trajectory and interactions. (3) Model selection based on the combined data was time consuming (**Box 1**).

To speed up the process we conducted model selection on a random sample of the combined data, and used the remaining data, as a "validation dataset", to evaluate the fit of the top selected models. In part these challenges reflect the fact that developing life-course trajectories from independent studies, by definition pools data from heterogeneous populations (e.g. they must have been born during different periods). A key strength of our approach is the ability to model trajectories over wide age-ranges and the sharing of information across studies. It also enables direct comparison of the same parts of the life-course in different geographical regions and time periods (e.g., the lower growth trajectory

between 9-18 years for a UK study of participants born in the early 1900s, compared to a UK cohort of participants born in the early-1990s and a Belarus cohort born in the mid-1990s, could be due to changes in diet between the mid-1900s and the later 1990s/early 2000s). Our approach can leverage several cohorts to inform about life-course trajectories and examine heterogeneity between cohorts to shed light on influences on trajectories.

Results

Table 1 shows the characteristics of the five cohorts contributing to the illustrative example.

Table 1: Characteristics of five cohorts used to generate cross cohort life-course trajectories

	ALSPAC	BCG	BiB	CHS	PROBIT
Region	England	Wales	England	England	Belarus
Calendar period	1990-2012	1972-1979	2007-2015	1936-1964	1996-2016
Age range	0-20	0-5	0-6	9-18	0-16
No. participants ^a	14,216	951	13,445	1,547	17,046
Sex					
male	51%	54%	52%	100%	52%
female	49%	46%	48%	0%	48%
missing	0%	0%	0%	0%	0%
Ethnicity					
White	79%	100% ^b	39%	100% ^b	100% ^b
South Asian	0%	0%	50%	0%	0%
Other	4%	0%	8%	0%	0%
missing	17%	0% ^b	3%	0% ^b	0% ^b
Maternal education					
left school at 15 or 16	55%	0%	43%	0%	4%
left school at 17 or 18	19%	0%	12%	0%	82%
degree	11%	0%	21%	0%	14%
missing	15%	100%	24%	100%	0%
Paternal occupation					
class I or II	22%	17%	13%	55%	11%
class III	36%	58%	19%	21%	60%
class IV, V or other	21%	23%	44%	3%	25%
missing	21%	2%	24%	21%	4%
Total no. weight measures	157,000	12,737	78,110	89,070	205,864
Median no. measures per child (IQR)	10 (10)	14 (1)	5 (3)	57 (18)	13 (5)
Median ALM ^c in years (IQR)	13.8 (13.1)	5 (0)	4.7 (2.7)	17.8 (1.5)	16.0 (1)

a: Included in the growth analysis. b: these cohorts had no record of ethnicity. Based on the populations from which they were recruited we assigned all participants to White European ethnicity. c: Age at last measurement. ALSPAC: Avon Longitudinal Study of Parents and Children, BCG: The Barry Caerphilly Growth study, BiB: Born in Bradford, CHS: The Christ Hospital School study, PROBIT: the Promotion of Breastfeeding Intervention Trial.

Figure 2 shows observed mean growth trajectories among the cohorts. The general trajectory shape was nonlinear: curving over in the first year, curving slightly under between ages 5 and 12 years and starting to plateau around age 17 years. Compared to ALSPAC and PROBIT, CHS had a lower growth trajectory.

The paper provides very detailed descriptions about model selection. In the illustrative example different potential models were compared using a random sample of 15,000 from all of the cohorts pooled data (the training dataset). From the model comparisons based on this training dataset, the first-

and second-best fitting fractional polynomials were two-degree models with powers 0.5 and 3, and powers 0 and 2, respectively, and the first- and second-best restricted cubic splines were models with 7 and 6 knots, respectively. **Figure 3** shows the mean weight trajectories according to the first- and second-best fitting fractional polynomial and restricted cubic spline models (generated from fitting the models to all of the combined data).

Figure 2: Observed mean trajectories for the five cohorts

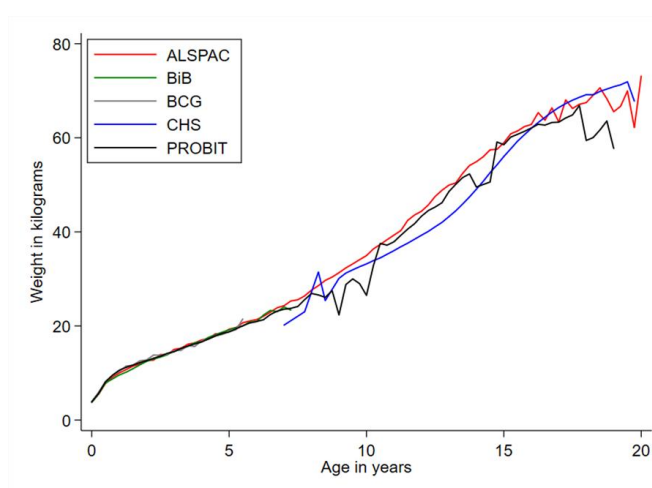


Figure 3: Predicted mean trajectories for the best two fitting fractional polynomial and best two fitting cubic spline models

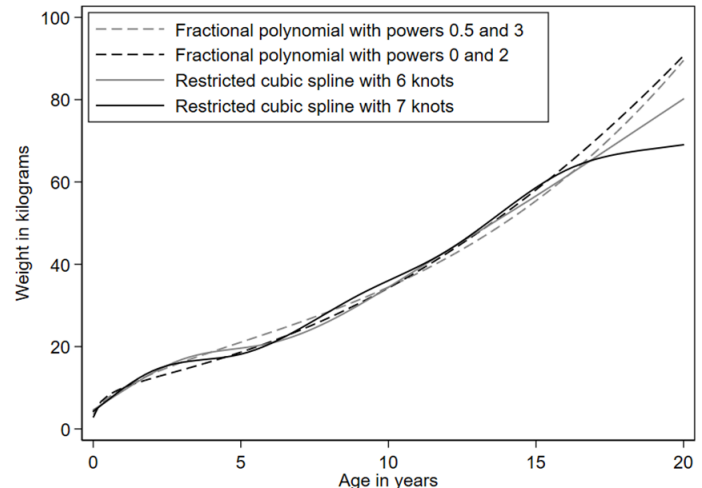


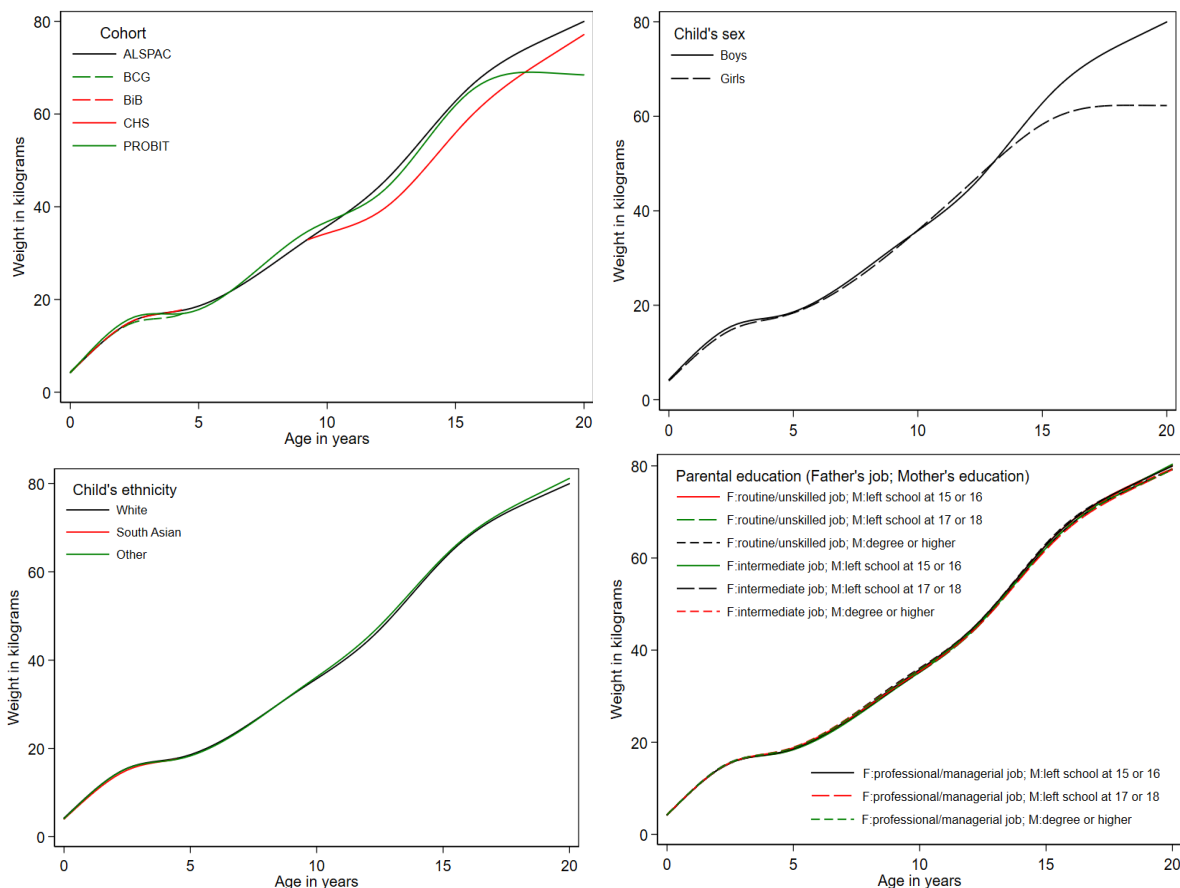
Figure 4 shows predicted mean weight trajectories between children of different cohorts, sexes, ethnic groups, and parental education groups (in each case holding all other covariates constant) from the final selected model. Weight trajectories were very similar between the cohorts in the first four years of life. They had a similar increase between ages 10-15 years for the three cohorts (ALSPAC, CHS and PROBIT) that contributed to these ages, but with ALSPAC participants being heaviest, PROBIT in between and CHD lightest across this period; for example, at age 15 years the predicted mean difference in weight between children from PROBIT and ALSPAC was -0.88 kg [95% confidence interval (CI) $-0.44, -1.33$ kg]. After age 15 there was a slight plateauing in ALSPAC weight increase and by age 20 years very little difference in weight between participants in CHS and ALSPAC (mean difference -6.83 kg [95% CI $-6.17, -7.49$ kg]). The marked plateauing effect after age 15 for the PROBIT cohort could be due to its limited number of measures between 15-20. The weight trajectories of boys and girls were similar until adolescence and start to diverge after age 15 years, such that by age 20 the predicted mean difference in weight between boys and girls was -2.84 kg [95% CI $-1.57, -4.10$ kg]. There were very little differences between children of different ethnic backgrounds or between those whose parents had different educational / SEP levels. However, for ethnicity the majority of participants across the cohorts were White European (81% out of the 44,354 children with observed ethnicity or ethnicity assumed to be White European). The high proportion of South Asian (mostly, Pakistani) participants in BiB contribute to trajectories from age 0 to 6 and it is possible that ethnic differences begin to emerge at older ages.

Conclusions

We have shown that careful analysis can harmonise and bring together information from different cohorts to inform life-course trajectories. We provide ways to overcome some of the main challenges in doing this, but as with all analyses, assumptions should be documented, and sensitivity analyses conducted. This approach can then leverage several cohorts to inform about life-course trajectories and examine heterogeneity between cohorts to shed light on which early life stressors influence trait development and degeneration. These methods will be extremely important to investigators in LifeCycle

exploring the impact of early life stressors on cardiometabolic, respiratory and neurocognitive and mental health life-course trajectories.

Figure 4: Predicted mean trajectories by cohort (top left), child sex (top right), ethnicity (bottom left) and parental SEP (bottom right).



Paper 2: Elhakeem A, Tilling K, Hughes R, Cousminer DL, Jackowski SA, Kwong ASF, Grant SF, Baxter-Jones A, Zemel BS, Lawlor DA. Spline, SITAR and mixture models for describing nonlinear growth trajectories: applications to bone mass in 3 different cohort studies. Currently being prepared for circulation to co-authors.

Overview

This extends the work described in Paper 1 above. It describes a wider range of methods that could be used for modelling repeatedly assessed data in the single- and multi-cohort setting, including latent class and Super Imposition by Translation and Rotation (SITAR) growth curve analysis. A different trait (bone mineral content) is used as an illustrative example of the different methods and how to interpret results from different methods. The choice of bone-mineral content was motivated by: (i) the importance of understanding of life-course trajectories of musculoskeletal measures is increasingly recognised as important to understanding healthy aging; (ii) an increasing number of cohorts have repeat measurements of bone composition and (iii) analyses of repeat measures of musculoskeletal outcomes are less common in the literature than are similar analyses for cardiometabolic, respiratory and mental health outcomes. This paper will provide a valuable education/tutorial piece for musculoskeletal researchers and researchers working in other health areas for whom analysing repeatedly assessed data is new.

Extended summary

Appropriate longitudinal data analysis can improve our understanding of influences on health trajectories across the life-course. This paper provides an overview of different approaches for describing nonlinear growth trajectories, exemplified by bone mineral content (BMC; grams) from age 5-40 years in 3 long-running cohort studies (>8,500 study subjects with >37,000 repeated scans). Model strengths and limitations, fitting and selection strategies, interpretation, and trajectory visualisation are discussed and illustrated, with a comparison of model performance between studies. Joint modelling of different cohorts as a single multicohort study is illustrated, and future developments allowing amalgamated multilevel modelling of remote data are discussed. Mixed-effects linear spline and natural spline models, and Super Imposition by Translation and Rotation (SITAR) growth curve analysis showed that BMC increased (nonlinearly) with age and that the levels and rate of change in BMC were greater for males than females. Both models showed that the greatest gains were in puberty, for example, the mean age at peak bone mineral accrual (from SITAR models with fixed effects for cohort) was 13.8 years in males and 12.1 years in females. Peak gains in BMC were followed by periods of decelerating growth lasting up to age 30 years. The modelling of heterogeneous growth curves (latent trajectories) identified possibly distinct trajectories, which might reflect effects of biology (e.g. puberty timing) and behaviour (e.g. exercise) on BMC trajectory. In summary, we present a useful resource for researchers interested in describing nonlinear growth. Scripts are provided along with synthetic replicas of the three studies which allow the reader to replicate all models and plots in R.

Results

Table 2 shows the characteristics of the three cohorts contributing to the illustrative examples.

Table 1: Characteristics of the 3 cohort studies included in the analysis: Avon Longitudinal Study of Parents and Children (ALSPAC), Bone Mineral Density in Childhood Study (BMDCS), Pediatric Bone Mineral Accrual Study (PBMAS)

Study name	ALSPAC	BMDCS	PBMAS
Design	Birth cohort study (started in 1990-1992)	Child cohort study (started in 2002-2003)	Child cohort study (started in 1991)
Region and country	Catchment area of 3 health authorities in Southwest England, UK	5 USA clinic centre: Los Angeles, Cincinnati, Omaha, Philadelphia	2 elementary schools, Saskatoon, Saskatchewan, Canada
Birth years	1990-1992	1985-1997	1983-1976
Ethnicity	98% white	Ethnically diverse	98% white
DXA device used to measure BMC	Lunar Prodigy	Hologic QDR-4500A	Hologic QDR-2000
Mean age at the baseline/youngest DXA scan (range)	9.9 years (8.8-11.7 years)	10.8 years (6.0-17.0 years)	11.8 years (8.0-15.1 years)
Mean age at the latest/oldest DXA scan (range)	24.6 years (22.4-26.5 years)	16.1 years (6.9-23.3 years)	37.3 years (34.3-40.2 years)
Frequency and maximum number of DXA scans	up to 6 repeat measures at mean ages 9.9, 11.7, 13.8, 15.4, 17.8, and 24.6 years	up to 7 yearly repeat measures	Up to 15 yearly repeats (1991-1998, 2003-2005, 2007-2011 and 2016-2017)
Number of study participants with ≥ 1 BMC measure*	3888 males 4007 females	465 males 488 females	112 males 127 females

*Analyses restricted to white ethnicity

Figure 5 shows observed BMC values among the cohorts, illustrating the age range and the dispersion of BMC, and the higher values in ALSPAC, which likely reflect the different device used to measure BMC in this cohort compared with the other 2 cohorts. The figure also shows expected evidence of nonlinear change in BMC with increasing age.

Figure 5 Scatterplot of observed bone mineral content (BMC) against age by sex and cohort.

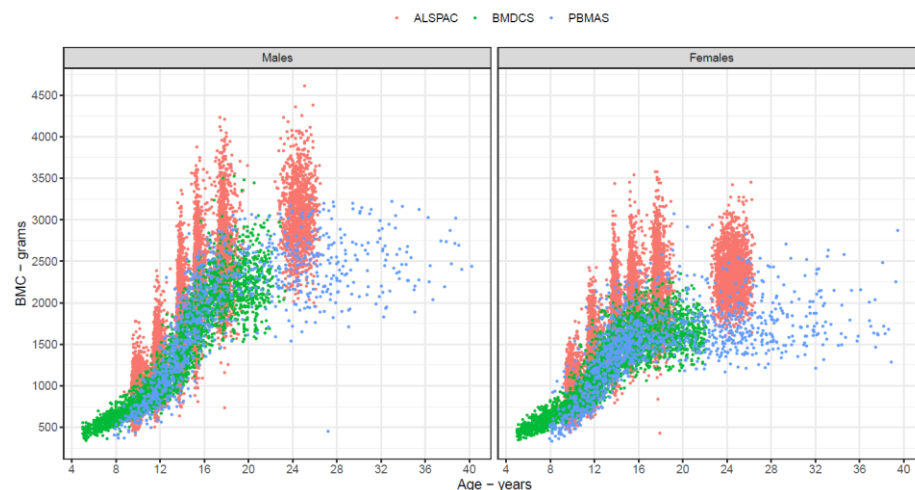


Figure 6 shows the estimated mean bone mineral content (BMC) trajectories from the best fitting multicohort linear and natural spline models with equally spaced and manually selected knots. Particularly for the linear spline mixed models, the models with manually selected knots provided a better fit and a more appropriate trajectory shape.

Figure 6 Estimated mean bone mineral content (BMC) trajectory from the selected multicohort mixed-effects linear spline and natural spline models. Mean trajectories are shown in black for males and in red for females. Shaded areas around the mean trajectories represent 95% confidence intervals.

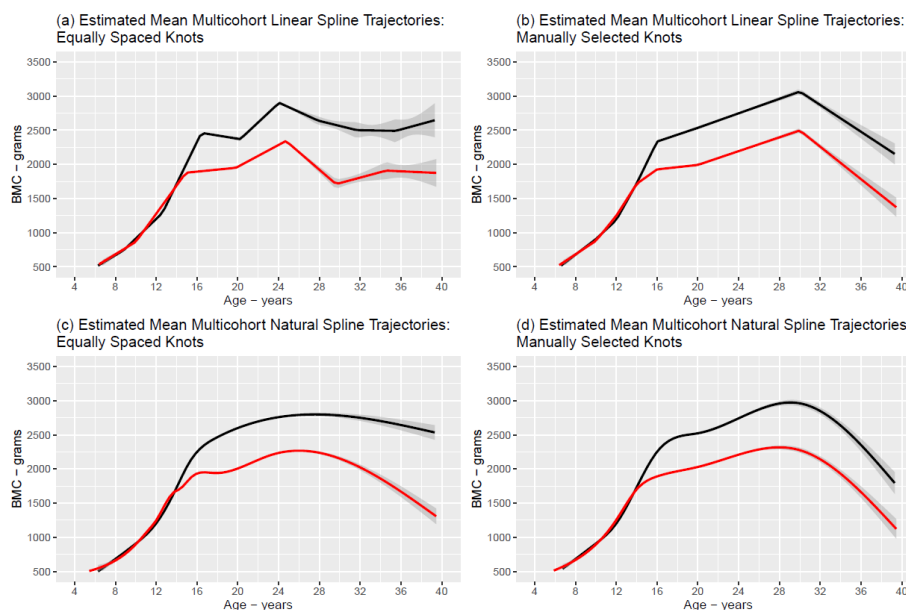


Figure 7 shows the estimated mean bone mineral content (BMC) trajectories from the best fitting multicohort SITAR models. The models show that BMC growth is nonlinear and peaks during puberty.

Figure 7 Estimated mean bone mineral content (BMC) trajectory from the selected multicohort selected SITAR (Super Imposition by Translation and Rotation) models. Mean trajectories are represented by the solid curves and are shown separately for males and females. The dashed blue curves represent BMC velocity (grams/year). The vertical dotted black line indicates age at peak BMC velocity.

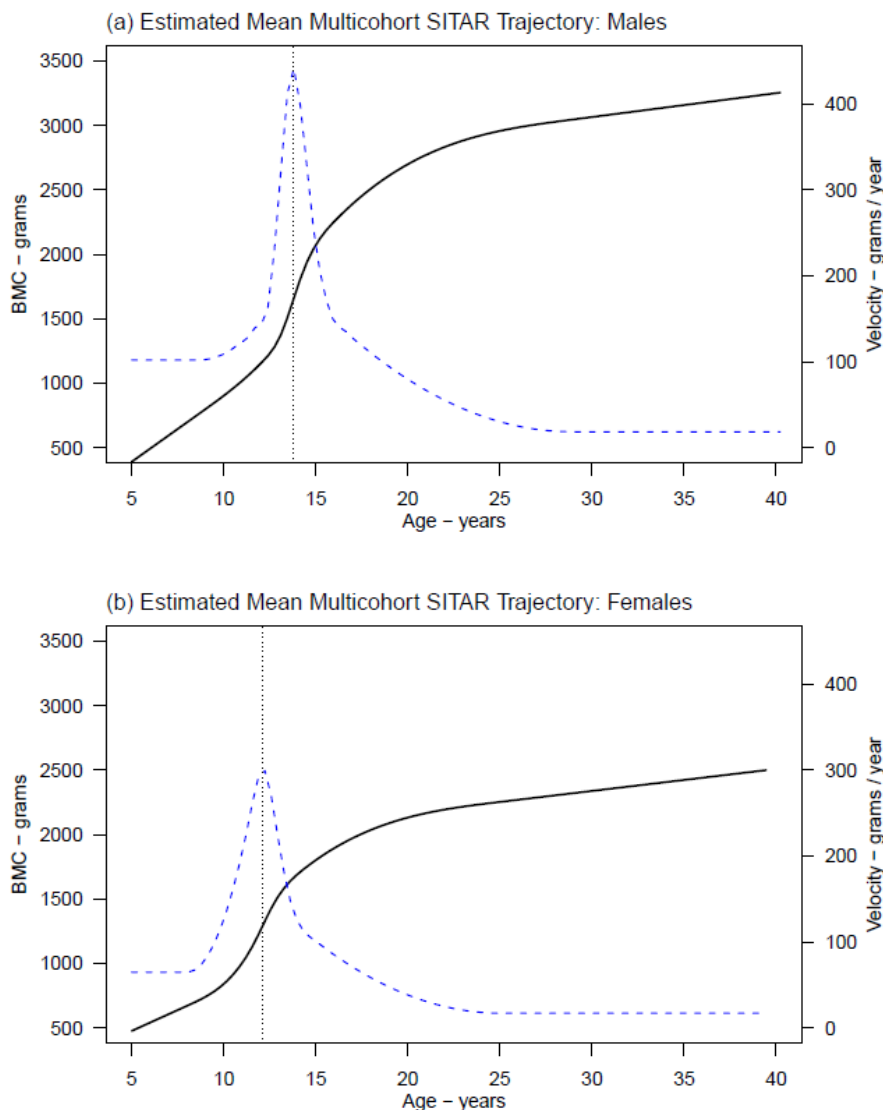


Table 3 shows the rates of change in bone mineral content (BMC) during each period from multicohort linear spline mixed-effect models with manually selected knots, and the features extracted from multicohort SITAR models. Estimates from the linear spline models with manually selected knots showed that BMC levels increased up to 30 years old and that gains were greater in males than females and peaked later in the males (between 14 to 16 years versus 12 to 14 years). This was consistent with SITAR estimated peak BMC accrual and age at peak BMC accrual.

Table 3 Rates of change in bone mineral content (BMC) during each period from multicohort linear spline mixed-effect models with manually selected knots, and the features extracted from multicohort SITAR models.

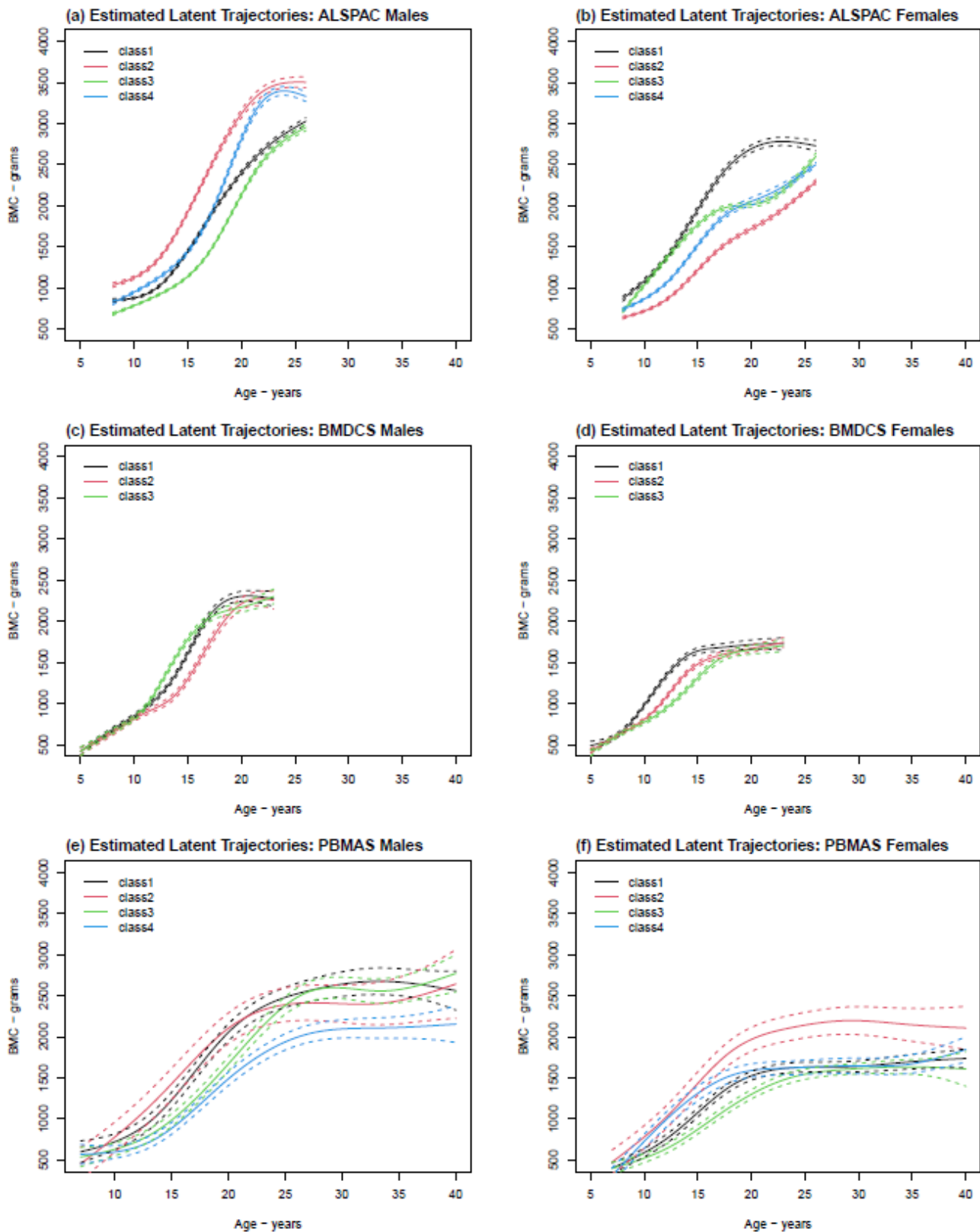
	Male participants	Female participants
<i>Rate of change in BMC (g/y) from multicohort linear spline mixed models with 6 manually selected knots [(mean (95%CI))]</i>		
5 to 10 years	51.2 (44.2 to 58.2)	51.4 (45.3 to 57.5)
10 to 12 years	157.1 (152.9 to 161.3)	205.6 (202.1 to 209.2)
12 to 14 years	271.2 (266.7 to 275.8)	236.2 (232.5 to 239.9)
14 to 16 years	306.1 (300.1 to 312.1)	104.2 (100.0 to 109.6)
16 to 20 years	88.3 (82.8 to 93.8)	35.0 (30.7 to 39.3)
20 to 30 years	59.8 (55.3 to 64.2)	49.3 (46.0 to 52.6)
30 to 40 years	-15.4 (-31.8 to 1.0)	-8.7 (-23.4 to 5.9)
<i>Features from multicohort SITAR models [mean (SD)]</i>		
age at peak BMC accrual (years)	13.8 (0.7)	12.1 (0.7)
peak BMC accrual (grams/year)	459.9 (137.4)	315.5 (105.7)
α – size	-4.2×10^{-14} (240.4)	2.0×10^{-13} (193.3)
β – timing	3.6×10^{-17} (0.7)	-5.2×10^{-16} (0.8)
γ – intensity	-4.8×10^{-17} (0.3)	3.2×10^{-16} (0.3)

Figure 8 shows the best fitting growth mixture models in each cohort (and sex). Overall, the latent trajectory subgroups within each cohort had similar mean levels of BMC at baseline and tended to diverge in terms of BMC levels during puberty and converge again in adulthood. Among PBMAS females, one group maintained higher levels of BMC from age 15 to 40.

Conclusion and current status of paper

We have demonstrated the application of four approaches to describing nonlinear trajectory modelling: using linear splines, natural splines, SITAR and latent trajectory models. Using BMC as an illustrative example the models were applied to each cohort separately and with the three cohorts combined into a single multicohort dataset. While we had access to individual-level data from all cohorts, it should be straightforward to apply these models to remotely held harmonised data using DataSHIELD, such as in LifeCycle (including to other growth processes). We are on course to submit the paper to a preprint server and the European Journal of Epidemiology in January 2021.

Figure 8 Estimated mean bone mineral content (BMC) latent trajectories for each cohort and sex from the selected growth mixture models. Dotted lines around the mean trajectories represent 95% confidence intervals. Colours distinguish between latent trajectories within subplots and should not be used to compare between subplots.



2.2.2 Reviews for the preparation of a tutorial

As detailed in Milestone 12, WP7 has been working to produce tutorials in the field of appropriate analyses of repeatedly measured traits (Deliverable 7.4). Five areas that WP7 will cover in the tutorials were chosen for their relevance to the field of life-course epidemiology and for gaps in the literature on how to implement the various methodological strategies. Four of these tutorials are relevant to Task 7.1 and preparation for those tutorials are described in deliverable 7.1 that is submitted at the same time as this deliverable. All five tutorials will aim at comparing different methods and the interpretation of their resulting estimates, by providing numerical case studies relevant to life-course epidemiology.

Below we provide a summary of the preparatory work conducted so far for the development of the tutorial on life-course trajectories, which will be delivered as part of Deliverable 7.4 (M60).

Developing Life-course trajectories of health outcomes

To fully understand the life-course epidemiology of health and disease one needs repeatedly assessed exposure and outcome data across life (ideally from when *in utero* to adulthood). This would enable the study of factors related to adverse development and adverse degeneration in order to fully understand how to promote and maintain health across life. No single study has data from being *in utero* to adulthood but across LifeCycle studies we do. Key aims of WPs 4, 5, 6 and 8, are to identify early life stressors that influence life-course trajectories of cardiometabolic, respiratory and mental health, and of DNA methylation, respectively. This tutorial will describe how to combine outcome data from multiple cohorts, where these cohorts each have at least two repeat measurements of the outcome and cover different, but overlapping, ages of collection. The methods that will be used are those described in Papers 1 and 2 in section 2.2 above.

We have already conducted considerable work on preparing for these tutorials, through working on Papers 1 and 2 (Section 2.2), running a LifeCycle workshop on repeatedly assessed data for life-course analyses at the end of the 4th LifeCycle General Assembly (24th October 2018, Barcelona). We are developing this further by working closely with DataShield experts. We will create a synthetic dataset hosted at UMGC which can be used by researchers in conjunction with the tutorial. The tutorial will provide practical guidance on how to combined repeatedly assessed data from multiple cohorts to enable exploration of early life stressors on life-course trajectories, both within a federated system (i.e. Datashield) where harmonised data across cohorts can be analysed without the researcher directly 'holding' any individual participant data, as well as without Datashield. The tutorial will demonstrate the application within DataSHIELD of the methods described in section 2.2. It will cover data preparation and quality control, model selection, sensitivity checks and the preparation of plots and tables. Bespoke R functions will be written to streamline these processes, and will be made available to all researchers. The tutorial will use the results from the papers described in section 2.2 as case studies and be made available to all LifeCycle researchers and partners via the data resource described in section 2.1.

2.3. Applied examples in LifeCycle

Some original research articles published by the LifeCycle project have a specific methodological component using repeated measurement analyses that has been supported by WP7. We provide three published examples below, which illustrating how we have supported repeat measures / trajectories of very types of different data, including repeat ultrasound scan measures of fetal growth, repeat

epigenomic (DNA methylation) and repeat metabolomic data. The first of these triangulates different causal methods and is therefore relevant to task 7.1 (as well as WP4), the second is relevant to WP8 and the third to WP4.

Applied methods example 1: Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Ellis Voerman E, Felix JF, Tilling K, Lawlor DA. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. *PLoS Medicine* 2019, 16(11): e1002972. doi: 10.1371/journal.pmed.1002972.

Abstract

Background: Maternal smoking during pregnancy is an established risk factor for low infant birth weight, but data on critical exposure windows and timing of fetal growth restriction are scarce and inconsistent. Here we investigate the effect of maternal quitting, reducing and continuing smoking during pregnancy on longitudinal fetal growth by triangulating evidence from three analytical approaches to strengthen causal inference.

Methods and findings: We analysed data from 8621 European liveborn singletons in two population-based pregnancy cohorts (the Generation R Study, The Netherlands 2002-2006 (n = 4682) and the Born in Bradford study, United Kingdom 2007-2010 (n= 3939)) with fetal ultrasound and birth anthropometric measures, data on parental smoking during pregnancy and maternal genetic data. Associations with trajectories of estimated fetal weight (EFW) and individual fetal parameters (head circumference (HC), femur length (FL), abdominal circumference (AC)) from 12/16 to 40 weeks (wks) gestation were analysed using multilevel fractional polynomial models. We compared results from 1). confounder adjusted multivariable analyses; 2). a Mendelian Randomization (MR) analysis using maternal rs1051730 genotype as an instrument for smoking quantity and ease of quitting; and 3). a negative control analysis comparing maternal and paternal smoking associations. In multivariable analyses, women who continued smoking during pregnancy had a smaller fetal size than non-smokers from early gestation (16-20 wks) through to birth (global p value for each parameter < 0.001). Fetal size reductions in continuing smokers followed a dose-dependent pattern (compared to non-smokers differences in mean EFW (95% CI) at 40 wks gestation were -144 g (-182 to -106), -215 g (-248 to -182) and -290 g (-334 to -247) for light, moderate and heavy smoking, respectively). Overall, fetal size reductions were most pronounced for FL. The fetal growth trajectory of women quitting smoking in early pregnancy was similar to that of non-smokers, except for a shorter FL and greater AC around 36-40 wks gestation. In MR analyses, each genetically determined 1-cigarette-per-day increase was associated with a smaller EFW from 20 wks gestation to birth in smokers (global p = 0.01, difference in mean EFW at 40 wks = -45.4 g (-80.7; -10.2)), but a greater EFW from 32 wks gestation onwards in non-smokers (global p = 0.03, difference in mean EFW at 40 wks = 25.6 (4.6; 46.7)). There was no evidence that paternal smoking was associated with fetal growth. Study limitations include measurement error due to maternal self-report and the modest sample size for MR analyses resulting in unconfounded estimates being less precise. The apparent positive association of the genetic instrument with fetal growth in non-smokers suggests that genetic pleiotropy may have masked a stronger effect in smokers.

Conclusions: A linear dose-dependent effect of maternal smoking with fetal growth was observed from the early second trimester onwards independently of observed and unmeasured confounding factors, while no major growth deficit was found in women quitting smoking early in pregnancy except for a shorter FL during late gestation. These findings reinforce the importance of smoking cessation advice in preconception and antenatal care. They provide stronger causal evidence for this advice and in particular show the benefit of smoking reduction to improve fetal growth in women who struggle to quit.

Selected results

Triangulating evidence from conventional multivariable regression (Figure 9), paternal negative control analyses (Figure 10) and Mendelian randomization (see publication) we showed that quitting smoking early in pregnancy resulted in fetal growth trajectories similar to non-smokers. For those who continued to smoke throughout pregnancy there was a clear dose response of faltering growth in all parameters across pregnancy (see publication).

Figure 9: Predicted differences in mean fetal size (95% CIs) across gestation comparing pre-pregnancy smokers who quit early in pregnancy & those continuing to smoke with non-smokers (reference)

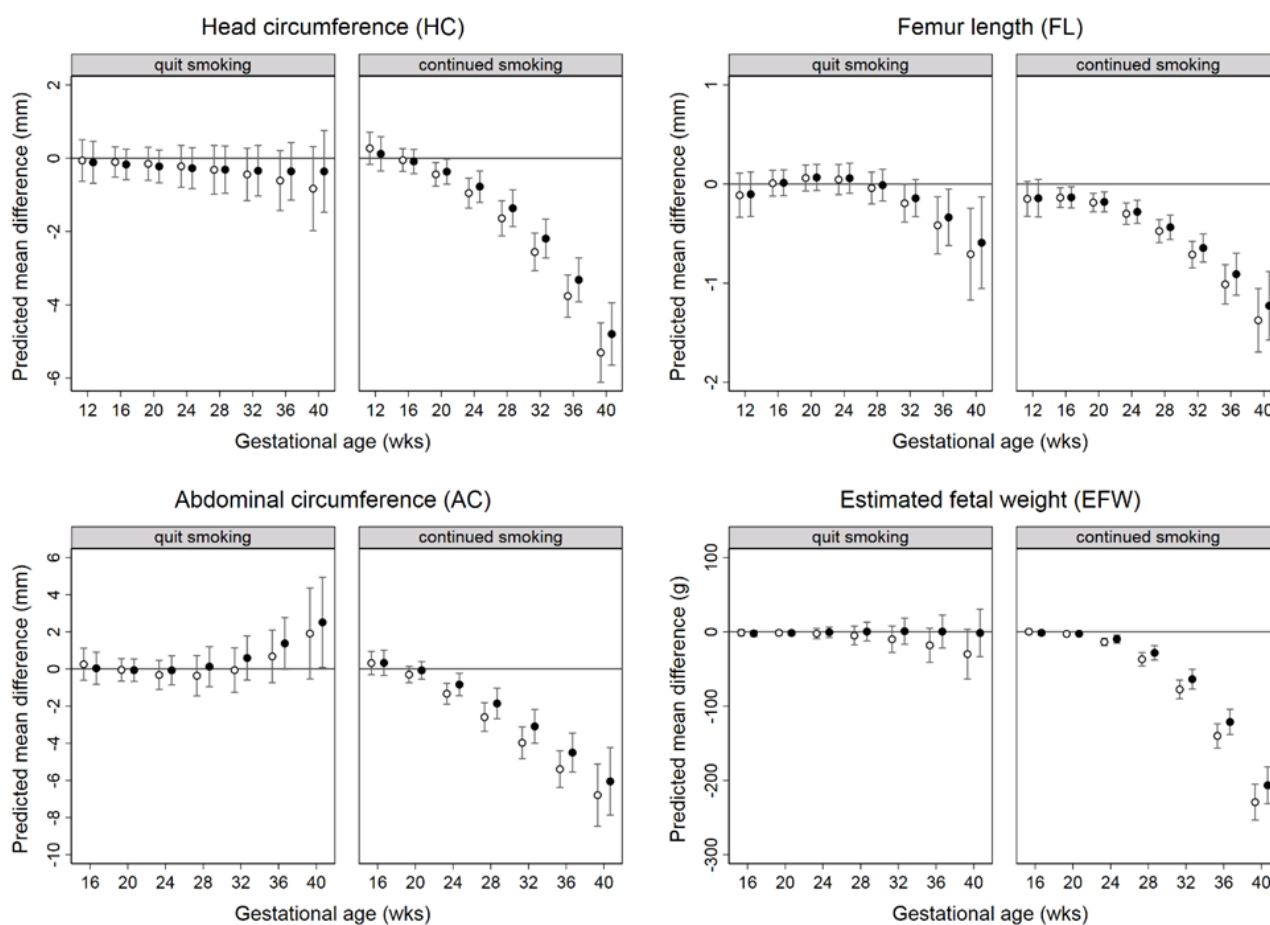
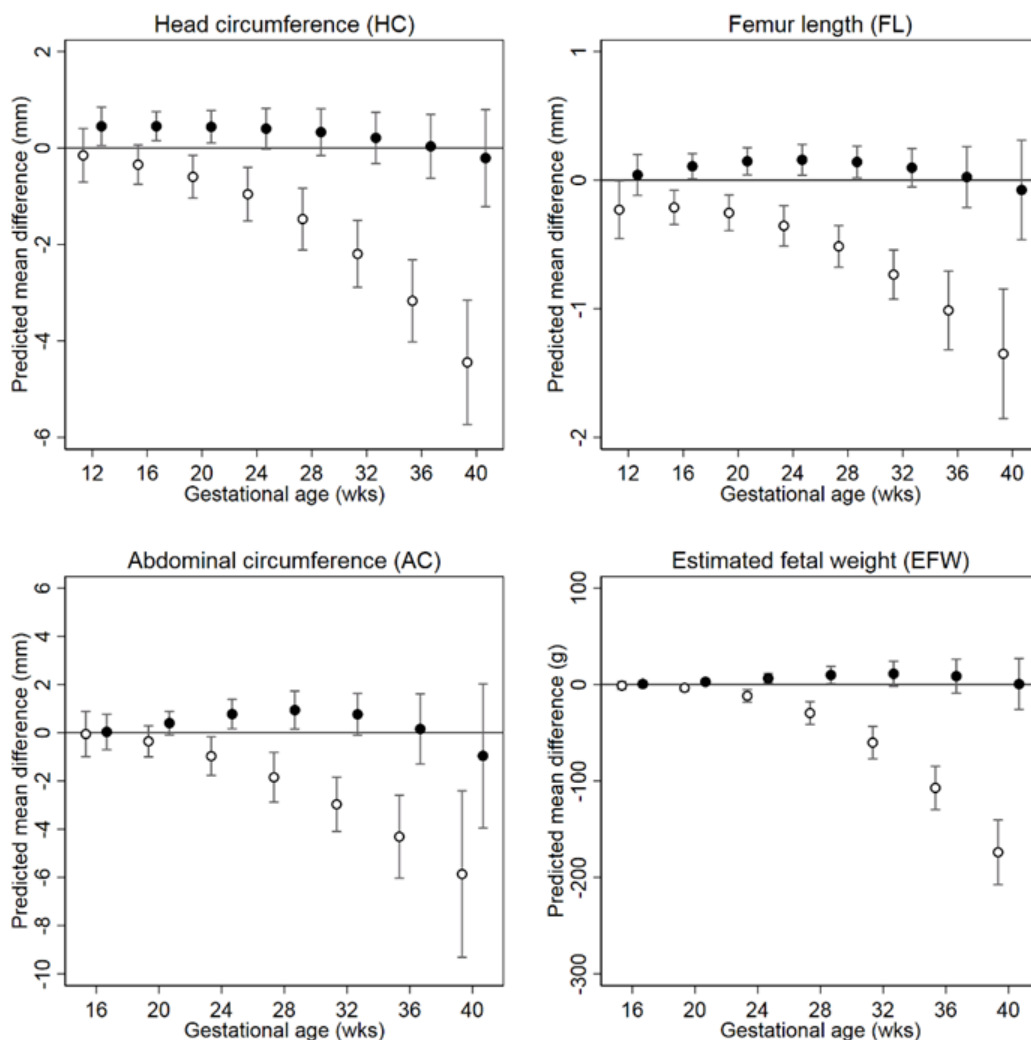


Figure 10: . Predicted differences in mean fetal size (95% CIs) across gestation comparing mothers and mother’s partners who smoke to non-smokers (reference)



Applied methods example 2: Kazmi N, Sharp GC, Reese SE, [37 additional co-authors] Hivert MF*, Gaunt TR*, Lawlor DA*, Relton CL* [*Joint Senior Authors]. Hypertensive disorders of pregnancy and DNA methylation in newborns. *Hypertension* 2019; 74:375-383

Abstract

Hypertensive disorders of pregnancy (HDP) are associated with low birthweight, shorter gestational age and increased risk of maternal and offspring cardiovascular diseases later in life. The mechanisms involved are poorly understood, but epigenetic regulation of gene expression may play a part. We performed meta-analyses in the Pregnancy And Childhood Epigenetics (PACE) Consortium to test the association between either maternal HDP (ten cohorts; n=5242 (cases=476)) or pre-eclampsia (PE; three cohorts; n=2219 (cases=135)) and epigenome-wide DNA methylation in cord-blood using the Illumina HumanMethylation450 BeadChip. In models adjusted for confounders, and with Bonferroni correction, HDP and PE were associated with DNA methylation at 43 and 26 CpG sites, respectively. HDP was associated with higher methylation at 27 (63%) of the 43 sites and across all 43 sites mean absolute difference in methylation was between 0.6 to 2.6%. Epigenome-wide associations of HDP with offspring DNA methylation were modestly consistent with the equivalent epigenome-wide associations of PE with offspring DNA methylation ($R^2=0.26$). In longitudinal analyses conducted in one study (n=108 HDP cases, 550 controls), with limited statistical power, there were similar age-related changes in DNA methylation in offspring of those with and without HDP up to adolescence. Pathway analysis suggested that genes located at/near HDP-associated sites may be involved in developmental, embryogenesis or neurological pathways. HDP is associated with offspring DNA methylation with potential relevance to development.

Trajectory methods and selected results

Here, we have focused on the longitudinal modelling of repeatedly assessed DNA methylation data. These data were available in one cohort – the Avon Longitudinal Study of Parents and Children (ALSPAC), which included up to three measures of white blood cell DNA methylation at birth (cord-blood) and mean ages 7 and 17 years, in 658 offspring in whom 108 had been exposed to HDP. The CpGs were first linked to the gene symbols using an Illumina mapping file and if unsuccessful were annotated to the nearest gene within 10Mb of each CpG. A multilevel model including a random intercept (to allow for between-offspring variability in methylation) and a linear regression spline term (to allow for linear change between two adjacent measures (e.g. birth and age 7) but differences in the magnitude of this over time between age periods (e.g. linear change between birth and age 7 could differ to that between age 7 and 17) was fitted to each of these CpGs separately. For example, for CpGs found on comparing HDP and normotensive mothers:

$$meth_{ij} = \beta_0 + \mu_{0i} + \beta_1 HDP_i + \beta_2 age_{ij} + \beta_3 (age_{ij} - 7)_+ + \beta_4 HDP_i age_{ij} + \beta_5 HDP_i (age_{ij} - 7)_+ + confounders + \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$$

$$\mu_{0i} \sim N(0, \sigma_\mu^2)$$

where $i = 1, \dots, 658$ indexes the children in ARIES, $j = 1, 2, 3$ indexes the measurement occasion and $(age_{ij} - 7)_+$ is equal to $age_{ij} - 7$ when $age_{ij} - 7$ is greater than 0, or equal to 0 if it is not, i.e. this cannot take a negative value. This term is used to construct a linear spline at age 7. The exact technical meaning of each beta coefficient in the above equation is provided in the box below. Once the model is fitted, we can calculate the change in methylation from 0-7 for children of non-HDP mothers (β_2), HDP mothers ($\beta_2 + \beta_4$) and the change from 7-17 for children of non-HDP mothers ($\beta_2 + \beta_3$) and HDP mothers

$(\beta_2 + \beta_3 + \beta_4 + \beta_5)$. To test whether there is a difference in methylation change between 7 and 17, for example, we test whether $\beta_4 + \beta_5$ is different from zero (the null). This can be done by comparing $(\beta_4 + \beta_5)/se(\beta_4 + \beta_5)$ to the standard normal distribution, where se is the standard error.

- β_1 = average difference in methylation between offspring of normotensive and HDP mothers at any age
- β_2 = average change in methylation from birth to adolescence
- β_3 = average difference between mean change from birth to 7 and mean change from 7 to 17 (i.e. a change in slope)
- β_4 = average difference in methylation change from birth to adolescence between HDP mother-offspring and non-HDP mother-offspring
- β_5 = average difference in the change in slope (i.e. the difference in mean change from 0-7 compared with mean change from 7-17) between offspring of HDP and non-HDP mothers

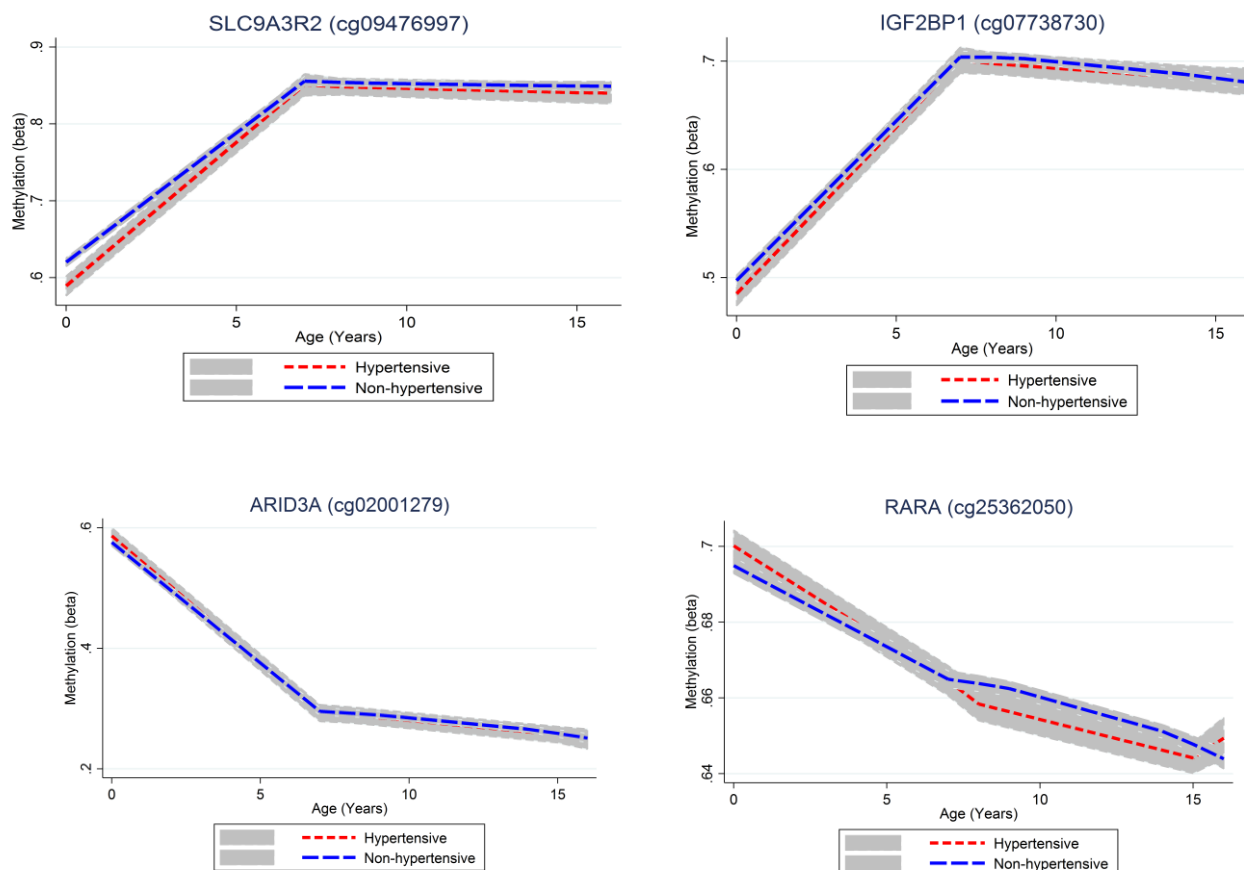
For each CpG, we used the above described multilevel model, adjusting for confounders (maternal age, parity, maternal smoking status, gestational diabetes, maternal pre-

pregnancy BMI, child sex and six cell counts (i.e. CD4 T cells, CD8 T cells, NK cells, B cells, monocytes and granulocytes estimated using the Houseman algorithm) at each of birth, age 7 and age 17. In these analyses we adjusted for cell-type using the adult blood reference and Houseman algorithm. While the adult reference panel may not be optimal for our cord-blood samples, we used it here to keep estimates consistent from birth to age 7 and 17.

As an illustration of the general pattern of observed results, **Figure 11** shows longitudinal changes in methylation for the top four CpGs that reached Bonferroni-corrected P-value thresholds in the main adjusted HDP cord-blood EWAS meta-analysis. There were similar increases in methylation levels between birth and adolescence for most of the 43 CpGs in offspring of mothers who experienced HDP and those who did not. For a small number of CpGs this age-related change was weaker and less consistent between 7 and 17 than between birth and 7 years. For all but one of the 43 CpGs, there was no strong statistical evidence that age-related change differed between offspring of cases and controls, suggesting that epigenetic differences persisted, but that this was due to general age-related change rather than any further long-term effect of exposure to HDP *in utero*. For the CpG cg08274637 (near *DLEU7* gene), there was evidence that offspring of HDP mothers (compared to those whose mothers did not experience HDP) had a slightly faster increase in methylation between birth and age 7 (0.27% increased methylation change per year, 95% CI 0.13 to 0.41% methylation change per year; P-value=0.0002).

Figure 11: . Longitudinal changes in methylation for four illustrative example CpGs

The figures show methylation (i.e. the proportion of methylated cells) over time for offspring of HDP mothers (dashed (red) line) compared with offspring of non-HDP mothers (dashed (blue) line). Ribbons indicate 95% confidence intervals.



Applied methods example 3: Mills HL, Patel N, White SL, Pasupathy D, Briley AL, Santos Ferreira DL, Seed PT, Nelson SN, Sattar N, Tilling K, Poston L, Lawlor DA, On behalf of the UPBEAT Consortium. The effect of a lifestyle intervention in obese pregnant women on change in gestational metabolic profiles: findings from the UK Pregnancies Better Eating and Activity Trial (UPBEAT) RCT. *BMC Medicine* 2018;17:15. doi: 10.1186/s12916-018-1248-7

Abstract

Background Pregnancy is associated with widespread change in metabolism, which may be more marked in obese women. Whether lifestyle interventions in obese pregnant women improve pregnancy metabolic profile change is unknown. The objectives of this study were to determine the magnitude of change in metabolic measures during obese pregnancy, and indirectly compare these to similar profiles in a general (unselected for BMI) pregnant population, and to determine the impact of a lifestyle intervention on change in metabolic measures in obese pregnant women.

Methods Data from an RCT of 1158 obese (BMI \geq 30 kg/m²) pregnant women who were recruited from six UK inner city obstetric departments were used. Women were randomised to either the UPBEAT intervention, a tailored complex lifestyle intervention focused on improving diet and physical activity or standard antenatal care (control group). UPBEAT has been shown to improve diet and physical activity during pregnancy and up to 6-months postnatally in obese women, and reduce offspring adiposity at 6-months; it did not affect risk of gestational diabetes (the primary outcome). Change in the

concentrations of 158 metabolic measures (129 lipids, 9 glycerides and phospholipids, and 20 low-molecular weight metabolites) quantified from Nuclear Magnetic Resonance on three occasions during pregnancy, were compared using multilevel models. We focused primarily on the magnitudes and precision (95% confidence intervals) of estimates of change and differences between trial arms when describing our results. The role of chance was assessed with false discovery rate of 5% adjusted p-values.

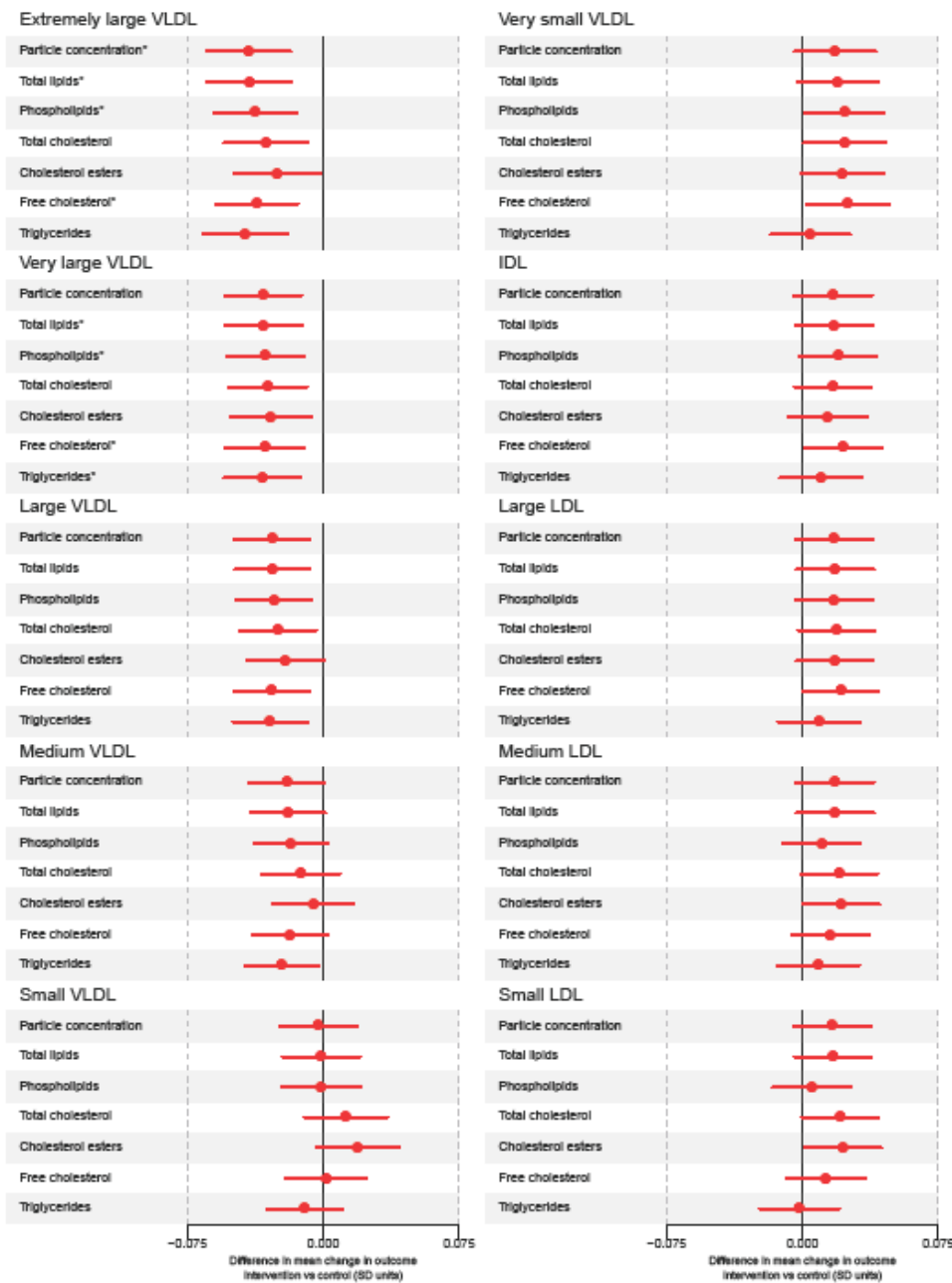
Results All (extremely large, very large, large, medium, small and very small) VLDL particles increased by 1.5 to 3 standard deviation units (SD), and IDL, and specific (large, medium and small) LDL particles increased by 1-2SD, between 16- and 36-weeks of gestation. Triglycerides increased by 2-3SD, with more modest changes in other metabolites. Indirect comparisons suggest that the magnitudes of change across pregnancy in these obese women were 2-3 fold larger than in unselected women (N = 4260 in cross-sectional and 583 in longitudinal) from an independent, previously published, study. The intervention reduced the rate of increase in extremely large, very large, large and medium VLDL, particularly those containing triglycerides.

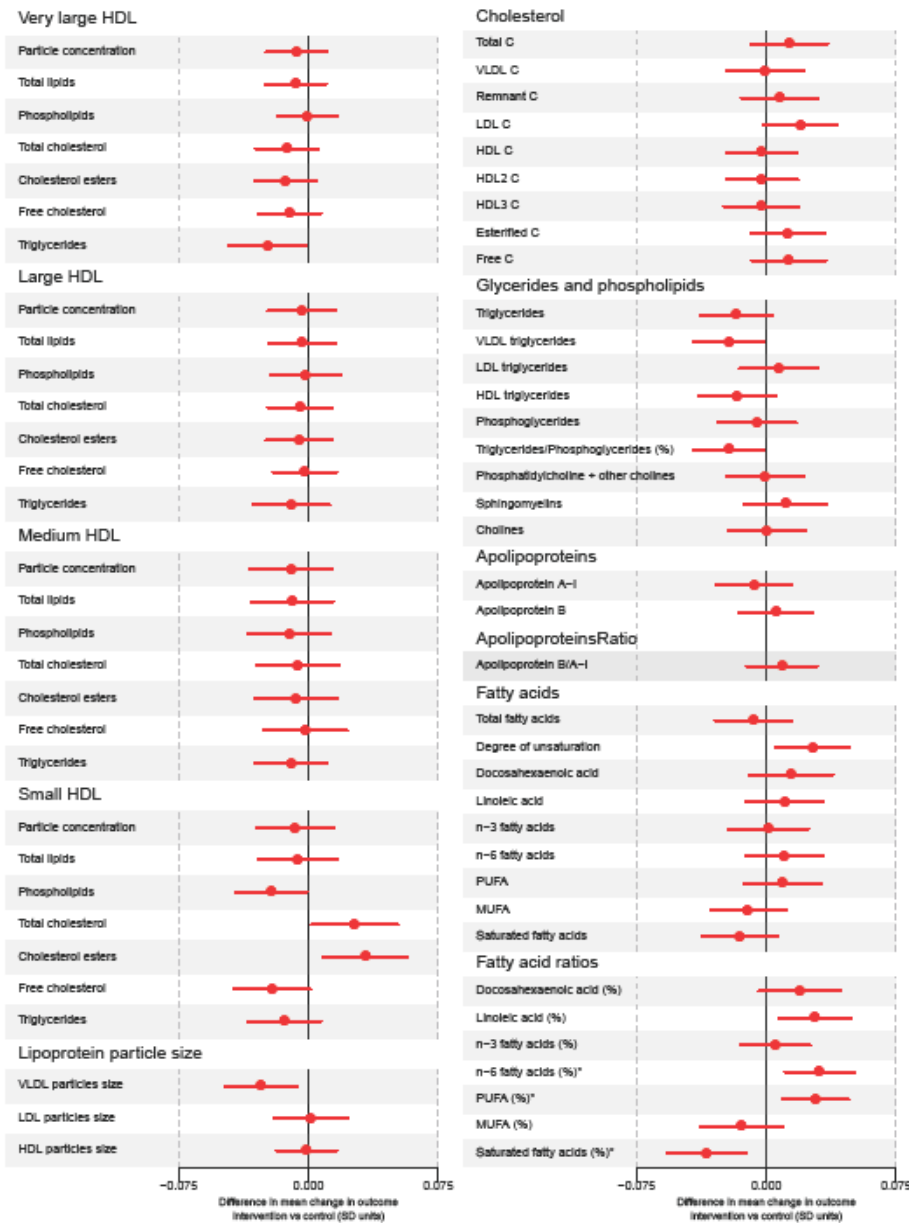
Conclusion There are marked changes in multiple lipids and lipoproteins and more modest changes in other metabolites across pregnancy in obese women, with some evidence that this is more marked than in unselected (for BMI) pregnant women. The UPBEAT lifestyle intervention may contribute to a healthier metabolic profile in obese pregnant women, but our results require replication.

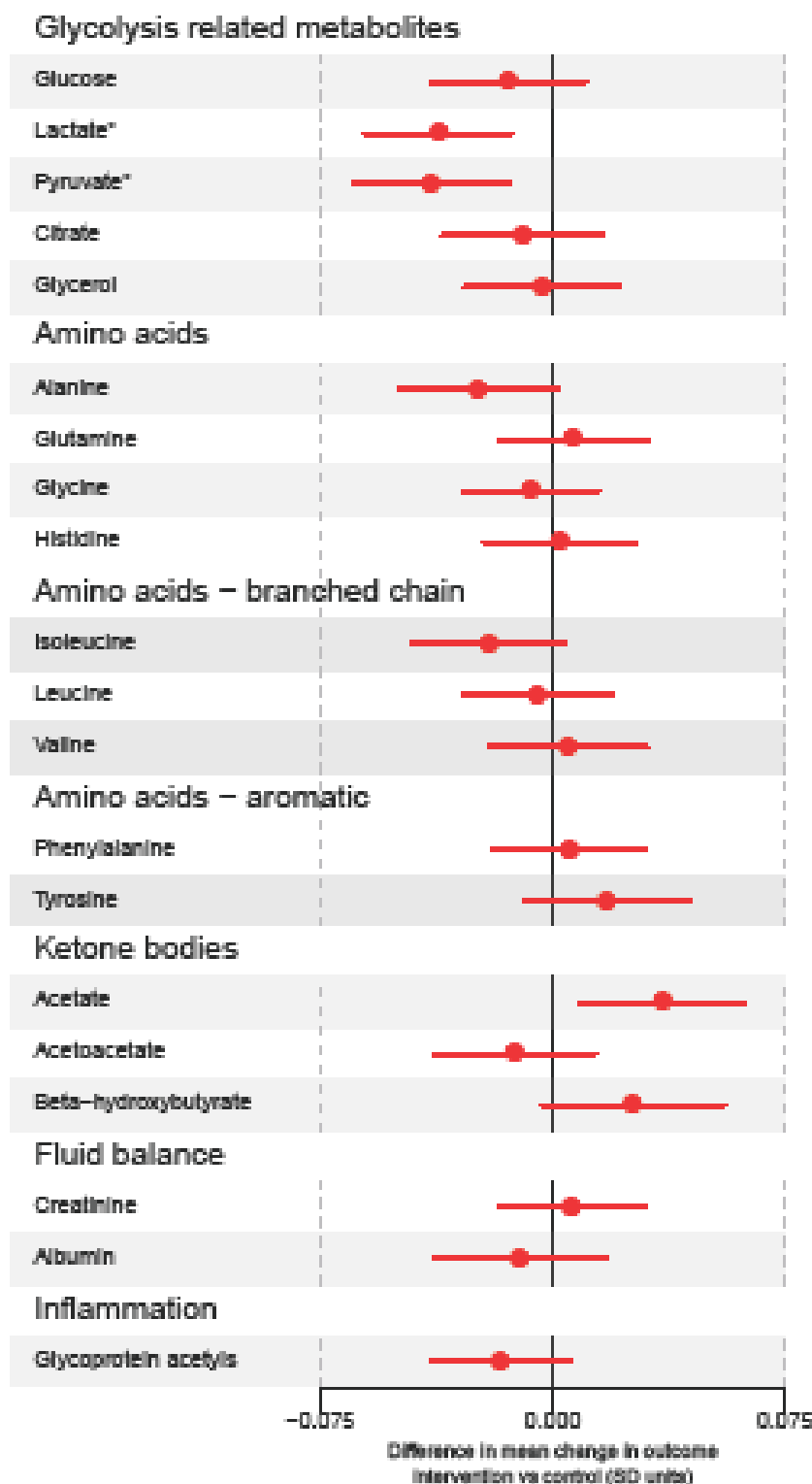
Selected results

Figure 12 shows the difference in mean change for each of the 158 metabolites in SD units per 4-weeks of gestation between 16- and 36-weeks of gestation comparing those who received the UPBEAT lifestyle intervention and those who did not.

Figure 12: . Differences in mean change in metabolites (SD per 4-gestational weeks) across pregnancy comparing women randomised to the UPBEAT lifestyle intervention to those randomised to standard care







3. Conclusions

We have provided in this report a detailed overview of the work that we have, and continue to undertake in relation to developing and promoting appropriate methods for analysing repeatedly assessed data across cohorts to understand life-course of different outcomes and the early life influences on these. Selected methods have been made available to the members of LifeCycle through a

web-based database, which will soon be disseminated within the EU Child Cohort Network and the broader scientific community. We describe methodological work contributing to three key publications relevant to task 7.2 and three illustrative examples of how we have worked with leads from other LifeCycle WPs to ensure these methods are appropriately used to address applied research questions.

We have also described the preparatory work for the tutorial that will form Deliverable 7.4. This, together with the tutorials on causal inference, which are described in the report for Deliverable 7.1, will provide the research community with detailed methods and motivating examples to encourage and facilitate the application of appropriate methods for trajectory analyses and causal inference in life course epidemiology.

Finally, all partners in WP7 are very active in disseminating its work through the organization of workshops and training courses, which will be fully described as part of Deliverable 7.5 (due M60).