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Caesarean Section Delivery and Childhood Obesity

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A thesis submitted to University College Cork

in fulfilment of the requirements for the degree of

Doctor of Philosophy

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LIST OF PEER REVIEWED PUBLICATIONS

Masukume G, O'Neill SM, Baker PN, Kenny LC, Morton SMB, Khashan AS. The Impact of Caesarean Section on the Risk of Childhood Overweight and Obesity: New Evidence from a Contemporary Cohort Study. *Scientific reports* 2018;8(1):15113.

Masukume G, McCarthy FP, Baker PN, Kenny LC, Morton SM, Murray DM, Hourihane JO, Khashan AS. Association between caesarean section delivery and obesity in childhood: a longitudinal cohort study in Ireland. *BMJ Open* 2019;9(3):e025051.

Masukume G, McCarthy FP, Russell J, Baker PN, Kenny LC, Morton SMB, Khashan AS. Caesarean section delivery and childhood obesity: evidence from the growing up in New Zealand cohort. *J Epidemiol Community Health* 2019;0:1–8.

Masukume G, Khashan AS, Morton SMB, Baker PN, Kenny LC, McCarthy FP. Caesarean Section Delivery and Childhood Obesity in a British Longitudinal Cohort Study. PLoS One. 2019;14(10):e0223856-e.

Publications from PhD-related modules: EH6044 Systematic Review and Meta-Analysis and PG6025 Community-based participatory research

Masukume G, O'Neill SM, Khashan AS, Kenny LC, Grech V. The Terrorist Attacks and the Human Live Birth Sex Ratio: a Systematic Review and Meta-Analysis. *Acta medica (Hradec Kralove)* 2017;60(2):59-65.

Aherne A, Barimo J, Barrett H, Burns K, Cargin R, Connolly B, et al. Community research report. 2018. http://hdl.handle.net/10468/9168

LIST OF PRESENTATIONS

Masukume G, McCarthy FP, Baker PN, Kenny LC, Morton SMB, Khashan AS. Caesarean Section Delivery and Childhood Obesity: Evidence from Growing Up in New Zealand. Society for Reproductive Investigation, 66th Annual Scientific Meeting, Paris, France (POSTER). 14 March 2019.

Presentations from PhD-related modules: EH6044 Systematic Review and Meta-Analysis and PG6025 Community-based participatory research

Masukume G, O'Neill SM, Khashan AS, Kenny LC, Grech V. The Terrorist Attacks and the Human Live Birth Sex Ratio: a Systematic Review and Meta-Analysis 7th World Congress on Women's Mental Health, Dublin, Ireland (ORAL). 9 March 2017.

Burns K, Crean A, Fons S, Galvin M, Hally R, Hegarty A, Macken S, **Masukume G**, McCarthy F, McGookin C, Murray M, Olmedo L, Mahony C, Racine E. Creating strong and sustainable partnerships between universities and community groups. 8th Living Knowledge Conference, Budapest, Hungary (POSTER). 31 May 2018. 2nd prize in the 'People's Choice' category.

LIST OF ABBREVIATIONS

AMSTAR - A measurement tool to assess systematic reviews

aRRR - adjusted relative risk ratio

BASELINE - Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints

BF% - body fat percentage

BMI - body mass index

CDC - Centers for Disease Control and Prevention

CS - Caesarean section

GUI – Growing Up in Ireland

GUiNZ - Growing Up in New Zealand

INFANT - The Irish Centre for Fetal and Neonatal Translational Research

IOTF - International Obesity Task Force

IQR - interquartile range

ISSDA - Irish Social Science Data Archive

LS - lower segment

MCS - Millennium Cohort Study

MOOSE - Meta-analysis Of Observational Studies in Epidemiology

NOS - Newcastle Ottawa Scale

NZ - New Zealand

PRISMA - Preferred Reporting Items for Systematic reviews and Meta-Analyses

RR - relative risk

SCOPE - Screening for Pregnancy Endpoints

SD - standard deviation

SDH - Social Determinants of Health

SGA, AGA and LGA - small, appropriate and large for gestational age

UK - United Kingdom

VBAC - vaginal births after Caesarean

VD - vaginal delivery

WHO – World Health Organization

DECLARATION

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

_____ _____

Signed Date

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It’s been a fairy tale.

I would like to express my deep appreciation to my primary supervisors Dr. Ali Khashan, Dr. Fergus McCarthy and Prof. Louise Kenny. In equal measure, I also convey this sentiment to my supervisory panel of Prof. Susan Morton, Dr Sinéad O’Neill and Prof. Philip Baker. Many thanks to Dr Jin Russell for providing pivotal input.

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THESIS ABSTRACT

Background and aims: Caesarean section (CS) birth, in particular elective/planned CS, has been found to be associated with an increased risk of childhood obesity. Various mechanisms that differ by birth mode, including differences in the vaginal and faecal microflora and stress hormone concentration have been suggested to underpin this association. The literature describing this association, often derived from non-nationally representative cohorts has been inconsistent, limited by small sample size, often unable to distinguish between elective and emergency CS, have publication bias favouring positive effects and often unable to adjust for key confounders like maternal pre-pregnancy body mass index (BMI). Given the rising global use of CS with some countries having CS rates above 50%, the aim of this thesis was to critically evaluate the association between CS birth and childhood obesity and to use three large contemporary nationally representative prospective longitudinal cohort studies and one smaller cohort, with detailed phenotypic data, to investigate this association.

Structure and methods: The existing published literature relating to CS birth and childhood obesity was critically evaluated and synthesised to identify major conceptual themes and research gaps (Chapter 1). Chapter 2 details and justifies the thesis' methodological approach. The following four longitudinal birth cohort studies were utilised: Screening for Pregnancy Endpoints (SCOPE) and Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE); Growing Up in Ireland (GUI); Growing Up in New Zealand (GUiNZ) and the Millennium Cohort Study (MCS) cohorts. In order to facilitate comparison between different cohorts (Chapters 3 through to 7), children were classified, on the basis of their BMI, as obese, overweight, normal or underweight according to the sex and age specific International Obesity Task Force criteria. A range of statistical analytic approaches including linear, multinomial and mixed-effects regression were employed. Multiple imputation was used to handle substantial missing data. In addition to our primary outcome BMI, which was modeled as continuous or categorical variable, the association between our exposure CS birth and BF% was investigated in cohorts that had this data available. Where the sample size permitted, the association between CS birth and transition into or out of obesity was examined. The overall results in the context of the

published literature were discussed including limitations and strengths and future research directions (Chapter 7).

***Results*:**

SCOPE-BASELINE cohorts: At two months of age, children born by CS, had a similar BF% to those born vaginally. At age six months, children born by CS had a significantly higher BMI, adjusted mean difference=0.24; 95% confidence interval (CI) 0.06 to 0.41, but this did not persist into future childhood, at age five years. There was no evidence to support an association between mode of delivery and long-term risk of obesity in the child.

GUI cohort: We found insufficient evidence to support a relationship between elective CS and childhood obesity at age three and five years. An increased risk of obesity in children born by emergency CS, adjusted relative risk ratio (aRRR) = 1.56; [95% CI 1.20 to 2.03], but not elective, suggests that the influence of vaginal microflora in developing childhood obesity was minimal. The association with emergency CS was likely due to its indications.

GUiNZ cohort: Planned CS was an independent predictor of obesity in early childhood at age two years aRRR=1.59; [95% CI 1.09 to 2.33] but this association was not apparent by four and a half years This suggests that birth mode is associated with early growth, at least in the short term. This association occurred during a critical phase of human development, the first two years of life. Given the developmental origins of health and disease hypothesis this may lead to long-term detrimental cardiometabolic changes.

MCS cohort: Infants born by planned CS did not have a significantly higher BMI at ages three, five, seven, eleven and fourteen years adjusted mean difference=0.00; [95% CI -0.10 to 0.10], or BF% at ages seven 0.13; [95% CI -0.23; 0.49] and fourteen compared to those born by normal VD. This may suggest that the association, described in the literature, could be due to the indications/reasons for CS birth or residual confounding.

Conclusions:

The hallmark finding of the thesis was an association between CS birth in general, elective CS in particular, and childhood obesity during the first two years of life. This association had dissipated by age three through to fourteen. Whether this association remerges in adulthood or is a risk factor for cardiometabolic disease is an area for future research. The association observed with emergency CS is possibly due to confounding by the underlying reasons for CS, confounding by indication. There is potential to improve consistency and

robustness in this research field by better and standardised definition particularly of the exposure, CS birth. Better consistency in the timing of obesity assessment is also warranted.

Chapter 1: Introduction

1.1. Obstetric mode of delivery and rising Caesarean section rates

Caesarean section (CS) has a history dating back to ancient times where it was practiced by various civilizations albeit mainly post-mortem [1]. Due to advances in fields such as surgery, anaesthesia, microbiology and transfusion medicine CS birth has generally become safe and as a result contemporary outcomes of the procedure are virtually identical when performed by either doctors or non-doctor health care workers in a low-income setting [2]. In such settings for every 1000 CS births, approximately 5.43 women die after surgery [3], an approximately 99.5% survival rate.

The operation is now one of the most common surgical procedures performed in the world, so much so that *The Economist* a prominent and influential weekly newspaper and *Forbes* a business magazine cover the ever increasing rate of this procedure with skepticism on their pages [4, 5]. This illustrates that this subject, beyond the scientific biologic aspects, has pertinent cultural, social and economic considerations.

In the United States (US) the CS rate rose from 20.7% in 1996 to a peak of 32.9% in 2009 [6, 7] This US hike and extant high rate is no exception. Globally CS rates for 169 countries, covering about 98.4% of births, rose from 6.7% in 1990 [7] to 21.1% in 2015 [8]. Some countries like the Dominican Republic, Egypt and Brazil have rates above 50% [8]. Beyond a CS rate of about 10%-15% at population level there is scarce evidence that it saves a mother's or baby's life, however data on other relevant outcomes of the procedure are also scarce like social and psychological outcomes [9, 10]. There is vigorous debate regarding the optimal rate with some authorities suggesting approximately 19% [11]. However, given the challenge, at times, of determining if a CS is/was necessary at individual level, suggested rates can only be a guide. Why the CS rate has risen is multifactorial and includes legal, socio-cultural and economic factors [9]. Some of the factors include having access to private health insurance, fear of sustaining pelvic injury, decline in vaginal births after CS, physician fear of litigation, maternal request, increasing age at first birth, more multiple pregnancies resulting from greater assisted reproductive technology use and specific delivery unit management [12].

It is worth noting that while some countries have too many CS births some still have far too few like South Sudan (0.6%) [11], Niger (1.4%), Chad (1.5%) and Ethiopia (1.5%) [13, 14]. Both too many and too few CS births have been associated with excess maternal morbidity and mortality [14].

1.2. Long-term complications of Caesarean section and proposed general underlying mechanisms

While Caesarean delivery has life-saving benefits for example with obstructed labour, fetal distress and numerous other conditions, it also has been associated with short term and long-term complications for both mother and infant [15]. Asthma, allergies, type 1 diabetes mellitus, gastrointestinal tract disease, autism spectrum disorder, acute lymphoblastic leukemia and cardio metabolic disease - including obesity - have variously been associated with Caesarean delivery [16-21]. The so-called non-communicable diseases which include cardio metabolic disease are becoming increasingly important causes of morbidity and mortality worldwide [22, 23]. Several meta-analyses have linked these before mentioned conditions with CS delivery [18, 19, 24-30]. These associations may or may not be causal given the underlying reason for the CS section taking place (confounding by indication) [31].

One of the factors that may underpin differences in development and susceptibility or resistance to disease between babies delivered vaginally or by CS might be differences in bacterial microbiota that colonize the neonate driven primarily by delivery mode [32, 33], although some dispute this and attribute differences to factors such as maternal obesity, absence of labour and gestational age [29, 34]. Other components of the microbiota like viruses, archaea, fungi and bacteriophages are under-studied, but this is beginning to change, including investigation of the role of the putative placental microbiota [35].

Another contributing factor to the reported differential short term and long-term offspring outcomes by delivery mode is the significant differential concentration of stress hormones between neonates born by various delivery modes [36, 37]. Infants born by planned/elective CS have lower stress hormone concentrations in comparison to those born by vaginal delivery with consequent downstream differences in the development of their

neuro-immuno-endocrine system [37]. Early term birth occurring between 37-38 completed weeks of gestation has been associated with higher odds of special educational needs in later life compared to delivery at 40 completed gestational weeks [38]. Thus earlier delivery is another mechanism underpinning difference in health status and development by mode of delivery. In summary, besides the underlying conditions that may have led to CS, exposure to vaginal microbiota, stress hormones and earlier delivery are the key three hypothesis postulated to drive the association between Caesarean delivery, in particular elective and various outcomes during the life course (Figure 1-1).

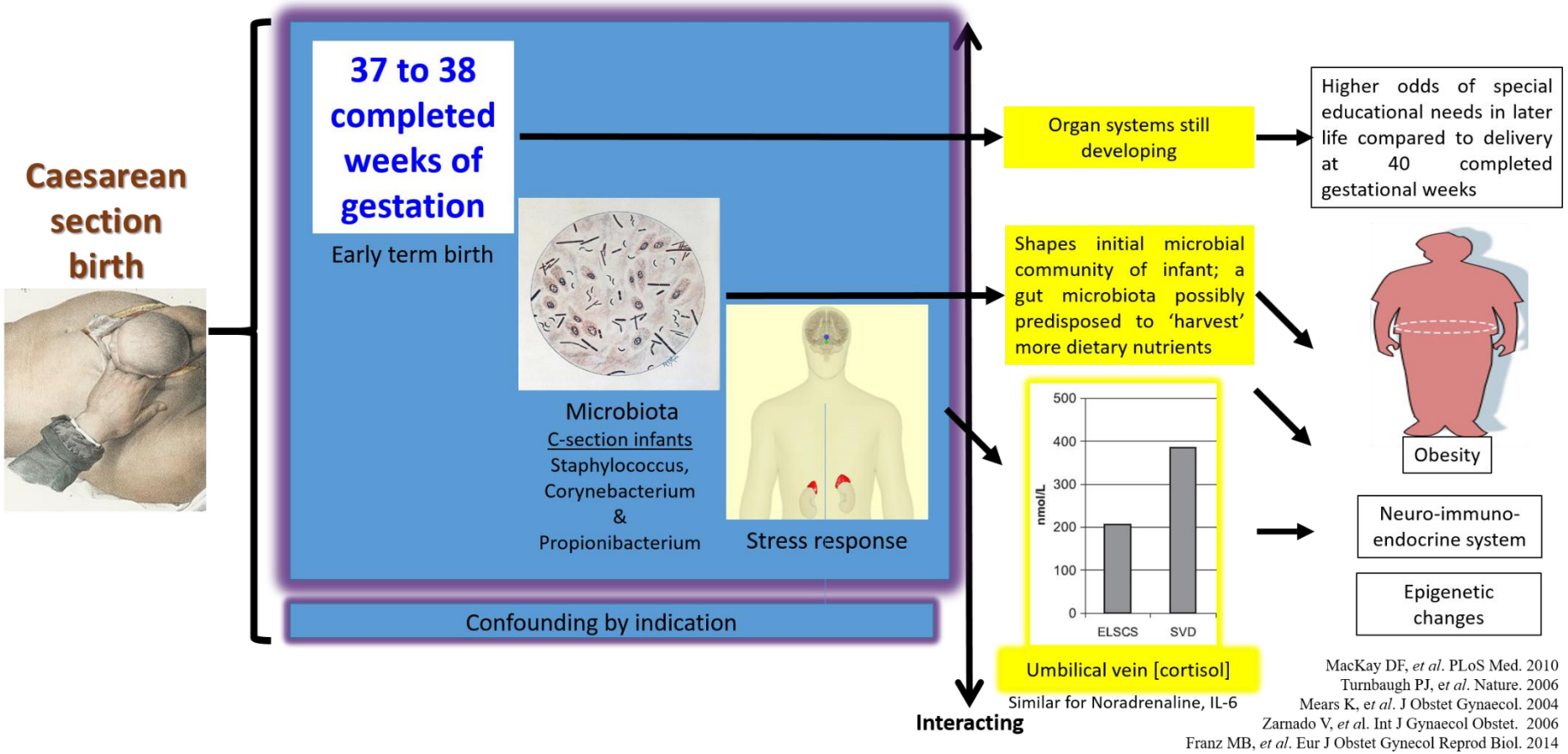


Figure 1- 1. Suggested biologic mechanisms by which Caesarean section birth results in obesity. C-section (Caesarean section), ELCS (elective caesarean section), SVD (spontaneous vaginal delivery), IL-6 (Interleukin 6). Adapted from [39] and images from [40, 41] or in the public domain. Umbilical vein [cortisol] figure reproduced with permission from Mears K et al. [37].

1.3. Caesarean section and post-natal growth mechanisms

Significant differences in the gut microbiota, neuro-immuno-endocrine system and timing of delivery between babies delivered vaginally compared to those born by CS can potentially translate into significant differences in postnatal growth via the mechanisms alluded to. These are considered in more detail:

Microbiota

This is the main mechanism implicated in the occurrence of childhood obesity following CS birth. Microbiota can influence energy balance, specifically, infants born by CS may have a microbiota that is more capable of harvesting dietary nutrients [42-44]. In fact animal studies and direct microbial studies suggest a potential causal role for CS delivery in the genesis of childhood obesity acting mainly via non exposure to vaginal and faecal microbiota [45, 46]. More specifically being overweight or obese has been linked to greater energy harvesting from short-chain fatty acids produced from the child's gut microflora when the bacterial phyla of the mostly gram-positive Firmicutes is greater than that of the gram-negative Bacteroidetes [47, 48]. Caesarean section birth is an important factor in infant gut microbiota dysbiosis namely late colonisation by *Bacteroides* [49, 50]. Higher gut levels of *Bacteroides* have been demonstrated in infants born vaginally [51]. The family Lachnospiraceae of the phylum Firmicutes has been found to be a major pathway via birth mode in the development of childhood obesity [44]. In mice, greater adiposity, body fat inflammation and a tendency of developing diabetes is promoted by the Lachnospiraceae family [52-54]. Microbiota is considered so essential that human trials on exposing infants born by CS to the mother's vaginal fluids by swabbing are being conducted [55].

Stress

Although the precise mechanisms are not well delineated, fetal physiologic stress, experienced during delivery may result in metabolic disorders, including obesity, in later life [56]. As illustrated by Figure 1-1 these mechanisms involve the immune and endocrine systems.

Pre-term birth

Although the proportion of infants that are born pre-term with low birth weight is relatively low, some of them exhibit excessive compensatory catch-up growth which portends the development of childhood obesity [57]. Pre-term birth rates have remained similar for at least the past three decades [58]. However during this time, as mentioned in the next section, childhood obesity rates rose substantially. This signifies that pre-term birth was not a key mechanism driving the surge in obesity.

Elective and emergency CS birth

Diagnosing the onset of labour is challenging [59]. Nevertheless, elective/planned CS is very often performed prior to the onset of labour, while emergency CS is very often performed during labour [60]. Their indications differ [60] and consequently so do their confounding structures. Rupture of the amniotic sac, more common before emergency CS, or labour's onset tilt the microbiota of infants born by emergency CS towards that of infants born vaginally [61]. Another difference between elective and emergency CS is the concentration of umbilical cord stress hormones, like cortisol, where elective CS born infants have the lowest levels [37].[59] Importantly, elective CS is potentially modifiable unlike emergency CS.

Although the separation of CS solely into elective and emergency is pivotal, this classification technique has its limitations [62]. It is for example not possible to determine if an elective CS was purely on maternal request; these can be different from other elective CSs. For instance, the burden of neurotic, stress-related, somatoform mood and other psychiatric disorders was found to be higher in women who gave birth by CS on maternal request [63]. Improving CS classification is an ongoing international effort [62, 64].

1.4. Determinants of childhood obesity

The World Health Organization (WHO) defines the social determinants of health (SDH) as the conditions and circumstances in which people are born, grow, live, work and age; and these are in turn influenced by political and economic factors [65, 66]. Mode of delivery is a condition or circumstance of birth. This thesis, which seeks to investigate if CS birth is a proximal/causal determinant of childhood overweight and obesity, is therefore located in the SDH framework. However, it is useful to distinguish between proximal and distal

determinants of childhood overweight and obesity. For instance, distal political factors can influence food prices, however the proximal childhood obesity determinant would be consuming cheap unhealthy processed calorie-dense food [67]. Some proximal determinants of childhood obesity include maternal diabetes, smoking and nutrition, fetal growth restriction, a large birth weight and parental obesity (Figure 1-2). How these childhood obesity determinants connect with each other, in relation to answering the thesis question, is considered further in Chapter 2.

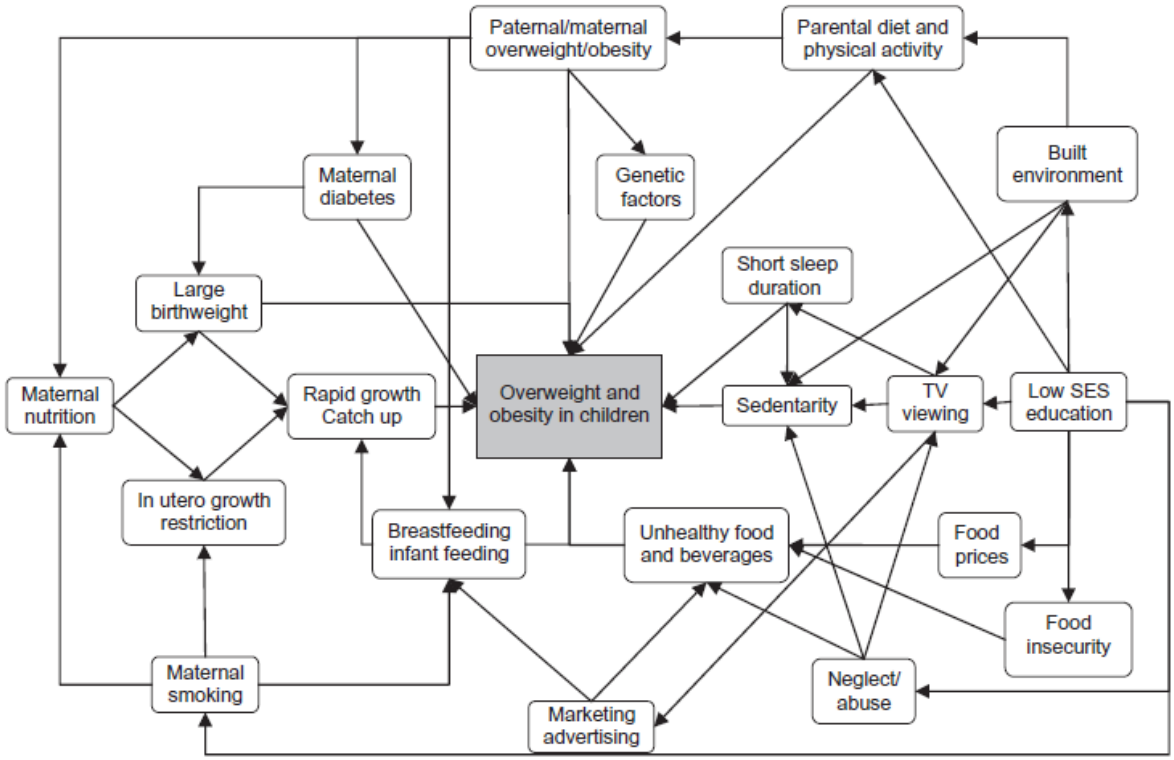


Figure 1- 2. The complex web of potential determinants of overweight and obesity in children. Reproduced with permission from Monasta et al. [68].

Studying the relationship between CS birth and post-natal growth is important given the growing worldwide epidemic of childhood obesity [69]. In 1975 obesity rates in children were less than 1% [70]. From then onwards, virtually all world regions had an upward trajectory in obesity rates, particularly in Polynesia and Micronesia. Nowadays rates of 10% are not uncommon [69]. By 2016, about 50 million girls and 74 million boys were obese worldwide [70]. Extant obesity rates for high-income regions are at a high plateau, with accelerating rates especially in some parts of Asia.

Childhood obesity has serious multisystem and often long-term complications that include obstructive sleep apnoea, hypertension, insulin resistance, fatty liver disease, gastro-oesophageal reflux, kidney hyperfiltration, lower-limb malalignment and adverse psychological effects [71]. If the link between CS birth and developing childhood obesity is causal, then these complications would be expected to increase.

Depending on where fat deposition occurs predominantly, obesity can be general or abdominal/central. The distinction between general and central obesity is more commonly made in adulthood. There is however growing recognition that central fat deposition in children ought to receive more attention in clinical and epidemiologic studies [72]. Both forms of obesity are independently associated with mortality, with central obesity being more predictive in certain populations [73].

Triangulation [74] of animal, direct microbial studies along with epidemiologic studies will help to answer the question regarding if CS birth causes overweight and obesity in children.

2. Literature review

The aim of the narrative literature review was to synthesise the published literature on the association between CS birth and childhood obesity and identify the gaps in the current literature [75]. A search for systematic reviews on the association between CS birth and childhood obesity was implemented through the electronic database MEDLINE via PubMed without language restriction, using Boolean search principles (AND or OR) and Medical Subject Headings terms (for example Caesarean Section OR Abdominal Delivery OR C-section). Childhood was defined as age < 18 years. Searches were performed from database inception up to 7 April 2020. The full search strategy is included in Appendix 3. In addition, a reference list hand search of retrieved articles and Google Scholar's cited by tool were employed. The output of this bibliographic search strategy was four systematic reviews and meta-analyses, from the 343 articles retrieved, which are summarized by Table 1-1 and subsequently critically evaluated. Small effects, odds ratio (OR) < 1.50, have been the general finding. The key differences between these reviews were, firstly, differences in the criteria utilised to include studies. The most recent, 2018 review [15], had arguably the most stringent criteria; namely only prospective cohort and randomised studies with greater than 1000 participants and with a minimum of a year's follow-up were deemed to be suitable for

inclusion [15]. No randomised studies were found. The second key difference was the stage across the life course considered; some studies considered only children [28] (up to 18 years), both children and adults [15, 27, 30] and only adults [29]. The final main difference was making a distinction between elective/planned and emergency/unplanned CS in the analysis. The most recent review by Keag *et al* did not make this distinction [15] as well as the review by Kuhle e*t al* [28]. Distinction between elective and emergency CS was made by Darmasseelane *et al* [29] (four studies), Sutharsan R *et al* [30] (two studies) and Li *et al* [27] (four data sets); these authors found no difference, an increased risk of being overweight or obese and no difference in those born by elective compared to emergency CS respectively. Geographically the underlying studies included in the aforementioned reviews were from all continents save for Africa and Antarctica.

Besides the limited capacity to distinguish between elective and emergency CS other limitations of the reviews include a limited ability to take into account pre-pregnancy BMI (a critical confounder), the presence of publication bias (which one study described as gross [30]), high to moderate heterogeneity and the ever present possibility of residual confounding [15, 28, 29].

Asymmetry of the funnel plot (Table 1-1) was assumed to indicate publication bias although there are other causes of funnel plot asymmetry [76]. Nevertheless, the possible impact of this presumed publication bias was assessed using the trim-and fill method by Li and colleagues [27]. The method imputes missing studies, to restore symmetry and calculates a 'true' effect measure [77]. The basis of this is the assumption that there is withholding of publications by authors and journal editors of small studies with results they consider to be unfavourable. Besides the assumptions mentioned, the trim-and-fill estimator employed to impute missing studies – whether R_0, L_0, or Q_0 – was not mentioned by Li et al. It is against this backdrop that the following discourse ensues. R_0, L_0, or Q_0 relate to different mathematical formula used to impute missing studies [77].

Before delving into a narrative critical appraisal of the individual systematic reviews and meta-analyses, in summary, there were small effects between CS birth and childhood obesity that emanated from a literature exhibiting (gross) publication bias favouring positive effects, an inability to account for pre-pregnancy BMI and not often distinguishing between elective and emergency CS birth. These limitations suggest that the true effect is closer to the null or is null. Critical evaluation of systematic review methodological quality was conducted with a measurement tool to assess systematic reviews (AMSTAR) (Table 1-1) and Appendix 2 that is reliable and valid [78, 79]. During AMSTAR development, calculation of an overall score was not factored in [80], thus there are no specific score cut off points for quality. However, to provide a gauge for review methodical rigour, the number of AMSTAR tool yeses were counted and presented and the following narrative review and qualitative assessment of quality was done. The rationale for conducting a narrative review was because it allows for wider scoping, synthesis, appraisal and identification of knowledge lack in the published literature [75]. In addition, considering the number of recent systematic reviews on this topic, what was needed is a narrative review to identify the gaps in the current literature.

Keag OE *et al (2018)*

This review was conducted by researchers from the United Kingdom and Australia according to a pre-specified registered protocol, the underlying studies were assessed for inclusion by two independent reviewers, and the authors of the original studies were contacted to clarify ambiguities. The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) recommendations were adhered to [81]. Only term infants (> 37 weeks gestation) recruited from prospective cohort or randomised studies with at least 1000 participants followed up for at least one year were considered for inclusion.

This review published in 2018, is comprehensive in that it had a remit that went beyond interrogating the association between CS birth and offspring overweight and/or obesity to also include maternal outcomes like pelvic floor dysfunction, offspring outcomes like asthma and subsequent pregnancy outcomes like perinatal death [15]. Six cohorts were included in the final meta-analysis: for CS birth and obesity up to age five years [82-85] and five cohorts

for children aged six to fifteen years [83, 85-87]. Reporting on an absolute scale in addition to a relative scale, by way of numbers needed to harm, was done to aid counselling in antenatal clinics: assuming causality 28 CSs, 95% confidence interval (CI) 16-82 would be needed to cause one additional obese child at six to fifteen years of age. An association between mode of birth and BMI that was of a greater magnitude for childhood obesity than it was for being overweight was a focal finding. However, there were limitations. The authors of this review specifically drew attention to being unable to analyse their results by the indication for CS or by whether the CS was elective or emergency. Substantial heterogeneity, defined as an I^2 statistic > 40%, was found suggesting that essentially apples and oranges were being compared, thus the systematic review's findings were unlikely to be generalizable to most settings. In addition, no sex-specific effects were explored.

Despite the limitations, a pattern of decreasing odds of obesity for CS born infants with increasing age was observed (Table 1-1). This suggests that other risk factors for obesity like a sedentary lifestyle and a high intake of calories grow in influence with increasing age and the putative influence of CS decreases.

Table 1- 1. Systematic reviews and meta-analyses comparing Caesarean and vaginal birth on the risk of childhood obesity.

Study [year]	**Search date up to**	**Studies included***	**Publication bias assessed**	**Key results**	**AMSTAR Number of Yeses****
Keag OE *et al* [2018] [15]	May 2017	n=12 [82-93]	No	Obesity up to 5 years [82-85]: OR 1.59, 95% CI 1.33-1.90, $p < 0.00001$, $I^2 = 68\%$; 6 cohorts Obesity 6-15 years [83, 85-87]: OR 1.45, 95% CI 1.15 to 1.83, $p = 0.002$, $I^2 = 63\%$; 5 cohorts	8
Kuhle S *et al* [2015] [28]	July 2014	n=24 [22, 83-90, 94-108]	Asymmetry of funnel plot*** Egger's test (P = 0.072)	Obesity 2-18 years: RR 1.34, 95% CI 1.18–1.51	8
Sutharsan R *et al* [2015] [30]	April 2014	n=14 [83-90, 92, 95, 97, 102, 109, 110]	Gross asymmetry of funnel plot***	Obesity up to 5 years: RR 1.80, 95% CI 1.34-2.44; 2 studies Obesity 5-18 years: RR 1.36, 95% CI 1.10-1.68; 5 studies	8
Li HT *et al* [2013] [27]	June 2012	n=8 + one unpublished [83, 84, 86, 90, 94-96, 109]	Asymmetry of funnel plot*** Begg's test (P= 0.009)	Obesity 3–8 years: OR 1.40, 95% CI 1.17-1.67, $I^2 = 50\%$; 6 estimates	8

*Overlap of reference numbers between the reviews indicates that the same study was included. **Maximum = 11. ***Favours positive effect. OR – odds ratio, CI – confidence interval, RR – risk ratio, AMSTAR - A measurement tool to assess systematic reviews.

Kuhle S *et al (2015)*

In this review by authors from Canada, published in 2015, it was not stated if the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) [111] or MOOSE recommendations were adhered to. In addition the review was not conducted according to a pre-specified registered protocol. This does not however suggest its quality was inherently low because during the time this review was conducted it was less common to register protocols [112]. Two independent investigators evaluated studies for inclusion according to a set of explicit criteria. There was no restriction by gestational age.

A total of 24 studies were included. Unlike the paper by Keag *et al*, case-control [94, 96, 101, 106] and cross-sectional studies, where the exposure and outcome are assessed simultaneously [98, 99, 104, 108], were included. Case-control studies are efficient for rare diseases and are prone to selection and recall bias; however given the prevalence of overweight (> 10%) which was not rare, including case-control studies might not have been efficient. With cross-sectional studies lack of temporality makes establishing any potential causal relationship challenging. Distinction between elective and emergency CS was not made as well as evaluating sex-specific effects.

The main result showing a small increase in the odds of obesity OR 1.34, 95% CI 1.18–1.51 in those born by CS compared to VD infants should be interpreted with caution given the aforesaid limitations and evidence of publication bias favouring positive effects. Subgroup analysis/sensitivity analysis by adjustment for pre-pregnancy BMI (Yes/No), study design (Cohort/Case control or cross sectional), country income (high/middle), exposure (CS/early life factors) and CS rate (≥ 30% or < 30%) was done. Attenuation of effects occurred mainly when pre-pregnancy BMI and early life factors were considered.

Sutharsan R *et al (2015)*

Like the article by Kuhle *et al*, a pre-specified protocol was not utilised, nonetheless two independent reviewers assessed studies for inclusion and the PRISMA recommendations

were followed [111]. Some of the salient aspects about this review, originating from Australia include that only longitudinal studies from the English literature with greater than a year's follow-up were included. No cut-off for gestational age was used. A total of 14 studies were included.

Most of the studies adjusted for more than three confounders, however six adjusted for pre-pregnancy BMI (a critical confounder). Importantly an analysis by planned versus unplanned CS was performed which found a higher risk of overweight and obesity in those born by planned CS. No sex-specific effect was observed of CS on the risk of becoming overweight or obese (four data sets).

To aid in a clinically meaningful discourse, the numbers needed to harm were calculated. Assuming causality 45 CSs, 95% CI 14-∞ would be needed to cause one additional overweight or obese child at five to eighteen years of age.

Like the review by Keag *et al* decreasing odds of obesity for CS born infants with increasing age was observed (Table 1) and an association that was of a greater magnitude for childhood obesity than it was for being overweight was found.

Evidence of gross publication bias in favour of positive effects was found and it was concluded that the overall small effects, albeit positive, are the cumulative result of biases. This conclusion is consistent with the landmark finding that the final results of most medical research are an accurate measure of bias rather than true effects [113].

In addition to the before mentioned sensitivity analysis by sex (female or male) and type of CS (planned or unplanned) analysis by country income (high or upper-middle) did not alter the results materially.

Li HT *et al (2013)*

This Chinese review which adhered to the MOOSE recommendations was broadly similar in terms of strengths and limitations to the aforementioned reviews save that by Keag OE *et al*. In particular it was similar to Kuhle and colleague's review wherein in addition to cohort studies, case-control studies - which have their aforementioned limitations - were included. Like the review by Sutharsan and colleagues language restriction to English was made. A

total of eight studies and one unpublished study were included after assessment by two independent reviewers.

Like the other reviews there was substantial heterogeneity (I^2 statistic > 50%) and evidence of publication bias favouring positive effects on a background of small effects OR < 1.50. This suggests reduced external validity of results which are close to the null.
For the association between CS birth and childhood obesity, no sex-specific effects were observed. Like the review by Keag *et al* an association between mode of birth and BMI that was of a greater magnitude for childhood obesity than it was for being overweight was found.

An interesting aspect was how there was no distinction made between confounders and mediators. By definition confounders can only be pre-exposure variables [114]. In this review and in the others, in the analysis, mediators like breastfeeding were treated as confounders. However mediation analysis is different from how confounders are handled [115]. Treating confounders and mediators synonymously might have influenced the final estimates.

Most of the covariates pertained to maternal characteristics. In this review, only two studies considered paternal BMI which is not unusual because often in obstetrics paternal data is lacking. This limitation applied to the other reviews as well. Adjustment for paternal factors would be expected to attenuate estimates towards the null.

Combining the results of the meta-analyses was deemed to be inappropriate because firstly, to a significant extent, the same underlying studies were included by each systematic review (Table 1-1). Secondly, there was substantial heterogeneity with an I^2 = 68% in some instances. Thirdly, on the basis of finding publication bias by multiple reviews, combining results would magnify bias.

Newly published studies not included in the extant systematic reviews

The before mentioned search strategy (Appendix 3), using Boolean operators, was used to systematically retrieve newly published original papers from the MEDLINE (via PubMed) and Scopus databases. A hand search was also made of the reference list of retrieved papers. Searches were performed from 1 April 2017 up to 26 March 2020. The output of this search strategy includes Table 1-2 and the subsequent critical assessment of this literature. Of the eighteen new papers retrieved from 708 articles, by the search strategy, approximately seven took into account the distinction between elective and emergency CS. Seven had large sample sizes (> 1500). Five, of these new studies made an adjustment for pre-pregnancy BMI. Geographically, of the permanently inhabited continents, Africa was not represented. Sutharsan and colleagues [30] proposed that studies, on this topic, which were prospective, adjusted for pre-pregnancy BMI and adjusted for a greater number of variables were of higher quality. Thus Table 1-2, in the absence of a formal validated tool to assess studies on this topic, also serves as a means to gauge the quality of the included studies on the basis of study design, sample size, splitting into elective or emergency CS, and which potentially confounding variables and time points were considered.

The Newcastle Ottawa Scale (NOS) is a generic tool for the quality assessment of non-randomised studies to be included in systematic reviews [116]. One of the NOS sections which pertains to adjustment for potential confounders, the comparability section, is awarded the least number of stars at two. In the following consideration of study quality, more than two important confounders were considered. In addition, the NOS may not be able to identify biased results and it has variable agreement [116].

Table 1- 2. New published papers since the last systematic review search in May 2017.

Paper #	Setting	Design	Sample size	Nationally representative	Pre-pregnancy BMI	Elective versus Emergency CS	Age (years)	Variables adjusted for	CS rate (%)	BMI reference	Key result
Zhou et al 2020 [117]	China	Prospective cohort	569	No	No	No	10-15	sex, birth weight, delivery mode, gestational age, children's history of hypertension, maternal pregnancy age, maternal BMI status, maternal schooling, and household income	28.5	BMI trajectory	Identification of five BMI trajectories, CS birth increased risk of the "progressive obesity" trajectory OR=2.50; 95% CI 1.42 to 4.41
Zhou et al 2019 [118]	China	Prospective cohort	1467	No	No	Yes	4-7	micronutrient supplementation, maternal age, educational level, ethnicity, occupation, BMI in early pregnancy, offspring sex, birth weight, and gestational age	N/A	Growth curves for Chinese children	Elective CS was associated with increased central obesity OR=1.33; 95% CI 1.02 to 1.72

Hawkins et al 2019 [119]	United States	Prospective cohort	55058	No	No	No	2, 5	child sex, maternal race/ethnicity, education, age, marital status, number of children in the household, year of birth, and the presence of multiple siblings per family by clustering on the family identifier	23.5	CDC	No association between CS delivery and BMI z-score after using sibling control analysis BMI z-score (SD) -0.13 (1.14)
Veile et al 2019 [120]	Mexico	Prospective cohort	128	No	No	No	0.1, 4	child age, population group, infant sex, birth order, child birth weight, maternal age, maternal height	47 & 20	WHO	At age 4 years, CS delivered Yucatec Maya girls and boys, and Toba/Qom boys, had a significantly higher weight-for-age compared to children delivered vaginally OR=4.167; 95% CI 0.937 to 18.538
Azcorra et al 2019 [121]	Mexico	Prospective cohort	256	No	No	No	6-8	place of residence, household crowding index, child's age, child's sex, birth weight, and maternal fat mass	43	Frisancho, A. R. (2008)	CS birth was associated with increased levels of childhood adiposity in girls but not boys OR=4.167; 95% CI 0.937 to 18.538

Cai et al 2018 [122]	Singapore	Prospective cohort	727	No	No	Yes	1	maternal ethnicity, age at delivery, educational level, parity, early pregnancy BMI, antenatal active or passive smoking, hypertensive disorders of pregnancy, GDM, and infant sex-adjusted BW-for-GA	30.5	WHO	Infants born by elective CS were more overweight at 1 year of age OR=1.93; 95% CI 1.07 to 3.48
Chojnacki et al 2019 [123]	United States	Retrospective survey	104	No	Yes	Yes	7-10	sex, birth weight, weight-for-gestational age percentile, maternal BMI pre-conception, feeding and duration type (i.e. breastfeeding vs. formula feeding)	28.6	CDC	Infants delivered by elective CS had increased adiposity in preadolescence compared to those born by VD and emergency CS=3.23 kg/m^2; 95% CI 0.50 to 5.96
Bar-Meir et al 2019 [124]	Israel	Historical prospective study	11001	No	Yes	No	17	maternal education, socioeconomic status, ethnicity, offspring sex, toxemia,	7.0	WHO adult BMI≥25kg/m^2	At age 17 years, CS birth was positively associated with being overweight or obese. This association was evident only in infants born to mothers who

								diabetes in pregnancy, multiple pregnancy, birth order, maternal age at delivery, smoking during pregnancy, pre- and post-term delivery, birth weight			were in the highest pre-pregnancy BMI quartile Overweight/Obese OR=1.44; 95% CI 1.14–1.82
Nunes et al 2019 [125]	Brazil	Cross sectional	475	No	No	No	6-7	No adjustment	41.3	WHO	There was no difference in mean BMI by delivery mode
Mueller et al 2018 [126] *	United States	Prospective cohort	563	No	Yes	No	0.25, 0.5, 0.75, 1	maternal age at delivery, race, marital status, highest educational achievement, household income, smoking status, pre-pregnancy BMI, and infant birth weight	31.8	WHO	CS birth was associated with increased weight gain during the first year of life, difference in adiposity by birth mode had appeared by age 3 months weight-for-length z score=0.26, 95% CI 0.05 to 0.47
Chu et al 2018 [127]	China	Cross-sectional survey.	13724	No	No	Yes	5-13	maternal education, paternal education, maternal history of gestational diabetes including	46.6	BMI curves for Chinese children and adolescents [128]	There was an increased risk of being overweight or obese in children born by CS overall and CS with no medical indication Obese OR=1.44; 95% 1.26 to 1.66

								impaired glucose tolerance, birth weight, gender, new-born resuscitation, family income, gestational age at birth, feeding within the first 4 months, children's passive smoking exposure			
Tun et al 2018 [44]	Canada	Prospective cohort	935	No	Yes	Yes	1, 3	location, infant sex, maternal race/ ethnicity, maternal prenatal asthma, maternal smoking during pregnancy and direct exposure of infants to antibiotics.	24.3	WHO	CS born infants of overweight mothers had a five times higher risk of being overweight at age 1 year OR=5.02; 95% CI 2.04 to 12.38
Lavin et al 2018 [129]	Vietnam	Prospective cohort	1937	No	No	Yes	8	wealth index, household primary education level, maternal anthropometry,	9.2	WHO	The odds of being overweight or obese in children born by planned or unplanned CS was similar to children delivered vaginally

								parity, birth weight, breastfed to 6 months, geographic location, smoker currently lives in household, total number of times child ate in last 24 hours, number of different food groups child ate in last 24 hours, and maternal age			Obese OR=2.2; 95% CI 1.2 to 4.0
Wang et al 2017 [130] (In Chinese)	China	Cross sectional	42758	No	-	-	6-17	-	-	-	Birth by CS section was associated with a higher risk of obesity
Vehapoglu et al 2017 [131]	Turkey	Cross sectional	4990	No	No	No	2-14	gender, birth weight, duration of breastfeeding, timing of solid foods initiation, maternal education level, and maternal smoking during pregnancy	44.5	IOTF	No association between CS birth and childhood obesity was found OR=0.98; 95% CI 0.64 to 2.87
Smithers et al 2017 [132]	Australia	Prospective Cohort	4099	No	No	Yes	3-6	maternal age, antenatal care, antenatal visits,	N/A Eligible	WHO	There was no association between CS birth and

								medical conditions during pregnancy (asthma, diabetes, hypertension), smoking in pregnancy, gestational age, birth weight for gestational, mother had a partner, maternal ethnicity, maternal occupation, neighbourhood-level indicators of socioeconomic disadvantage and remote residence	women were those who had a prior CS and then had an elective CS or spontaneous vaginal delivery		anthropometric measurements ATE=0.11; 95% CI -0.25 to 0.46
Mueller et al 2017 [133]	United States	Prospective Cohort	1441	No	Yes	No	2-8	maternal age, maternal race/ethnicity, maternal education, 2nd trimester exposure to air pollution, maternal pre-pregnancy BMI, gestational	33.3	CDC	There was an association between CS delivery together with pre-pregnancy overweight and obesity with childhood overweight or obesity OR=1.4; 95% CI 1.1 to 1.8

								weight gain, birth weight. smoking during pregnancy, diabetes, maternal marriage status, prenatal and intra-partum antibiotics, household income			
Chen et al 2017 [134]	Taiwan	Prospective Cohort	19269	Yes	No	No	5.5	gestational age, infant gender, maternal age, maternal education, family monthly income, GDM, gestational weight gain	33.2	Taiwan Bureau of Health Promotion criteria	There was a robust association between CS birth and childhood obesity. OR 1.18; 95% CI 1.07 to 1.30

BMI – Body-mass-index; CS – Caesarean section; GDM – Gestational diabetes mellitus; OR – Odds ratio; RRR – Relative risk ratio; ATE Average treatment effect; OWOB – Overweight/Obese; N/A – Not applicable; WHO – World Health Organization; CDC – Centers for Disease Control and Prevention; IOTF – International Obesity Taskforce.

* subscapular and triceps skinfold thickness included, SD – standard deviation

Excluding papers from this thesis

For the new papers, substantial differences existed in terms of study setting, sample size, adjustment for pre-pregnancy BMI, considering elective versus emergency CS and the age at evaluation of the outcome (Table 1-2). It is consequently not surprising that the final study results are heterogeneous. Although more studies, on the topic, are beginning to consider elective versus emergency CS this is often at different ages: for example at age one year [122], two years [135], three to six years [132] and seven to ten years [123]. In childhood, half a year is enough to alter the classification criteria for being overweight or obese [136]. Therefore, combining these disparate ages in a meta-analysis would be incongruous. Nevertheless, what may be considered to be the most robust studies, in terms of analytic approach [119] [137] - including use of a sibling cohort design, found no positive association between CS birth and childhood obesity. This accords with findings from the aforementioned systematic reviews and meta-analyses, that studies which adjusted for more confounders had results closer to the null. A pattern emerged where a positive association was generally evident in studies that considered the first two years of childhood [44, 122, 126, 138]. Given the pivotal importance of events during the first two years of life in predisposing one to adulthood disease [139], this pattern requires further exploration by future studies. In summary the main limitations and gaps in the literature are a triad of not infrequently small sample sizes, commonly not distinguishing between elective and emergency CS birth and often not adjusting for pre-pregnancy BMI.

The rationale for conducting this research, which gives rise to the aims and objectives, was to fill the aforementioned frequent gaps in the literature like the inability to distinguish between emergency and elective CS, not adjusting for maternal pre-pregnancy BMI, not considering infant macrosomia as a potential confounder, not investigating sex specific effects, not allowing for the movement of children from one BMI category to another, small sample sizes (< 1500), publication bias favouring positive effects, disparate handling of confounders and mediators, not using statistically principled techniques to handle missing data, use of non-nationally representative cohorts, differing time points at which childhood obesity was evaluated and not measuring body fat percent by robust techniques like air displacement plethysmography. Filling these research gaps would contribute to the body of knowledge by helping to establish if the association between CS birth and childhood obesity is more than a mere correlation.

3. Aims and objectives

The general aim of this thesis, by publication, was to assess the association between CS delivery, particularly elective, and childhood obesity.

In particular the objectives were to:

a) Perform a literature review to synthesise the evidence examining the effect of a Caesarean section birth on subsequent offspring childhood obesity.
b) Investigate the relationship between Caesarean section birth and childhood obesity using three nationally representative prospective longitudinal cohorts and one hospital-based prospective cohort.
    i. To examine whether emergency and elective CS had different effects on the outcome and to investigate sex-specific effects.
    ii. To examine the potential confounding effect of maternal BMI and macrosomia.
    iii. To determine whether the exposure had an influence on transitioning.
c) Update the literature review in the context of the findings from the present thesis and any newly published research articles on the topic.

4. Hypothesis

The null hypothesis (H_0) would state that:

a) There is no association between Caesarean section delivery, particularly elective, and childhood obesity.

Chapter 2: Methods

This chapter begins with thoughts regarding how the research question could be best answered. Next is consideration of the cohorts used and this is followed by the ethical framework employed, and finally the analysis plan together with the basis for selecting the statistical approaches.

A well conducted clinical trial randomising pregnant women to give birth by CS or vaginally, and following up children to assess their weight, would provide a definitive answer to the thesis question [140]. In order to preserve the benefit of randomisation, an intention-to-treat analysis would be particularly warranted [141]. This is because the final mode of delivery, for some women, will unquestionably differ from that assigned to them initially. For instance, a woman allotted to the vaginal birth group may develop an emergency which requires a CS.
Conducting such a randomised clinical trial may however be morally and ethically indefensible. In the absence of such a trial, observational studies provide the best available evidence.

The thesis question was investigated by using prospective longitudinal data obtained from four contemporary birth cohorts whose main characteristics have been summarized in Table 2-1. Permission to utilise the data was obtained from and granted by the study gatekeepers after signing data sharing agreements. These agreements governed research data usage. The large sample size, nationally representative Growing Up in Ireland (GUI), Growing Up in New Zealand (GUiNZ) and UK Millennium Cohort Studies (MCS) [142, 143] were designed in a very similar way with broadly comparable time points at participant follow-up that facilitated harmonized analysis. The diverse contexts of the populations for example, different CS rates, Northern/Southern hemisphere, extent of ethnic diversity and the particular nature of healthcare systems helped to capture more nuanced aspects. The hospital-based Babies After the Screening for Pregnancy Endpoints Study Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) Study had

mothers and infants that were richly phenotyped, with multiple time points available for analysis in early childhood [144]. All anthropometric measurements were robust because they were obtained by trained interviewers using standardized protocols and medically approved instruments with audit procedures [142-145]. Because the cohort data had already been collected and outcomes observed, calculation of post hoc power would have been misleading [146].

Table 2- 1. Summary of included cohort studies.

Cohort*	**Number of children recruited**	**Period of recruitment and 'inclusion criteria'**	**Number of main sweeps/waves**	**Caesarean section rate %**
SCOPE-BASELINE** [144]	1,537	Antenatal recruitment from SCOPE study between November 2007 and February 2011	6 (at 2 days, 2 and 6 months, 1, 2 and 5 years)	27.8
Growing Up in Ireland [145]	11,134	Born during December 2007 through to June 2008	3 (at 9 months, 3 and 5 years)	26.0
Growing Up in New Zealand [142]	6,853	All pregnant women living in the three contiguous DHB areas with an estimated delivery date between 25 April 2009 and 25 March 2010 were eligible for inclusion	5 (antenatal, perinatal linkage, 9 months, 2 and 4 ½ years)	23.2
UK Millennium Cohort Study [143]	18,827	"born throughout the UK between September 2000 and January 2002"	6 (at 9 months and 3, 5, 7, 11 and 14 years)	21.4

* Linkage to civil registration and vital systems has been done in most cases. ** Not nationally representative. UK (United Kingdom), DHB (District Health Board), BASELINE (Babies After the Screening for Pregnancy Endpoints Study – SCOPE - Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints) birth cohort.

Ethics

The ethical principles espoused by the Declaration of Helsinki [147] guided the approach to secondary analysis of the before mentioned cohort data. Written informed consent was obtained from parents or guardians of the children by the primary investigators. Further details are furnished in the respective cohort chapters. The secondary analysis was conducted with ethical values like respect, dignity, integrity and the Kaitiaki principle - the Māori expression for guardianship. Pragmatic examples include keeping data confidential and storing it on an encrypted device.

Participants were not involved in establishing the research question, outcome measures including the study design and interpretation or writing of this paper. The results will be disseminated via the study websites, social media, information evenings and by newsletter. While secondary data was used, the principle of patient public involvement, where research is conducted 'with' instead of 'on' participants was borne in mind [148].

Exposure variable

Obstetric mode of delivery was classified according to the respective cohort definitions as Vaginal (spontaneous/normal/unassisted or instrumental/assisted/operative) and Caesarean (elective/planned/pre-labour or emergency). This variable was defined using synonyms, for example, elective/planned CS were before the onset of labour. Because establishing the onset of labour is challenging [59], elective and emergency CS were conducted mainly pre-labour or in labour respectively. The onset of labour contractions is important because infant microbial colonisation generally begins afterwards [149].

Outcome variables

The age and sex specific International Obesity Task Force (IOTF) criteria [136, 150, 151] were used to classify the primary outcome, body mass index (BMI) measured in kg/m^2, as thin/underweight, normal, overweight or obese. These criteria have the advantage of being best adapted for population studies [152]. The WHO BMI criteria were also used [153]. Although BMI is the most commonly used clinical index of adiposity, it does not discriminate between fat, bone and muscle mass [154]. A more objective adiposity index, body fat percentage (BF%), available only for the BASELINE and MCS cohorts, was the secondary outcome.

Confounders

Confounders were defined as pre-exposure variables associated with both the exposure and the outcome, but were not on the causal pathway or were not a common effect of the exposure or outcome [114]. In addition, a variable that altered the measure of association by a minimum of 10% was considered to be a confounder [126, 129, 133].

Macrosomia can be defined as a birth weight > 4000g or > 4500g [155]. Which of these two cut-offs to use was determined by whether the measure of association still changed by a minimum of 10% i.e. confounding was still occurring. Figure 2.1 depicts the conceptual framework adopted for confounding, where post-exposure variables were regarded as potential mediators. To a high degree, there was harmony in the variables available to adjust for confounding among the cohorts as follows:

SCOPE-BASELINE: maternal age, education, ethnicity, marital status, smoking, infant sex, birth weight including macrosomia, gestational age, pre-eclampsia and BMI at the first antenatal visit

GUI: maternal age, education, ethnicity, marital status, infant sex, birth weight including macrosomia, gestational age, parity, pre-eclampsia, gestational diabetes and weight gain during pregnancy

GUiNZ: maternal age, education, ethnicity, marital status, smoking, infant sex, birth weight including macrosomia, gestational age, parity, gestational diabetes, pre-pregnancy BMI

MCS: maternal age, education, ethnicity, marital status, smoking, couple income, infant sex, birth weight including macrosomia, gestational age, parity, diabetes mellitus including gestational and pre-pregnancy BMI

Statistical analysis

Stata versions 14 and 15 SE, College Station Texas, were used for statistical analyses. A p-value < 0.05 was considered to be statistically significant.

Depending on the nature of the outcome variable; continuous, categorical with four components, repeated measures: linear, multinomial and mixed-effects linear regression models were fitted respectively.

In a simplified mathematical form, linear regression describes the relationship between a continuous dependent variable Y and one or more continuous, binary or categorical independent variables X [156]. Multivariable linear regression is denoted by the following equation: $Y = a + b_1 \times X_1 + b_2 \times X_2 + \ldots + b_n \times X_n$. Where

Y = dependent variable

X_1 = independent variables

a = constant (y-intersect)

b_1= regression coefficient of the variable X_1

Simultaneous investigation of potential confounding between multiple independent variables is possible with multivariable linear regression, via adjustment of their regression coefficients [156]. The major assumptions of linear regression, using estimation techniques like ordinary least squares, include a linear relationship, normality, equal variance, independence and no or limited multicollinearity. In the current thesis, linear regression was used in the SCOPE-BASELINE and MCS cohorts where BMI or BF% were modelled as continuous variables.

Multinomial logistic regression describes the relationship between a non-ordered categorical dependent variable, for which there are three or more categories, and one or

more continuous, binary or categorical independent variables [157]. Nomination of one of the dependent variable categories as the baseline occurs and the log-odds of the other categories are calculated relative to baseline. These log-odds are then assumed to follow a linear model. Exponentiation of the log-odds yields an estimate of the odds. Our primary dependent variable, BMI, classified as thin, normal, overweight and obese was a non-ordered categorical variable.

Mixed-effects linear regression is an extension of linear regression especially useful with repeated measures of the same variable. These models allow for fixed and random effects. Measurement of BMI from the same individual was done at different times, for example at age three, five, seven, eleven and fourteen years in the MCS. Because these measurements were not independent of each other, mixed-effects linear regression was used to control for this non-independence [158].

When outcome prevalence is greater than 10%, logistic regression based techniques noticeably overestimate adjusted effect measures [159]. Under such circumstances, alternatives to logistic regression like log-binomial and Poisson regression provide better estimates [160]. Because the prevalence of childhood obesity was less than 10%, logistic regression based techniques were appropriate, parsimonious and were thus utilised.

Sub-group analysis

Pre-specified sub-group analysis was conducted for mothers aged > 35 years, preterm births (< 37 weeks) and by infant sex.

Population attributable fraction

The population attributable fraction is defined as the proportion of all cases of a disease or health condition in people that is due to a particular exposure [161]. The population attributable fraction was calculated for significant associations only. Although a positive

association does not mean a causal relationship, the population attributable fraction allows the maximum possible proportion of obese children attributable to planned CS birth in the population to be estimated.

Missing data

Depending on the cohort, where a variable had a small amount of missing data (less than 5% of the sample), the data was either dropped or a missing data category was created. This was because it has been suggested that if the number of missing cases is less than 5% of the sample they can be dropped from the analysis without unduly biasing the results [162]. It has also been suggested that where missing data is minimal adding it as a missing category has a minimal impact on effect estimates [163].

For substantial missing BMI and BF% data (> 5%), multiple imputation a statistically principled flexible approach was performed, making the assumption that this data was missing at random [162]. Rubin classified missing data into three categories [164]. The terms used to denote the three categories are misnomers because they do not convey the actual meaning [165]. Thus their technical meaning is clarified: First - missing completely at random, is defined as there being no systematic differences between missing and observed data. Second - missing at random, is defined as differences in observed data, but not unobserved data, being able to explain systematic differences between missing and observed data. Third - missing not at random, is defined as unobserved data being able to explain systematic differences between missing and observed data.

Although multiple imputation is a popular and robust technique, it has some limitations [165]. First, multiple imputation is computationally intensive. Second, if data was not normally distributed and a transformation process has not been performed, there is the possibility of generating bias. Third, some data may inherently be missing not at random.

This chapter began with thoughts regarding how the research question could be best answered. Next was consideration of the cohorts used and this was followed by the ethical framework employed, and finally the analysis plan together with the basis for selecting the statistical approaches.

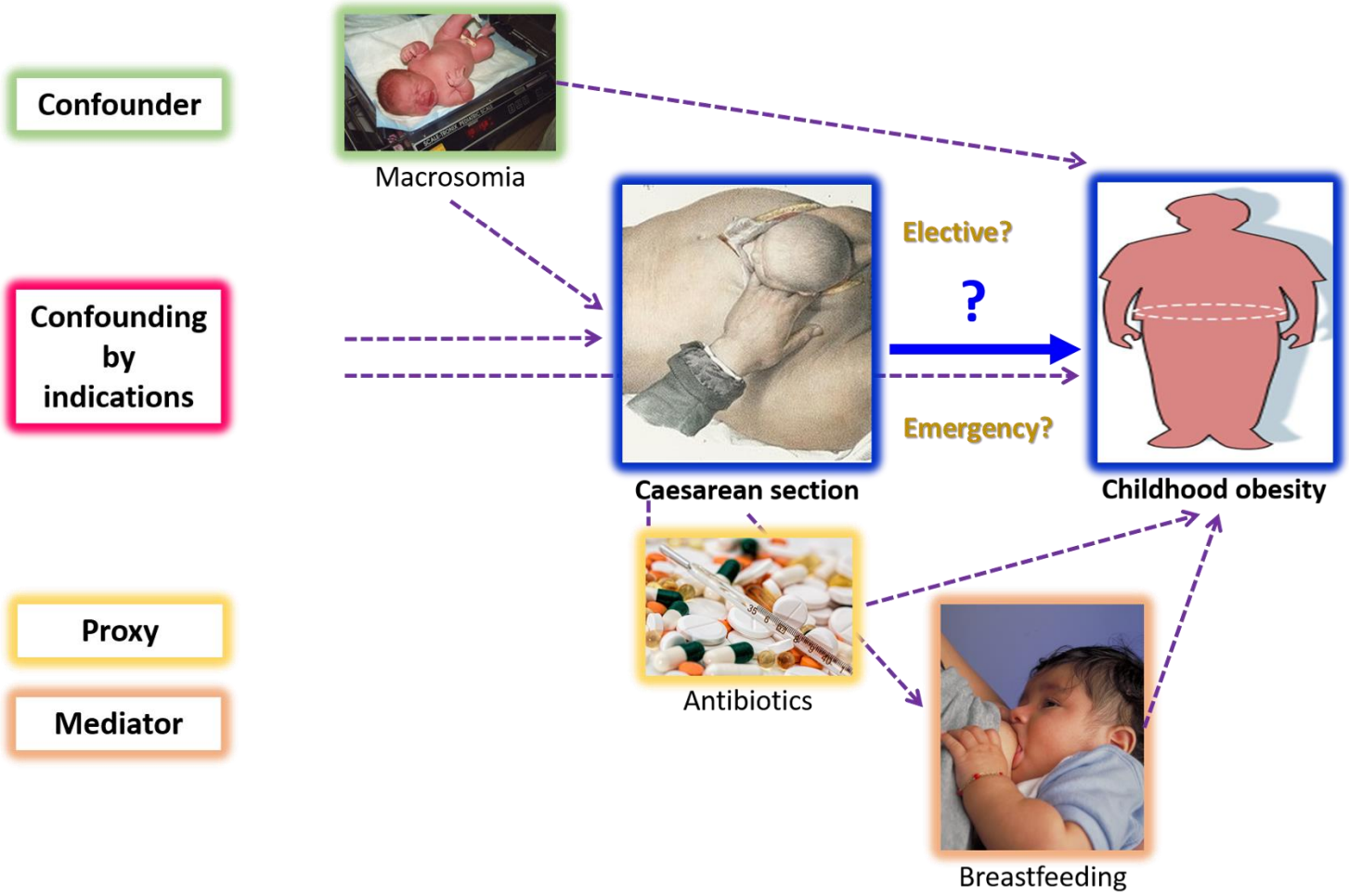


Figure 2- 1. Conceptual framework of the relationship between the determinants linking Caesarean section birth and childhood obesity. Images from [166] or in the public domain.

Chapter 3: The association between Caesarean Section Delivery and Obesity in Childhood: A Longitudinal Cohort Study in Ireland

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(Appendix 1)

3.1 Abstract

Objectives To investigate the association between Caesarean section (CS) birth and body fat percentage (BF%), body mass index (BMI) and being overweight or obese in early childhood.

Design Prospective longitudinal cohort study.

Setting Babies After Screening for Pregnancy Endpoints: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE) cohort.

Participants Infants born to mothers recruited from the Screening for Pregnancy Endpoints (SCOPE) study, Cork University Maternity Hospital between November 2007 and February 2011.

Outcome measure Overweight or obese defined according to the International Obesity Task Force criteria.

Results Of the 1305 infants, 362 (27.8%) were delivered by CS. On regression analysis, BF% at two months did not differ significantly by delivery mode. Infants born by CS had a higher mean BMI at six months compared with those born vaginally (adjusted mean difference=0.24; [95% confidence interval (CI) 0.06-0.41], p-value = 0.009). At two years no difference was seen across the exposure groups in the risk of being overweight or obese. At five years, the association between pre-labour CS and the risk of overweight or obesity was not statistically significant (adjusted relative risk ratio (aRRR) =1.37; [95% CI 0.69-2.69]) and the association remained statistically non-significant when children who were macrosomic at birth were excluded from the model (aRRR=0.86; [95% CI 0.36-2.08]).

Conclusion At six months of age children born by CS had a significantly higher BMI but this did not persist into future childhood. There was no evidence to support an association between mode of delivery and long-term risk of obesity in the child.

Key words

Caesarean section; body composition; body fat; obesity; childhood; Ireland

Article summary

Strengths and limitations of this study

- Data was obtained from a well phenotyped contemporary prospective longitudinal cohort study.
- Body fat percentage was measured by air displacement plethysmography which is regarded as the gold standard method.
- A limitation was the unavailability of maternal pre-pregnancy body mass index.
- The number of overweight and obesity cases at two and five years of age was limited.

3.2 Introduction

Over recent decades Caesarean section (CS) rates have risen considerably worldwide and in some countries rates now exceed 50%.[7] The aetiology of the global CS rate increase is multifactorial and includes a decline in vaginal births after Caesarean (VBAC), physician fear of litigation, maternal request, more multiple pregnancies resulting from greater assisted reproductive technology use and access to private health insurance.[167-172]

Although a timely CS can be both necessary and life-saving, for example, in cases of obstructed labor, transverse lie and fetal distress/compromise, it nevertheless conveys complications. For the mother, these include an increased length of hospital stay, infection and haemorrhage, as well as a higher risk of respiratory complications in the infant and consequent admission to the neonatal intensive care unit.[60]

Birth weight is the most commonly used indicator of *in utero* growth, however, body composition at birth, the relative proportion of fat and fat-free mass, can provide a more accurate picture.[173] We have shown retrospectively that neonatal body fat percentage is more closely linked to risk of CS than birth weight.[174] Therefore conversely changes in body fat percentage could be an early and more sensitive indicator of future health. It has been hypothesized that the described association between abnormal birth weight and future cardio-metabolic disease[175] across the life course, can be more closely attributed to differences in early life body composition than to birth weight differences.[173]

CS itself has been consistently associated with an increased risk of obesity later in life, although studies have been inconclusive.[28-30] It is also unclear whether this increased risk pertains to elective/prelabour CS or emergency CS/CS in labour. Making this distinction is challenging because of limited literature so much so that the latest systematic review and meta-analysis on the topic (2018) performed an analysis including all CS and did not

differentiate.[15] Several research papers have been able to distinguish between elective and emergency CS but these have been limited by small sample sizes.[56, 84, 100] With CS in labour, membranes are more likely to have ruptured thereby exposing the infant to vaginal microflora.[149] However lack of exposure to the vaginal microflora among infants born by elective CS, where membranes are more likely to be intact, has been suggested as the main causal mechanism for the increased risk of obesity later in life.[42-44] Some have disputed this,[34, 46] nevertheless robust data from animal experiments demonstrates a potential causal role for CS delivery in the development of childhood obesity.[45]

Given the worldwide increase in non-medically indicated prelabour CS[60], this type of CS represents a potentially modifiable risk factor for childhood obesity. The aim of this study was to investigate the relationship between CS delivery, particularly prelabour CS, and childhood body composition and growth, using a well phenotyped prospective longitudinal birth cohort with detailed clinical phenotyping of both mothers and their children. We wanted, in particular, to examine the potential confounding effect of macrosomia, as this is both a risk factor for CS, and for long-term obesity.

3.3 Methods

Data source and population sampled

Data was obtained from the Irish cohort of the prospective Screening for Pregnancy Endpoints (SCOPE) study of 'low risk' nulliparous women with singleton pregnancies (ACTRN12607000551493, www.scopestudy.net/) and its follow-up prospective Irish birth cohort, the Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE) study (NCT01498965, www.baselinestudy.net/).

The SCOPE and BASELINE study methodology are reported in detail elsewhere.[144, 176] Briefly, the aim of the SCOPE study was to develop screening approaches, clinical and molecular, to predict fetal growth restriction, pre-eclampsia, and spontaneous preterm birth in healthy nulliparous women during early gestation. Exclusion criteria included: 1) considered to be at high risk of fetal growth restriction, pre-eclampsia, or spontaneous preterm birth due to underlying medical conditions (chronic hypertension, diabetes, renal disease, systemic lupus erythematosus, anti-phospholipid syndrome, sickle cell disease, HIV), previous cervical knife cone biopsy, ≥ 3 previous terminations or ≥ 3 miscarriages, current ruptured membranes; 2) had a major uterine anomaly, a known major fetal anomaly or abnormal karyotype; or 3) received an intervention that could modify pregnancy outcome (e.g. aspirin therapy, cervical suture).

In brief, the BASELINE cohort participant's mothers were recruited at 15 ±1 weeks of pregnancy from Cork University Maternity Hospital between November 2007 and February 2011. Of the 2579 women approached to participate, 1774 (69%) gave their written informed consent. From those, 1537 (87%) had infants recruited into the BASELINE study. The socio-demographic, lifestyle and physical measurements were collected by trained research midwives. A complete audit trial was available for the data that was entered into a centrally accessed internet database (MedSciNet AB, Stockholm, Sweden).

Exposure and outcome ascertainment

Delivery mode was grouped into four categories, namely unassisted vaginal delivery (VD), operative VD, prelabour lower segment (LS) CS and LSCS in labour. Operative VD constituted delivery by either vacuum extraction or forceps.

Whole body density was calculated from naked weight measured by an electronic scale (seca 384; seca, Birmingham, UK) to the nearest gram divided by body volume estimated by the

PEA POD air displacement plethysmography system (COSMED, Concord, California, USA) within the first four days of life and also at age two months. The PEA POD agrees highly with the gold standard four-compartment model and is non-invasive, fast and safe.[174, 177, 178]

Based on body density and a two-compartment model of body composition (fat and fat-free mass), using values established by Fomon[177], body fat percentage (BF%), the primary outcome, was calculated as [(Fat mass (kg)/body mass (kg))×100].

The child's height and weight were measured by a trained interviewer using standardised protocols and medically approved instruments. At birth, two months, six months, one year, two years and five years of age, body mass index (BMI) in kg/m^2 was calculated for each child. At age two and five years, BMI was classified as thin, normal, overweight or obese, according to the International Obesity Task Force (IOTF) criteria.[136, 150] The IOTF classification begins at age two years.

The following potential confounders as reported in the literature[27-30, 91] were included *a priori*: maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia. For instance smoking cigarettes is a potential confounder because it is a risk factor for both CS birth[179] and for childhood obesity.[180]

Statistical analysis

Stata version 14SE (StataCorp LP College Station, TX) was used for statistical analysis. Categorical variables were described using frequency (n) and percent (%). Numeric variables were described using the mean (standard deviation-SD) or median (interquartile range-IQR).

Crude and adjusted linear regression models were used to examine the association between mode of delivery and BF%. Linear regression models were also used to evaluate the association between delivery mode and BMI as a continuous measure.

Crude and adjusted multinomial logistic regression models were used to examine the association between mode of delivery and the risk of being overweight or obese. Adjusted mean differences and adjusted relative risk ratios (aRRR), for the linear and multinomial logistic regression models respectively, were calculated with 95% confidence intervals (CIs). Unassisted VD was the reference category and normal BMI was the base outcome for the multinomial logistic regression models. Models were stratified by whether infants were macrosomic or not which was defined as a birth weight > 4000g or ≤ 4000g respectively. We also explored interaction by infant sex. Statistical significance was defined as a p-value < 0.05.

Patient involvement

Participants were not involved in establishing the research question, outcome measures including the study design and interpretation or writing of this paper. The results will be disseminated via the study website, social media, information evenings and by newsletter.

3.4 Results

Of the 1305 infants, 943 (72.3%) were delivered vaginally. The remainder of the deliveries (27.8%) were by CS; prelabour LSCS (12.0%) and LSCS in labour (15.8%) respectively (Figure and Table 3-1). At birth, 13.0% of infants were macrosomic (> 4000g); 11.0% were large for gestational age (> 90th percentile for customised birth weight centiles). At two years

of age, 116 (10.9%) children were overweight or obese (using IOTF cut-offs). At age five, the respective number was 118 (14.5%). At age two months, the mean (SD) BF% was calculated at 21.8% (±4.3%). BF% approximated to the normal distribution.

The average BMI, by the four birth modes, at each of the six time points is depicted by Figure 3-2 and for all vaginal and CS births by Figure 3-3. The maximum divergence in BMI by delivery mode occurred at six months of age. At six months, the mean BMI of infants delivered vaginally and those born by CS was 17.3 kg/m^2 and 17.6 kg/m^2 respectively.

Across delivery mode missing data was distributed equally for the primary and secondary outcomes, BF% and BMI respectively. Thus missing data was unlikely to have affected the results or conclusions (Supplementary Table 3-1).

Mode of delivery and body fat percentage at age two months

At two months' age there was no association between prelabour CS and BF% (adjusted BF% mean difference=0.46; [95% CI -0.46-1.40]) and LSCS in labour (adjusted BF% mean difference=0.07; [95% CI -0.88-0.73]) in comparison to the reference group of children delivered by unassisted VD (Table 3-2).

Mode of delivery and body mass index at age six months, two years and five years

Infants born by CS had a significantly higher mean BMI at six months compared with those born vaginally, adjusted BMI mean difference=0.24; [95% CI 0.06-0.41], p-value = 0.009. Limiting analysis to non macrosomic infants resulted in an adjusted BMI mean difference=0.26; [95% CI 0.07-0.45], p-value = 0.008.

There was, however, no statistically significant differential effect by sex (p-value for the interaction term was 0.70) – Supplementary Figure 1-3).

There was no statistically significant association between prelabour CS (aRRR=1.38; [95% CI 0.73-2.62]) or LSCS in labour (aRRR=0.88; [95% CI 0.48-1.61]) and the risk of being overweight or obese at age two years, as compared to the reference group (Table 3-3). Limiting analysis to non-macrosomic infants at age two resulted in the association between prelabour CS and the risk of overweight and obesity being (aRRR=0.95; [95% CI 0.44-2.05]) and for LSCS in labour (aRRR=0.89; [95% CI 0.44-1.82]) (Supplementary Table 3-2.

At age five years, there was a non-significant association between prelabour CS and the risk of being overweight or obese (aRRR=1.37; [95% CI 0.69-2.69]) (Table 3-4). There was also no association between LSCS in labour and the risk of being overweight or obese (aRRR=1.69; [95% CI 0.92-3.08]). Limiting analysis to non-macrosomic infants at age five resulted in the association between prelabour CS and the risk of overweight and obesity being (aRRR=0.86; [95% CI 0.36-2.08]) and for LSCS in labour (aRRR=2.37; [95% CI 1.19-4.68]) (Supplementary Table 3-3).

3.5 Discussion

Main findings

There was no significant difference in BF% at age two months between modes of delivery. A statistically significant difference in BMI at age six months was observed between infants born by CS and VD. Infants born by CS had a higher mean BMI. There was no evidence to support a link between prelabour CS and our secondary outcome, being overweight or obese, at two and five years of age.

Strengths and limitations

A major strength was the availability of data from a well phenotyped prospective longitudinal cohort that is among those with the most data available for BF%. This allowed us to investigate the role of factors such as cigarette smoking prior to conception, which is often not available from prior or extant cohorts. In addition, we used robust measures of body composition obtained by air displacement plethysmography, which is regarded as the gold standard method.

A homogenous sample where 98% of the cohort's participants were Caucasian, primiparous and 'low risk'[144] could limit the generalizability of these findings to heterogeneous populations. However, the cohort reflected the Republic of Ireland's demographics of reproductive age women (15-49 years), where 93% are Caucasian women.[181] The variable pre-pregnancy BMI was unavailable; this variable attenuated effect size estimates towards the null[28] in previous studies. Body mass index at 15 weeks' gestation, a good proxy for pre-pregnancy BMI, was used because 15 weeks is prior to the occurrence of most weight gain in pregnancy. It has been suggested that any association between CS birth and childhood obesity is due to antibiotics administered during CS, with CS delivery serving as a proxy, nonetheless this proposition has not been supported by evidence.[133, 182] The major limitation was the low number of cases at two and five years of age. Moving from four to two birth mode groups led to increased power, due to increased numbers of infants in each group, however this change resulted in wider effect size confidence intervals.

Interpretation

The relationship between CS delivery and offspring being overweight or obese has been explored by several systematic reviews and meta-analyses.[15, 28, 29, 183] A positive

association was the most common finding. Our findings are similar to those of infants, born in 2010, from a Danish prospective cohort study which found that the largest BMI difference by delivery mode, from birth to five years of age, occurred at six months' age and that this difference did not track into later childhood at age five.[182] In addition, similar to this study, no significant difference in BF% by delivery mode, was found. It is worth highlighting that the first two years of life have been identified as a critical developmental window during which perturbations in growth and development are more likely to result in lifelong sequelae.[139] This Danish study, like ours and also as reported by the systematic reviews and meta-analyses[27, 30], did not find a sex-specific growth pattern by mode of birth. This suggests that in humans CS birth might not influence sex-specific growth patterns as has been observed in mouse studies.[45]

Childhood fat mass index data from a Brazilian longitudinal cohort also showed no significant difference between children born by CS and VD at six years of age.[184] The declining influence of CS birth on the risk of obesity as children grow older has been attributed to the increasing influence of other risk factors for obesity like physical inactivity, family dietary habits, watching television (and the use of other electronic devices).[85] Indeed a study which utilized a sibling-pair design attributed the observed association between CS birth and childhood obesity to unmeasured confounding.[185] Unmeasured confounding from unmeasured variables such as some sociocultural factors can lead to biased effect estimates [186].

Our results are dissimilar to those of children from a Boston, US cohort study which found a positive association between delivery mode and being overweight or obese at age five.[133] The Boston study, unlike ours, did not sub classify CS births into elective and emergency for

example, and unusually there were more girls delivered by CS,[187] this might indicate reduced external validity for the US study. This means the conclusions of this US study are applicable particularly to its specific context because in the general population more boys than girls are delivered by CS.

A few studies have been able to differentiate between elective/prelabour CS and emergency/ LSCS in labour and they have been limited by small sample sizes.[84, 100] However a higher risk of childhood obesity for infants born by emergency CS than elective CS was reported.[84] Finding an association at age five between LSCS in labour, when membranes are more likely to have ruptured, and being overweight or obese, but not with prelabour CS suggests an attenuated role for vaginal flora in the genesis of children being overweight or obese. A possible explanation for the LSCS in labour association is confounding by the indications for CS. The exact indications for CS were not available for this cohort. However, a divergent BMI trajectory in mid-infancy which then converges by age five between VD and CS babies may suggest a transient role for the vaginal microflora. Further exploration, around mid-infancy, of the association between CS birth and BMI is required.

The CS rate of 27.8% in this cohort, is consistent with published national estimates of 27.1% to 28.6% that prevailed during the study's recruitment period from 2007 to 2011.[188] This suggests the generalizability of findings to the Irish population, particularly 'low risk' first time mothers. A macrosomia (> 4000g) prevalence of 13.0% is almost double that of another high income country, the US at 7.5% during a similar time period, and suggests high baseline Irish rates of excess adiposity.[189] The general Irish population had at age three and five years a prevalence of 24% and 20% respectively for obesity and being overweight[190] which is higher than that observed in this cohort. This cohort's low risk population likely

explains its lower prevalence of being overweight or obese compared to the general Irish population. Although the prevalence of the outcome, obesity, was low consistency of results with the before mentioned Danish cohort suggests their merit.

3.6 Conclusion

We have found no evidence to support a relationship between prelabour CS and offspring being overweight or obese in early childhood. No significant differences in outcome at two months and two years, and an increased risk of being overweight or obese in children born by CS in labour, but not prelabour CS at five years, suggests that the previously hypothesized causal effects due to vaginal microflora are also unlikely at least in the long-term.

Acknowledgements

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Author contributions

GM, FPM, PNB, LCK, SMBM, DMM, JOH, ASK conceived and designed the study. GM and ASK analysed the data and all authors interpreted the results. GM wrote the first draft of the article and FPM, PNB, LCK, SMBM, DMM, JOH, ASK revised it critically for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work.

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Competing interests

No, there are no competing interests for any author.

Participant consent

Obtained.

Ethics approval

Clinical Research Ethics Committee of the Cork Teaching Hospitals (Ref: ECM5 (9) 01/07/2008.

Data sharing statement

Data may be accessed by request from the Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE) study. Contact details are available on the study website http://www.baselinestudy.net/.

Table 3- 1. Characteristics of the study population at two months.

Characteristic	**Overall n (%)**	**Unassisted vaginal n (%)**	**Operative vaginal [a] n (%)**	**Prelabour LSCS n (%)**	**LSCS in labour n (%)**
N	1305 (100)	470 (36.0)	473 (36.2)	156 (12.0)	206 (15.8)
Maternal age (years), median IQR	30 (28-33)	30 (27-32)	30 (28-33)	32 (29.5-34)	31 (29-33)
< 20	19 (1.5)	9 (1.9)	9 (1.9)	1 (0.6)	0 (0.0)
20-24	111 (8.5)	57 (12.1)	38 (8.0)	4 (2.6)	12 (5.8)
25-29	388 (29.7)	157 (33.4)	139 (29.4)	34 (21.8)	58 (28.2)
30-34	615 (47.1)	215 (45.7)	214 (45.2)	85 (54.5)	101 (49.0)
35-39	155 (11.9)	31 (6.6)	66 (14.0)	28 (17.9)	30 (14.6)
≥40	17 (1.3)	1 (0.2)	7 (1.5)	4 (2.6)	5 (2.4)
Ethnicity					
Caucasian	1,287 (98.6)	463 (98.1)	466 (98.5)	155 (99.4)	203 (98.5)
Other	18 (1.4)	7 (1.5)	7 (1.5)	1 (0.6)	3 (1.5)
Schooling (years primary and secondary), median IQR*	13 (13-14)	13 (13-14)	13 (13-14)	13 (13-14)	13 (13-14)
Marital status					
Single	123 (9.4)	52 (11.1)	49 (10.4)	11 (7.1)	11 (5.3)
Married	920 (70.5)	321 (68.3)	330 (69.8)	115 (73.7)	154 (74.8)
Stable relationship not married	261 (20.0)	97 (20.6)	94 (19.9)	29 (18.6)	41 (19.9)
Sex of baby					
Male	666 (51.0)	221 (47.0)	252 (53.3)	81 (51.9)	112 (54.4)
Female	639 (49.0)	249 (53.0)	221 (46.7)	75 (48.1)	94 (45.6)
Pre-eclampsia	48 (3.7)	17 (3.6)	7 (1.5)	16 (10.3)	9 (4.4)
Maternal BMI at 15 weeks (kg/m^2), median IQR	24.0 (22.1-26.9)	23.9 (21.5-26.4)	23.7 (22.1-26.7)	24.9 (22.3-28.7)	24.7 (23.0-27.9)
Gestational age (weeks), median IQR	40.3 (39.3-41.0)	40.3 (39.3-41.0)	40.6 (39.6-41.1)	39.3 (38.6-40.1)	40.6 (39.6-41.3)

Number of cigarettes per day at 15 weeks SCOPE visit, mean (±SD)	0.5 (±2.1)	0.7 (±2.4)	0.4 (±2.1)	0.5 (±2.3)	0.3 (±1.4)
Smokers	114 (8.7)	57 (12.1)	35 (7.4)	10 (6.4)	12 (5.8)
Birth weight (g), median IQR	3460 (3160-3770)	3400 (3120-3690)	3510 (3200-3800)	3345 (2915-3670)	3650 (3300-4000)
Macrosomia (> 4000g)	169 (13.0)	32 (6.8)	65 (13.7)	21 (13.5)	51 (24.8)
Baby size according to customized centile					
SGA < 10th centile	135 (10.3)	59 (12.6)	40 (8.5)	22 (14.1)	14 (6.8)
AGA ≥ 10th centile ≤ 90th centile	1,027 (78.7)	383 (81.5)	374 (79.1)	110 (70.5)	160 (77.7)
LGA > 90th centile	143 (11.0)	28 (6.0)	59 (12.5)	24 (15.4)	32 (15.5)
Body composition (at two months)					
Body fat (%), mean SD	21.8 (±4.3)	21.8 (±4.3)	21.6 (±4.4)	22.3 (±4.6)	21.6 (±4.2)
missing	272 (20.8)	98 (20.9)	93 (19.7)	39 (25.0)	42 (20.4)
Body mass index (kg/m^2) at 2 years**					
Thin	77 (5.9)	28 (6.0)	34 (7.2)	6 (3.8)	9 (4.4)
Normal	812 (62.2)	289 (61.5)	286 (60.5)	101 (64.7)	136 (66.0)
Overweight	96 (7.4)	29 (6.2)	39 (8.2)	12 (7.7)	16 (7.8)
Obese	10 (0.8)	4 (0.9)	2 (0.4)	3 (1.9)	1 (0.5)
Missing	310 (23.8)	120 (25.5)	112 (23.7)	34 (21.8)	44 (21.4)
Body mass index (kg/m^2) at 5 years**					
Thin	38 (2.9)	13 (2.8)	17 (3.6)	3 (1.9)	5 (2.4)
Normal	656 (50.3)	236 (50.2)	232 (49.0)	83 (53.2)	105 (51.0)
Overweight	97 (7.4)	22 (4.7)	42 (8.9)	12 (7.7)	21 (10.2)
Obese	21 (1.6)	10 (2.1)	6 (1.3)	3 (1.9)	2 (1.0)
Missing	493 (37.8)	189 (40.2)	176 (37.2)	55 (35.3)	73 (35.4)

LSCS (Lower segment Cesarean section), SD (Standard deviation), IQR (Interquartile range), SGA (Small for gestational age), AGA (Appropriate for gestational age), LGA (Large for gestational age).

[a] Vacuum or forceps

* Total years of schooling (primary and secondary, not pre-school or tertiary)
** International Obesity Task Force age and sex-specific cut-offs

Table 3- 2. Mode of delivery and body fat percent at age two months.

Delivery mode	Cases n	Coef. (95% CI)	p-value	AdjCoef. (95% CI)**	p-value
Unassisted vaginal	372	reference		reference	
Operative vaginal	380	-0.16 (-0.78-0.46)	0.614	-0.10 (-0.72-0.52)	0.743
Prelabour LSCS	117	0.50 (-0.40-1.40)	0.278	0.46 (-0.46-1.40)	0.325
LSCS in labour	164	-0.19 (-0.9-0.61)	0.642	0.07 (-0.88-0.73)	0.864

N for adjusted model = 1,033. Linear regression. BMI – Body mass index, Coef. (β-Coefficient), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia

Table 3- 3. Mode of delivery and body mass index at age two years.

BMI category (normal BMI – base outcome)	Cases n	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin					
Unassisted vaginal	30	reference		reference	
Operative vaginal	37	1.23 (0.74-2.05)	0.417	1.42 (0.83-2.41)	0.199
Prelabour LSCS	6	0.59 (0.24-1.47)	0.259	0.65 (0.26-1.62)	0.352
LSCS in labour	9	0.65 (0.30-1.41)	0.279	0.86 (0.39-1.87)	0.696
Overweight or Obese					
Unassisted vaginal	37	reference		reference	
Operative vaginal	41	1.11 (0.69-1.78)	0.670	0.95 (0.58-1.56)	0.853
Prelabour LSCS	17	1.45 (0.79-2.65)	0.233	1.38 (0.73-2.62)	0.324
LSCS in labour	20	1.18 (0.66-2.10)	0.583	0.88 (0.48-1.61)	0.680

N for adjusted model = 1,062. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia

Table 3- 4. Mode of delivery and body mass index at age five years.

BMI category (normal BMI – base outcome)	Cases n	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin					
Unassisted vaginal	13	reference		reference	
Operative vaginal	18	1.45 (0.69-3.02)	0.324	1.82 (0.84-3.96)	0.131
Prelabour LSCS	3	0.68 (0.19-2.44)	0.553	0.46 (0.14-1.56)	0.212
LSCS in labour	5	0.86 (0.30-2.47)	0.777	1.06 (0.36-3.09)	0.915
Overweight or Obese					
Unassisted vaginal	36	reference		reference	
Operative vaginal	52	1.51 (0.95-2.40)	0.079	1.64 (1.00-2.67)	0.050
Prelabour LSCS	17	1.39 (0.74-2.60)	0.305	1.37 (0.69-2.69)	0.368
LSCS in labour	26	1.61 (0.93-2.80)	0.090	1.69 (0.92-3.08)	0.090

N for adjusted model = 856. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia

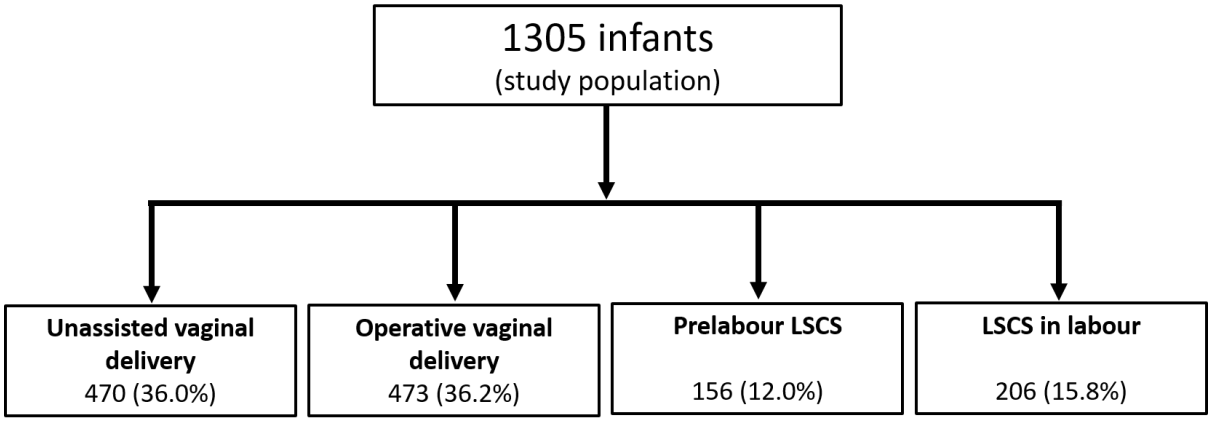


Figure 3- 1. Participant flow chart. Lower segment Caesarean section (LSCS).

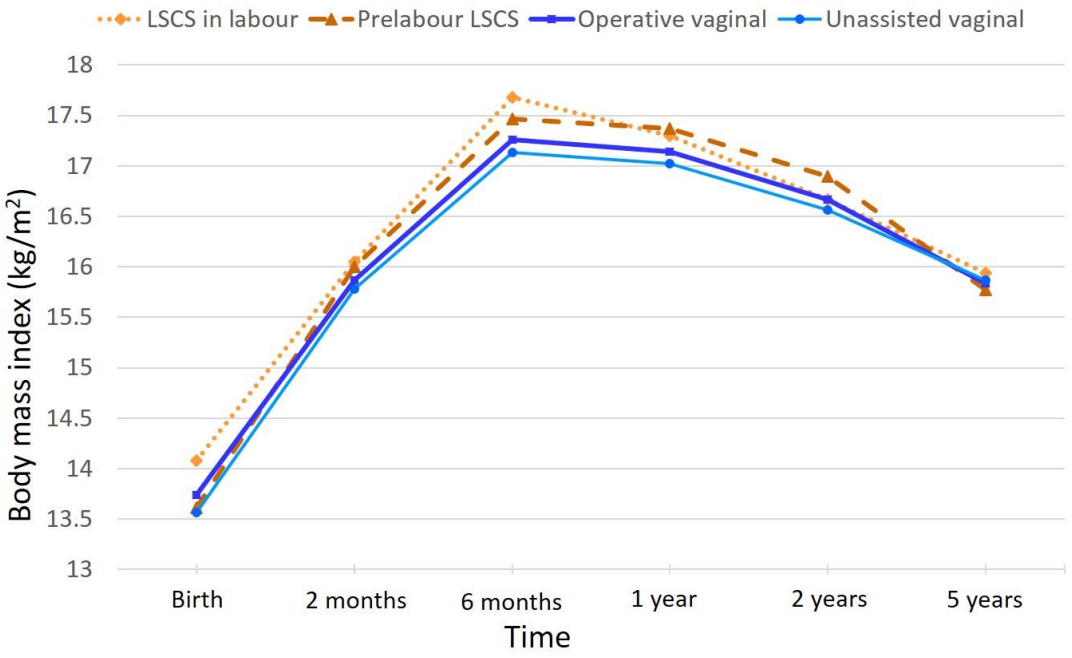


Figure 3- 2. Mean body mass index (BMI) from birth to five years of age. Lower segment Caesarean section (LSCS). Please note that the time axis has been expanded below age one year to permit clearer visualisation.

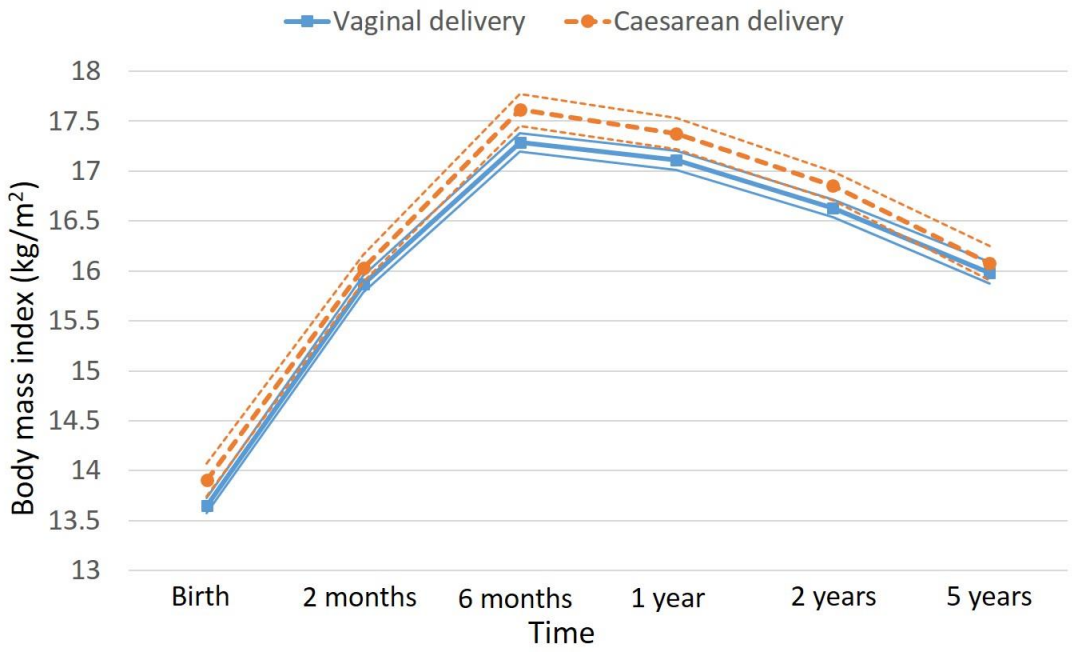
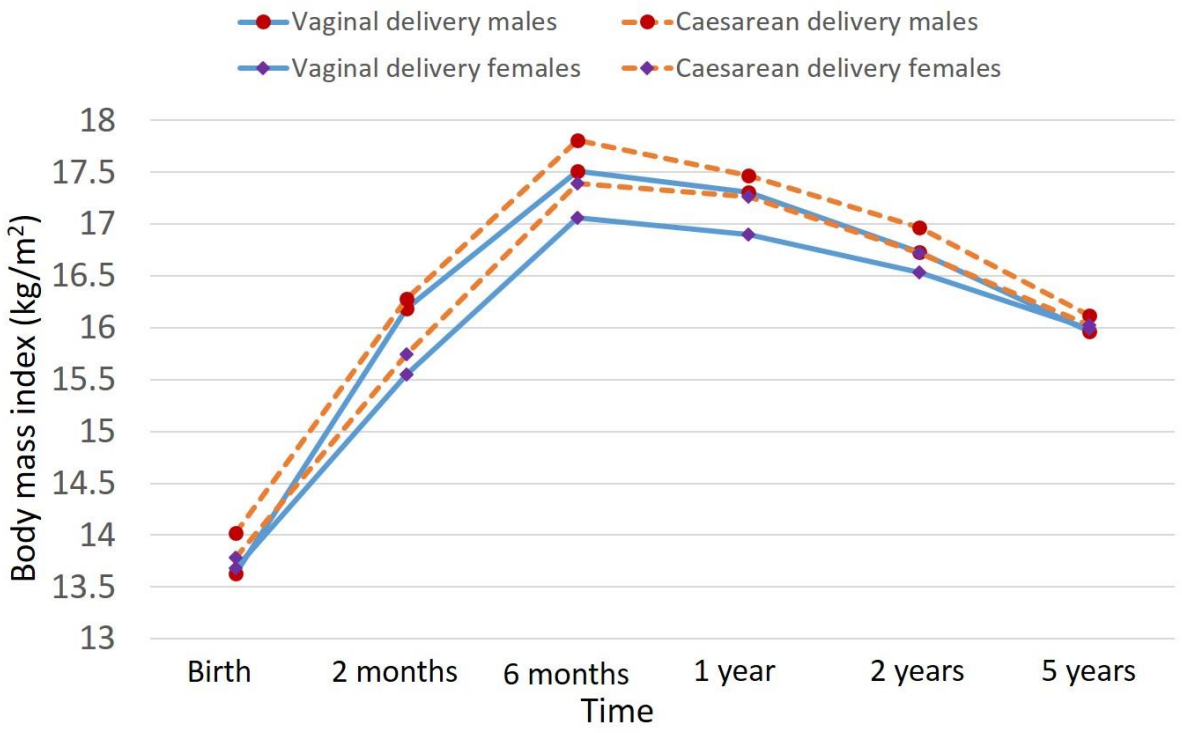


Figure 3- 3. Mean body mass index (BMI) from birth to five years of age with 95% confidence intervals (CIs) around the mean BMI – thin lines. There is no overlap of the 95% CIs at six months of age. Please note that the time axis has been expanded below age one year to allow clearer visualisation.



Supplementary Figure 1. Mean body mass index (BMI) from birth to five years of age by delivery mode and sex. Please note that the time axis has been expanded below age one year to allow clearer visualisation.

Supplementary Table 3-1. Missing data for body fat % at age two months.

Characteristic	**Body fat % data available at age two months (n %) n=1033**	**Body fat % data missing at two age months (n %) n=272**	**p-value[a]**
Maternal age (years), median IQR	31 (28-33)	30 (28-33)	0.6021
Ethnicity[b]			0.558
Caucasian	1018 (98.5)	269 (98.9)	
Other	15 (1.5)	3 (1.1)	
Schooling (years primary and secondary), median IQR	13 (13-14)	13 (13-14)	0.5227
Marital status[b]			0.879
Single	100 (9.7)	23 (8.5)	
Married	725 (70.2)	195 (71.7)	
Stable relationship not married	207 (20.0)	54 (19.9)	
Sex of baby[b]			0.081
Male	540 (52.3)	126 (46.3)	
Female	493 (47.7)	146 (53.7)	
Pre-eclampsia[b]	40 (3.9)	9 (3.3)	0.664
Maternal BMI at 15 weeks (kg/m^2), median IQR	24.1 (22.1-26.9)	23.7 (22.0-26.7)	0.2455
Gestational age (weeks), median IQR	40 (39-41)	40 (39-41)	0.4624
Number of cigarettes per day at 15 weeks SCOPE visit, mean (±SD)[c]	0.5 (±2.2)	0.4 (±2.0)	0.2517
Birth weight (g), median IQR	3460 (3150-3770)	3475 (3160-3750)	0.9099

IQR – Interquartile range, BMI – Body mass index, SD – standard deviation, SCOPE – Screening for pregnancy endpoints.

[a] Mann-Whitney test

[b] Pearson's χ^2 test or Fisher's exact

[c] Two-sample t test

Supplementary Table 3-2. Mode of delivery and body mass index at age two years. Non-marosomic.

BMI category (normal BMI – base outcome)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin				
Unassisted vaginal	reference		reference	
Operative vaginal	1.41 (0.84-2.36)	0.188	1.51 (0.89-2.58)	0.130
Prelabour LSCS	0.26 (0.24-1.62)	0.357	0.67 (0.27-1.68)	0.398
LSCS in labour	0.73 (0.32-1.64)	0.443	0.83 (0.37-1.90)	0.664
Overweight or Obese				
Unassisted vaginal	reference		reference	
Operative vaginal	0.98 (0.58-1.64)	0.929	0.93 (0.54-1.59)	0.789
Prelabour LSCS	0.93 (0.44-1.95)	0.842	0.95 (0.44-2.05)	0.891
LSCS in labour	1.01 (0.51-1.98)	0.982	0.89 (0.44-1.82)	0.747

N for adjusted model = 921. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia

Supplementary Table 3-3. Mode of delivery and body mass index at age five years. Non-macrosomic.

BMI category (normal BMI – base outcome)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin				
Unassisted vaginal	reference		reference	
Operative vaginal	1.59 (0.76-3.32)	0.221	1.85 (0.85-4.04)	0.120
Prelabour LSCS	0.73 (0.20-2.63)	0.629	0.46 (0.14-1.55)	0.209
LSCS in labour	1.09 (0.38-3.14)	0.880	1.14 (0.39-3.34)	0.815
Overweight or Obese				
Unassisted vaginal	reference		reference	
Operative vaginal	1.43 (0.87-2.36)	0.161	1.77 (1.03-3.04)	0.038
Prelabour LSCS	0.89 (0.41-1.95)	0.768	0.86 (0.36-2.08)	0.750
LSCS in labour	1.59 (0.85-2.98)	0.150	2.37 (1.19-4.68)	0.014

N for adjusted model = 741. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, infant sex, maternal smoking during pregnancy, maternal BMI at the first antenatal visit, gestational age (at delivery), birth weight and pre-eclampsia

Chapter 4: The Impact of Caesarean Section on the Risk of Childhood Overweight and Obesity: New Evidence from a Contemporary Cohort Study

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(Appendix 1)

4.1 Abstract

Caesarean section (CS) rates are increasing globally and exceed 50% in some countries. Childhood obesity has been linked to CS via lack of exposure to vaginal microflora although the literature is inconsistent. We investigated the association between CS birth and the risk of childhood obesity using the nationally representative Growing-Up-in-Ireland (GUI) cohort.

The GUI study recruited randomly 11134 infants. The exposure was categorised into normal vaginal birth (VD) [reference], assisted VD, elective (planned) CS and emergency (unplanned) CS. The primary outcome measure was obesity defined according to the International Obesity Taskforce criteria. Statistical analysis included multinomial logistic regression with adjustment for potential confounders.

Infants delivered by elective CS had an adjusted relative risk ratio (aRRR)=1.32; [95% confidence interval (CI) 1.01-1.74] of being obese at age three years. This association was attenuated when macrosomic children were excluded (aRRR=0.99; [95% CI 0.67-1.45]). Infants delivered by emergency CS had an increased risk of obesity aRRR=1.56; [95% CI 1.20-2.03]; this association remained after excluding macrosomic children.

We found insufficient evidence to support a causal relationship between elective CS and childhood obesity. An increased risk of obesity in children born by emergency CS, but not elective, suggests that there is no causal effect due to vaginal microflora.

Key words

Caesarean section, obesity, overweight, childhood, microbiota

4.2 Introduction

Estimates from 121 countries reveal that Caesarean section (CS) rates increased from 6.7% in 1990 to 19.1% in 2014.[7] In 2015 the United States had a 32.0% CS rate[191], Brazil 55.5% [192] and England 26.5%.[193] Ireland experienced a similar rise in CS rates with an increase from 10.5%[7] in 1990 to 31.4%[188] in 2015.

There is no consensus regarding the optimal population-level CS rate, however, a systematic review suggested that rates up to 16% were associated with reduced maternal, neonatal and infant mortality[194] and a further review reported reduction in mortality up to a 19% rate.[11] Multiple factors have driven the CS rate increase, including advanced maternal age at first childbirth, a decrease in vaginal births after Caesarean (VBAC)[167], physician fear of litigation, maternal choice and access to private health insurance.[168-172]

Babies delivered by CS, particularly elective CS, are generally not exposed to their mother's vaginal and faecal microbiota, which helps to shape the initial composition of an infant's microbiota including that of the gut.[32] Infants born by elective CS have been found to have a gut microbiome that has low diversity and richness.[195] Some studies suggest that infants born by CS might have a gut microbiota that has a tendency to harvest more dietary nutrients, thereby predisposing them to being overweight or obese.[42-44]

There is epidemiologic evidence of an association between CS birth and subsequent excess body mass index (BMI) across the life course.[27-30] Although heterogeneity, confounding, publication bias and inability to account for elective versus emergency CS delivery were limitations in trying to unpack this association, a study using a sibling-control design found that those born by CS had significantly higher odds of being obese later in life compared to

their siblings born vaginally.[91] It was, however, not possible in this sibling-control study to completely rule out confounding by the indications for CS, although the observed association was unlikely to be due to familial or genetic confounding.[31]

Childhood obesity and overweight are at epidemic levels globally.[69] Although the aetiology of childhood excess adiposity is multifactorial, given its serious complications, the aim of this study was to investigate the relationship between obstetric mode of delivery and childhood overweight and obesity. We hypothesised that infants born by elective CS, because of the aforementioned reduced exposure to their mother's vaginal and faecal flora would be at higher risk of being overweight or obese. In the most recent (2018) systematic review and meta-analysis considering the association between CS birth and childhood obesity (six cohorts), distinction between elective and emergency CS was not made.[15] In addition, small sample sizes have previously limited the evaluation of elective CS.[100, 196] We aimed to investigate the potential confounding effect of macrosomia and/or large for gestational age (LGA) on the association between CS delivery and obesity. To our knowledge one previous study investigated this confounding effect.[97]

4.3 Methods

Data source and population sampled

The Growing Up in Ireland (GUI) study is a nationally representative infant longitudinal cohort (http://www.esri.ie/growing-up-in-ireland/), which recruited randomly 11134 infants born in Ireland from 1st December 2007 to 30th June 2008.[145, 197, 198] (Infants born during the months of July to November, inclusive, were not part of the GUI cohort.)
These children and their families had a baseline face-to-face questionnaire-based interview conducted by trained interviewers in participating households when the infants were

approximately nine months old. Mother-infant pairs were subsequently followed-up by home interview when infants were three and five years old; follow-up continues. The response rates were as follows relative to most recent contact: at baseline interview (nine months) 64%, second interview (at three years) 91%, and at the third interview (at five years) 87%.[145, 197, 198] Children lost to follow-up tended to have unmarried mothers or mothers with lower educational attainment. In this study, children whose primary caregivers were not their mothers (*n* = 40, 0.36%) were excluded because the availability of potentially confounding variables such as age, maternal weight gain during pregnancy and health status predominantly pertained to mothers. In addition, children born by vaginal breech delivery (*n* = 41, 0.37%) and whose mode of delivery was unknown (*n* = 4, 0.04%) were also excluded, leaving 11,049 (99.2%) mother-infant pairs at baseline. Children born by vaginal breech delivery were excluded as they may differ from those born by vaginal cephalic delivery in important ways, for instance, they have a higher neonatal mortality rate[199], moreover, we did not have enough numbers to include them as a separate category. Further details regarding the GUI study have been reported previously.[145, 197, 198]

Exposure and outcome ascertainment

The primary exposure variable was obtained from mothers during the initial face-to-face interview when infants were nine months old by asking them, "What was the final mode of delivery?", which has been demonstrated to be a robust method.[200] If this method to ascertain exposure based on maternal recall, was not robust enough, there would be substantial bias of the outcome risk estimate deviating towards or away from the null.[201] Unfortunately this cohort was not linked to birth data to ascertain mode of birth. The delivery mode was grouped into four categories, namely normal vaginal delivery (VD), assisted VD and elective/planned and emergency/unplanned CS. Elective/planned and

emergency/unplanned CS were mainly pre-labour or in labour respectively. The onset of labour contractions is significant because offspring microbial colonisation generally begins afterwards.[149] Children born by pre-labour CS would have had little to no exposure to vaginal microflora while children born by CS in labour were likely to have been exposed. Assisted VD constituted delivery by forceps or vacuum extraction. We used this classification system because it is well accepted clinically, and importantly, it allows us to test the main hypothesis that the association between CS and the increased risk of childhood obesity is due to differential exposure to vaginal microflora by mode of birth. The GUI study did not collect data on individual CS indications. Although the main focus of the present study is CS compared to normal VD, the assisted VD group is included in the analysis for completeness.

The child's height and weight were measured by a trained interviewer using a validated standard measuring stick (Leicester portable height measure) and a medically approved weighing scale (SECA 835 digital weighing scales).[145, 197, 198] BMI in kg/m^2 was calculated for each child and each child was then classified as thin, normal, overweight or obese, according to the International Obesity Task Force (IOTF) - now World Obesity Policy & Prevention - system for boys and girls at age three and five years (please see Table 4-1 for the cut-offs for each category).[136, 150]

Potential confounders

Data on the following potential confounders as reported in the literature[27-30, 91] were collected and included *a priori* in the analyses as presented in Table 4-2: maternal age, ethnicity, educational level, marital status, infant sex, birth weight, gestational age, parity, weight gain during pregnancy, preeclampsia and gestational diabetes. Parity defined as the

total number of stillbirths and live births a woman has had was not available, however, we used the number of individuals currently in the study household who were a son/daughter of the mother as a proxy for parity. Birth weight centiles, adjusted for sex and gestational age, were calculated using the Bulk Centile Calculator for Ireland (please see Table 4-2 for the classification criteria into small, appropriate and large for gestational age; SGA, AGA and LGA respectively).[202]

Breast feeding can be considered to be a mediator because mothers who gave birth by CS, particularly elective CS, are less likely to breastfeed[203] and babies not breast fed are prone to future excess adiposity.[204] Variables such as the number of antibiotic courses during the last year, typical time to bed and the presence of a television in the child's room have been associated with an increased risk of childhood obesity.[205, 206] These variables including breast feeding were, however, not considered as confounders because they came after CS and cannot by definition confound the association between mode of birth and childhood obesity.[114]

Missing data

Variables with missing data are as depicted in Table 4-2. The majority of key covariates had a low proportion of missing data. Importantly our outcome variable, BMI, had missing data either due to non-response or loss to follow-up which was equally distributed across the mode of delivery categories. Where a variable had a small amount of missing data (in this study all the key variables had < 2% data missing) an extra category was added for example, 'Ethnicity' (1=White; 2=Other; 3=Missing). It has been suggested that where missing data is minimal adding it as a missing category has a minimal impact on effect estimates.[163]

Statistical analysis

Statistical analysis was conducted using Stata version 14SE (StataCorp LP College Station, TX). Frequency (n) and percent (%) were used to report categorical variables. The mean (standard deviation-SD) or median (interquartile range-IQR) were used to report numeric variables.

To evaluate the study hypothesis at ages three and five, we used multinomial logistic regression to calculate the adjusted relative risk ratio (aRRR) with 95% confidence intervals (CIs) with normal VD as the reference category and normal BMI as the base outcome. We also considered the association between mode of birth and transition of IOTF BMI category from three to five years (two time points); 0=remained normal (base outcome), 1=remained obese, 2=became obese, 3=became non-obese and 4=other transition. For the multinomial regression models because the IOTF childhood BMI classification starts at two years of age[207], we thus did not examine the association between mode of delivery and BMI at nine months age.

To explore if any associations could be explained by other factors we conducted sensitivity analyses by restricting analysis to SGA, AGA, LGA or non-macrosomic infants. Secondly we combined vaginal breech delivery with normal vaginal birth to form the reference category. We also performed subgroup analyses by infant sex, preterm birth (< 37 weeks), restricting analysis to infants whose mothers did not have pre-eclampsia and to mothers < 35 years old. Statistical significance was defined as a p-value < 0.05.

Ethics statement

The GUI study received independent ethics approval from a Research Ethics Committee convened by the Department of Health and Children. Written informed consent was obtained from parents or guardians. All methods were performed in accordance with the relevant guidelines and regulations.

Data availability statement

The data that support the findings of this study are available from the Irish Social Science Data Archive (ISSDA), www.ucd.ie/issda, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Bona fide researchers can apply for the data from ISSDA.

4.5 Results

Descriptive statistics

Of the 11049 infants, 8175 (74.0%) were delivered vaginally; most of these deliveries were by normal VD (59.5%) and the remainder were by assisted VD (14.4%) (Figure 4-1). The rest of the deliveries (26.0%) were by CS; elective CS (12.7%) and emergency CS (13.3%) respectively (Table 4-2). The cohort had 51.1% boys and 48.9% girls; approximately 55% of deliveries by assisted VD and emergency CS were of boys. Of women who gave birth by elective CS just over half, 50.4%, were 35 years and older.

At birth, 13.9% of children were macrosomic (> 4000g); 10.9% were large for gestational age (population centiles). At three years of age, there were 1767 (18.7%) overweight and 506 (5.3%) obese children. At age five, the respective numbers were 1389 (15.8%) and 437 (5.0%).

Most children (n=5030, 57.0%) remained within a normal BMI category between age three and five years whilst 175 (2.0%) remained obese. Two hundred and fifty six (2.9%) children who were obese at age three became non-obese (overweight, normal or thin) at age five and 262 (3.0%) children who were not obese at age three became obese at age five. Of the mothers who delivered vaginally, 13.2% were obese and of those who delivered by CS 21.5% were obese.

Mode of delivery and BMI at age three years

There was an association between elective CS (aRRR=1.32; [95% CI 1.01-1.74]) and emergency CS (aRRR=1.56; [95% CI 1.20-2.03]) and the risk of obesity at age three years compared to the reference group of children delivered by normal VD (Table 4-3). The risk of being overweight at age three years was associated with emergency CS (aRRR=1.23; [95% CI 1.04-1.44]) but not elective CS (aRRR=1.06; [95% CI 0.90-1.25]).

There was no statistically significant association between elective CS and the risk of obesity at age three among AGA infants, (aRRR=1.15; [95% CI 0.81-1.64]) (Supplementary Table S4-1). The analysis of AGA infants who were not macrosomic suggested that there was no association between elective CS and child obesity at age three years, (aRRR=0.99; [95% CI 0.67-1.45]) (Supplementary Table S4-2). Among LGA infants there was an association between elective CS and the risk of obesity at age three years (aRRR=2.01; [95% CI 1.10-3.67]) (Supplementary Table S4-3). The median birth weight for these LGA infants was 4200g and their median birth centile was 97.6. SGA infants also drove the overall association, albeit just falling short of reaching statistical significance, (aRRR=2.73; [95% CI 0.99-7.51]) (Supplementary Table S4-4). The median birth weight for these SGA infants was 3000g and their median birth centile was 7.6. The p-value for the interaction term between

delivery mode and birth centile categories in relation to obesity at age three years was < 0.001.

There was an association between emergency CS (aRRR=1.77; [95% CI 1.26-2.47]) and obesity when restricting to AGA non-macrosomic children (Supplementary Table S4-2).

For the observed elective CS effect, there was no statistically significant differential effect by sex, however, girls tended in the direction of having a greater effect size (p-value for interaction term was 0.093). Combining vaginal breech delivery with normal vaginal birth to form the reference category did not alter the results overall (data not shown). Excluding children of pregnancies complicated by pre-eclampsia or preterm birth and children of mothers less than 35 years of age did not affect the results overall (Supplementary Table S4-5).

Mode of delivery and BMI at age five years

At age five, the association between elective CS and obesity was of borderline significance (aRRR=1.30; [95% CI 0.98-1.73]) (Table 4-4); this association was not changed materially when the analysis was restricted to AGA non-macrosomic infants (aRRR=1.26; [95% CI 0.86-1.84] (Supplementary Table S4-6), thus an association cannot be completely ruled out. Furthermore, there was an association between emergency CS and the risk of obesity (aRRR=1.46; [95% CI 1.10-1.93]) (Table 4-4). There were no other statistically significant associations between mode of delivery and the remaining BMI categories. Restricting the analysis to AGA non-macrosomic children did not alter the observed association between emergency CS and the risk of obesity (Supplementary Table S4-6).

Mode of delivery and BMI transition between ages three and five years

There was no association between elective CS and any BMI category transition (Supplementary Table S4-7). Those born by emergency CS had an increased risk of remaining obese from the age of three to five years (aRRR=1.74; 95% CI 1.14-2.69]). Infants born by emergency CS also had an increased statistical risk of becoming non-obese (aRRR=1.74; [95% CI 1.21-2.49]). Finally, emergency CS infants had an increased risk of making any other BMI category transition (aRRR=1.20; [95% CI 1.04-1.38]).

Adding maternal weight gain in pregnancy (13.6 % missing data) did not alter the interpretation of our results materially at age three or five years and transition between these ages.

4.5 Discussion

Main Findings

We investigated the association between CS birth, particularly elective CS, and the risk of childhood obesity using a large, prospective, nationally representative, longitudinal cohort study. In the multinomial logistic regression analysis we found insufficient evidence to support a causal relationship between elective CS and childhood obesity. Indications for emergency CS likely explained the increased risk of obesity observed in infants delivered via this mode, but not elective CS, suggesting that there is no causal effect due to vaginal microflora.

Strengths and limitations

Firstly, the GUI study is a large and nationally representative sample due to the application of sampling weights. The major strength was that our main outcome, BMI, based on height and weight was collected prospectively by trained personnel using validated techniques thus

minimising measurement error. In addition, BMI was classified using widely accepted international criteria which allows comparison with other populations. We did not assume that once an individual is classified as obese, they remain so at a future time point. This allowed us in addition to evaluate if the mode of delivery was associated with transition into or out of obesity between time points. The availability of an ample suite of variables to adjust for confounding also strengthened our study. For example, we included gestational diabetes which was not included by several previous studies.[28]

A limitation was the unavailability of maternal pre-pregnancy BMI which has been highlighted to attenuate effect estimates when included in models.[28] However this limitation was partially ameliorated because we had access to maternal gestational weight gain, an important variable in its own right, which has been suggested to be significantly correlated with maternal pre-pregnancy BMI.[208] Recall bias remains a concern because some key variables were collected sometimes a year after pregnancy. This bias refers to the accuracy or differential recall of exposures/predictors or outcomes by participants.[201] These two recall bias elements often co-exist simultaneously. The extent and nature of recall bias determines the confidence with which a study's results can be regarded. Our main predictor, mode of delivery, relied on maternal recall nine months post-partum. We can be confident however that this is likely to be accurate in the vast majority of cases given that a similarly designed and conducted population-based study from the United Kingdom, the Millennium Cohort Study reported that 94% of mothers recalled their mode of delivery nine months post-partum when compared to their hospital records.[200] Another aspect worth mentioning is that infants born during the months of July to November, inclusive, were omitted from the GUI cohort. This is a constraint because month of birth can serve as a proxy for specific seasonal environmental circumstances that can significantly influence future

health.[209] This means this study's results are generalisable to those born from December to June, inclusive. It is challenging to predict how inclusion of a sample from July to November may have affected the results. These results could have remained the same or moved closer or further from the null.

The classification of CS into elective and emergency, although addressing a limitation of previous studies, did not allow sufficient granularity of issues like whether the CS was purely on maternal request; these may differ from other elective CSs, or if membranes had ruptured prior to surgery (exposure of the fetus to vaginal microbiota). All the women classified in the elective CS group had pre-labour CS. Although it is likely that women in the emergency CS group mostly had in labour CS, we cannot rule out the possibility that some of them had pre-labour CS. This is unlikely to have influenced the elective CS result, especially in terms of our hypothesis which is based on pre-labour CS. Improving CS classification is an ongoing worldwide effort that is only gaining traction during this century.[64] There was lack of statistical power for some analyses, like the overweight analysis, however the RRRs were similar to previously reported associations. Given the consistency of our results we thus think there is merit in them.

Our proxy measure for parity, the number of individuals in the study household who were a son/daughter of the mother, assumed for instance that the mother had no biologic children outside the household. Despite the assumptions we made, the average number of children a mother had in the GUI cohort, infants born circa 2008, was 1.97 which is close to the 2008 reported total fertility rate for Ireland of 2.06.[210] Thus the proxy parity variable was likely to be accurate in most cases and capture birth order sufficiently in the models.

Interpretation

The CS rate in this cohort was 26.0%, and is consistent with published national estimates of 25.6%.[168] This corroborates the national representativeness of the GUI cohort and the likely external validity of our findings. The 13.9% prevalence of macrosomia (> 4000g) however, was almost twice the 7.6% prevalence for the United States, another high-income country, during a similar time period circa 2008.[211] This suggests a highly obesogenic Irish milieu with high baseline levels of excess adiposity from birth.

We found high rates of childhood obesity and overweight, for comparison global obesity rates for girls and boys in 1975 were less than 1%.[70] The slightly lower prevalence of obesity at age five (5.0%) than at age three (5.3%) was in keeping with the natural obesity prevalence decline observed from approximately age two to 14 years.[212]

Approximately 80 studies of various designs (cohort, case control, cross sectional) and several systematic reviews have investigated the association between CS and offspring obesity.[28, 29, 183] Most of these studies found a positive association, however evaluation of this association was limited by publication bias, potential for residual confounding and moderate heterogeneity.[28] Studies which accounted for maternal pre-pregnancy weight and adjusted their analyses for a greater number of potential confounders reported effect sizes closer to the null.[28]

As reported by the previous systematic reviews and meta-analyses, we also found a small effect size (odds ratio/RRR < 1.50) before accounting for macrosomia in the association between CS birth and subsequent overweight and obesity.[28, 30] We too found a greater association between CS birth and being obese than with being overweight.[30]

Few studies have been able to differentiate between emergency and elective CS.[28, 30, 91] However our finding that elective/planned CS is a risk factor for obesity at three years has been found previously in an American prospective cohort from Boston followed up largely during this century.[84] Nevertheless this study did not explore the potential confounding effect of macrosomia. Inability to account for elective and emergency CS calls into question the findings and conclusions of a sibling-control study[91] which suggested a causal link between CS birth and future obesity. Another study with a sibling-control design, albeit also limited by inability to distinguish between elective and emergency CS, did not find an association between CS birth and higher BMI z score at age five years.[185] Unfortunately, the GUI cohort did not have data that allows sibling-cohort analysis.

The association between CS and obesity generally dissipates with increasing age, which can be attributed to attrition, greater interference by external factors such as antibiotic therapy or because of the natural decline in obesity prevalence from two to 14 years.[30, 91, 212] A study with follow-up to age twenty found higher overweight and obesity rates as well as higher concentrations of total and low-density lipoprotein cholesterol, leptin and apolipoprotein B in those born by CS.[196] It however remained unsettled if these unfavourable rates and markers of cardiometabolic disease could be attributed to CS birth itself or to the underlying reasons that necessitated CS birth.

Most studies have adjusted for birth weight[30], however, a Canadian population-based survey is to the best of our knowledge the only study to specifically consider macrosomia, defined in that study as > 4080g.[97] Although a non-modifiable risk factor, it is important to highlight that emergency CS was associated with being overweight and obese at three years and being obese at five years. In addition, infants delivered by emergency CS were more

likely to ‘transition’ between ages three and five, namely: remain obese, become non-obese (normal, overweight or thin), or have any other transition between the IOTF BMI categories.

As mentioned in the introduction, infants born by CS may have a microbiota that is more capable of harvesting dietary nutrients.[42-44] With emergency CS, membranes are more likely to have ruptured with consequent exposure of the infant to vaginal microbiota resulting in reduced odds of future obesity compared with elective CS infants. However finding a greater effect size for obesity following emergency CS, as previously reported[84], suggests other mechanisms may be at play with emergency CS namely confounding by indication. Indeed a recent study suggested that the main mechanism driving the microbiota’s structure and function in infancy is body site and not mode of delivery.[46] At birth, Chu and colleagues found that the neonatal microbiota structure and function was generally similar across different body sites (human anatomical locations), regardless of vaginal or CS delivery [46]. However by six weeks of age, infant microbiota structure and function was no longer similar across different body sites. There was no marked body site difference in the microbiota’s structure and function between infants born vaginally and by CS. This meant it was body site and not mode of birth which drove microbiota reorganisation.

Like we mentioned the natural history and drivers of being overweight or obese differ significantly by age. Although there is literature on adults,[29] some of which supports our findings, we focused our discussion on childhood at ages comparable to those in our study.

4.6 Conclusion

We did not find enough evidence to support a causal relationship between elective CS and childhood obesity. An increased risk of obesity in children born by emergency CS, but not

elective, suggests that there is no causal effect due to vaginal microflora and the association is likely to be explained by the underlying indications of emergency CS.

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Author contributions statement

G.M., S.O.N., P.N.B., L.C.K., S.M.B.M., A.S.K. conceived and designed the study. G.M., S.O.N., A.S.K. analysed the data and all authors interpreted the results. G.M. wrote the first draft of the article and S.O.N., P.N.B., L.C.K., S.M.B.M., A.S.K. revised it critically for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work.

Competing interests

The author(s) declare no competing interests.

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Table 4- 1. International body mass index cut-off values by age and sex.

	3 years		5 years	
	Boys	**Girls**	**Boys**	**Girls**
Body mass index (kg/m^2)				
Thin	<14.74	<14.47	<14.21	<13.94
Normal	≥14.74-<17.89	≥14.47-<17.56	≥14.21-<17.42	≥13.94-<17.15
Overweight	≥17.89-<19.57	≥17.56-<19.36	≥17.42-<19.30	≥17.15-<19.17
Obese	≥19.57	≥19.36	≥19.3	≥19.17

Table 4- 2. Characteristics of the study population.

Characteristic	Overall n (%)	Normal vaginal delivery n (%)	Assisted vaginal delivery[a] n (%)	Elective Caesarean section n (%)	Emergency Caesarean section n (%)
N	11049 (100)	6579 (59.5)	1596 (14.4)	1402 (12.7)	1472 (13.3)
Maternal					
Age, (years) median IQR	32 (28-36)	32 (28-35)	31 (27-35)	35 (31-37)	32 (28-35)
Ethnicity					
White	10266 (92.9)	6060 (92.1)	1530 (95.9)	1319 (94.1)	1357 (92.2)
Other	739 (6.7)	489 (7.4)	62 (3.9)	80 (5.7)	108 (7.3)
Missing	44 (0.4)	30 (0.5)	4 (0.3)	3 (0.2)	7 (0.5)
Marital status					
Married and living with husband	7421 (67.2)	4317 (65.6)	1007 (63.1)	1110 (79.2)	987 (67.1)
Married and separated from husband	210 (1.9)	131 (2.0)	27 (1.7)	24 (1.7)	28 (1.9)
Divorced/Widowed	134 (1.2)	78 (1.2)	16 (1.0)	20 (1.4)	20 (1.4)
Never married	3148 (28.5)	1955 (29.7)	534 (33.5)	235 (16.8)	424 (28.8)
Missing	136 (1.2)	98 (1.5)	12 (0.8)	13 (0.9)	13 (0.9)
Number of people in the household who are a son/daughter to the mother – 'Parity'					
1	4508 (40.8)	2104 (32.0)	1208 (75.7)	325 (23.2)	871 (59.2)
2	3643 (33.0)	2424 (36.8)	274 (17.2)	583 (41.6)	362 (24.6)
3+	2898 (26.2)	2051 (31.2)	114 (7.1)	494 (35.2)	239 (16.2)
Missing	14 (0.1)	12 (0.2)	0 (0.0)	2 (0.1)	0 (0.0)
Gestational age, (weeks) mean (±SD)	39.5 (±2.1)	39.7 (±1.9)	40.1 (±1.6)	38.7 (±1.7)	38.9 (±3.0)
Missing	37 (0.3)	24 (0.4)	4 (0.3)	3 (0.2)	6 (0.4)
Weight gain during pregnancy, (kg) mean (±SD)	13.6 (±6.6)	13.4 (±6.6)	14.0 (±6.3)	13.8 (±6.4)	14.2 (±6.9)
Missing	1500 (13.6)	884 (13.4)	236 (14.8)	178 (12.7)	202 (13.7)
Pre-eclampsia	765 (6.9)	354 (5.4)	127 (8.0)	107 (7.6)	177 (12.0)
Gestational diabetes	316 (2.9)	151 (2.3)	42 (2.6)	61 (4.4)	62 (4.2)
Offspring					
Sex					
Male	5644 (51.1)	3253 (49.4)	885 (55.5)	702 (50.1)	804 (54.6)
Female	5405 (48.9)	3326 (50.6)	711 (44.5)	700 (49.9)	668 (45.5)
Birth weight, (g) mean (±SD)	3485 (±534)	3507 (±502)	3551 (±466)	3431 (±562)	3369 (±672)
Macrosomia (> 4000g)	1539 (13.9)	899 (13.7%)	228 (14.3%)	183 (13.1%)	229 (15.6%)
Missing	124 (1.1)	70 (1.1)	12 (0.8)	26 (1.9)	16 (1.1)
Birth weight centiles adjusted for sex and gestational age					
SGA < 10th centile	1552 (14.0)	910 (13.8)	236 (14.8)	175 (12.5)	231 (15.7)
AGA ≥ 10th centile ≤ 90th centile	8138 (73.7)	4932 (75.0)	1214 (76.1)	983 (70.1)	1009 (68.5)

LGA > 90th centile	1199 (10.9)	643 (9.8)	130 (8.1)	215 (15.8)	211 (14.3)
Missing	160 (1.4)	94 (1.4)	16 (1.0)	29 (2.1)	21 (1.4)
Body mass index (kg/m^2) at 3 years*					
Thin	445 (4.0)	275 (4.2)	56 (3.5)	48 (3.4)	66 (4.5)
Normal	6748 (61.1)	4037 (61.4)	1000 (62.7)	866 (61.8)	845 (57.4)
Overweight	1767 (16.0)	1038 (15.8)	249 (15.6)	227 (16.2)	253 (17.2)
Obese	506 (4.6)	280 (4.3)	67 (4.2)	73 (5.2)	86 (5.8)
Missing	1583 (14.3)	949 (14.4)	224 (14.0)	188 (13.4)	222 (15.1)
Body mass index (kg/m^2) at 5 years*					
Thin	534 (4.8)	318 (4.8)	78 (4.9)	55 (3.9)	83 (5.6)
Normal	6459 (58.5)	3860 (58.7)	954 (59.8)	834 (59.5)	811 (55.1)
Overweight	1389 (12.6)	798 (12.1)	215 (13.5)	187 (13.3)	189 (12.8)
Obese	437 (4.0)	252 (3.8)	48 (3.0)	65 (4.6)	72 (4.9)
Missing	2230 (20.2)	1351 (20.5)	301 (18.9)	261 (18.6)	317 (21.5)

SD (Standard deviation), IQR (Interquartile range), SGA (Small for gestational age), AGA (Appropriate for gestational age), LGA (Large for gestational age).

[a] Vacuum or forceps

* International Obesity Task Force age and sex-specific cut-offs

Educational level not shown because of up to 14 overlapping categories that were challenging to recode into coherent mutually exclusive groups, missing data 10 (0.1%)

Table 4- 3. Mode of delivery and body mass index at age three years.

BMI category (normal BMI – base outcome)		Cases n (%)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin						
	Normal vaginal delivery	275 (2.9)	reference		reference	
	Assisted vaginal delivery	56 (0.6)	0.82 (0.61-1.10)	0.194	0.77 (0.56-1.05)	0.098
	Elective Caesarean	48 (0.5)	0.81 (0.59-1.11)	0.200	0.84 (0.61-1.16)	0.299
	Emergency Caesarean	66 (0.7)	1.15 (0.87-1.52)	0.336	1.11 (0.84-1.48)	0.456
Overweight						
	Normal vaginal delivery	1038 (11.0)	reference		reference	
	Assisted vaginal delivery	249 (2.6)	0.97 (0.83-1.13)	0.684	1.02 (0.87-1.20)	0.787
	Elective Caesarean	227 (2.4)	1.02 (0.87-1.20)	0.815	1.06 (0.90-1.25)	0.467
	Emergency Caesarean	253 (2.7)	1.17 (1.00-1.36)	0.056	1.23 (1.04-1.44)	0.013
Obese						
	Normal vaginal delivery	280 (3.0)	reference		reference	
	Assisted vaginal delivery	67 (0.7)	0.97 (0.73-1.27)	0.806	1.05 (0.78-1.39)	0.764
	Elective Caesarean	73 (0.8)	1.22 (0.93-1.59)	0.154	1.32 (1.01-1.74)***	0.045
	Emergency Caesarean	86 (0.9)	1.47 (1.14-1.89)	0.003	1.56 (1.20-2.03)	0.001

N for adjusted model = 9466. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity. ***1.45 (1.10-1.91) when birth weight added.

Table 4- 4. Mode of delivery and body mass index at age five years.

BMI category (normal BMI – base outcome)		Cases n (%)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin						
	Normal vaginal delivery	318 (3.6)	reference		reference	
	Assisted vaginal delivery	78 (0.9)	0.99 (0.77-1.28)	0.954	0.95 (0.73-1.24)	0.697
	Elective Caesarean	55 (0.6)	0.80 (0.60-1.08)	0.140	0.78 (0.57-1.06)	0.115
	Emergency Caesarean	83 (0.9)	1.24 (0.96-1.60)	0.093	1.18 (0.90-1.54)	0.238
Overweight						
	Normal vaginal delivery	798 (9.0)	reference		reference	
	Assisted vaginal delivery	215 (2.4)	1.09 (0.92-1.29)	0.310	1.15 (0.97-1.37)	0.114
	Elective Caesarean	187 (2.1)	1.08 (0.91-1.29)	0.366	1.13 (0.94-1.35)	0.190
	Emergency Caesarean	189 (2.1)	1.13 (0.95-1.34)	0.181	1.18 (0.99-1.42)	0.066
Obese						
	Normal vaginal delivery	252 (2.9)	reference		reference	
	Assisted vaginal delivery	48 (0.5)	0.77 (0.56-1.06)	0.107	0.84 (0.60-1.16)	0.279
	Elective Caesarean	65 (0.7)	1.19 (0.90-1.58)	0.219	1.30 (0.98-1.73)	0.072
	Emergency Caesarean	72 (0.8)	1.36 (1.04-1.79)	0.027	1.46 (1.10-1.93)	0.009

N for adjusted model = 8819. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).

** Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity.

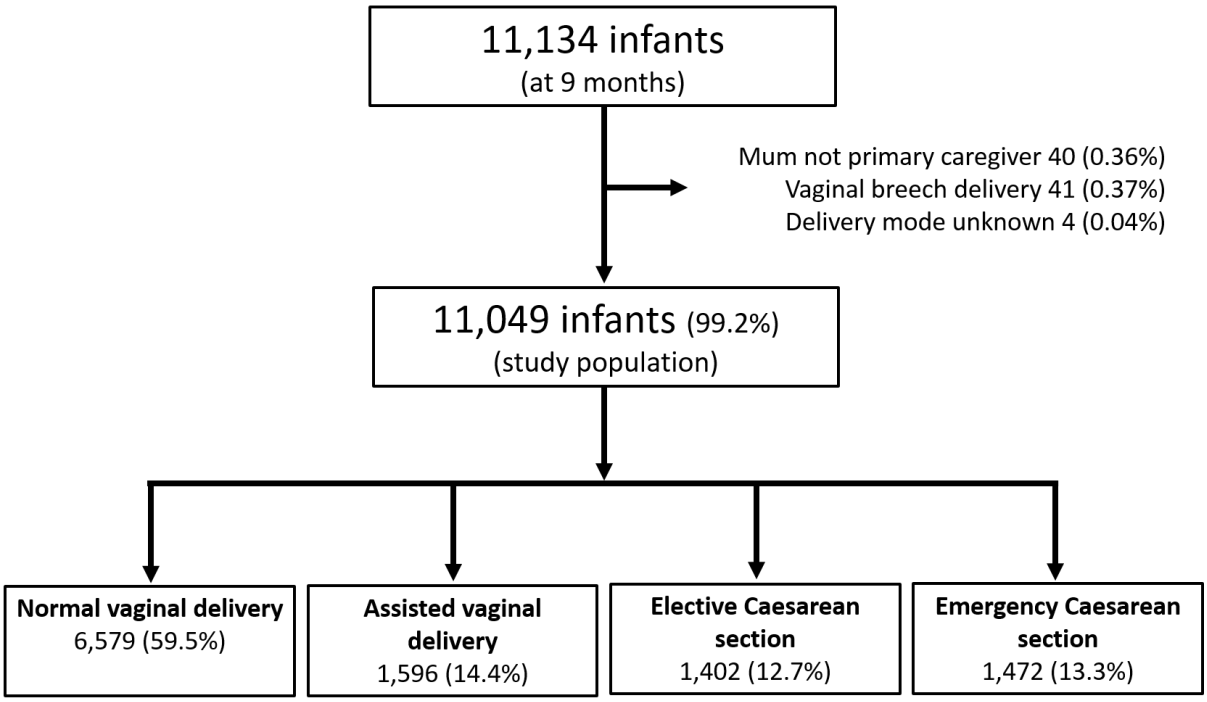


Figure 4- 1. Participant flow chart.

Supplementary Table S4-1. Mode of delivery and body mass index at age 3 years, Appropriate for Gestational Age (AGA).

BMI category (normal BMI – base outcome)	n	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin					
Normal vaginal delivery	188	reference		reference	
Assisted vaginal delivery	32	0.71 (0.48-1.04)	0.050	0.62 (0.42-0.92)	0.018
Elective Caesarean	27	0.63 (0.41-0.96)	0.067	0.62 (0.40-0.94)	0.023
Emergency Caesarean	36	0.92 (0.65-1.33)	0.977	0.84 (0.58-1.21)	0.354
Overweight					
Normal vaginal delivery	776	reference		reference	
Assisted vaginal delivery	203	1.04 (0.87-1.24)	0.601	1.08 (0.90-1.30)	0.384
Elective Caesarean	151	0.99 (0.81-1.20)	0.408	1.00 (0.82-1.21)	0.962
Emergency Caesarean	170	1.17 (0.97-1.41)	0.178	1.21 (0.99-1.47)	0.057
Obese					
Normal vaginal delivery	205	reference		reference	
Assisted vaginal delivery	52	1.02 (0.74-1.39)	0.923	1.03 (0.74-	0.861
Elective Caesarean	42	1.15 (0.69-1.37)	0.862	1.44)	0.426
Emergency Caesarean	61	1.66 (1.15-2.08)	0.004	1.15 (0.81-1.64)	0.001
				1.67 (1.22-2.29)	

N for adjusted model = 7001. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity.

Supplementary Table S4-2. Mode of delivery and body mass index at age 3 years, AGA, restricted to non-macrosomic.

BMI category (normal BMI – base outcome)		AdjRRR (95% CI)**	p-value
Thin			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	0.65 (0.44-0.97)	0.036
	Elective Caesarean	0.62 (0.40-0.94)	0.025
	Emergency Caesarean	0.83 (0.57-1.22)	0.341
Overweight			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	1.07 (0.88-1.31)	0.473
	Elective Caesarean	1.00 (0.82-1.23)	0.991
	Emergency Caesarean	1.15 (0.93-1.42)	0.195
Obese			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	1.08 (0.76-	0.682
	Elective Caesarean	1.53)	0.953
	Emergency Caesarean	0.99 (0.67-1.45)***	0.001
		1.77 (1.26-2.47)	

N for adjusted model = 6321. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity. ***0.96 (0.66-1.41) when birth weight added.

Supplementary Table S4-3. Mode of delivery and body mass index at age 3 years, Large for Gestational Age.

BMI category (normal BMI – base outcome)	n	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin					
Normal vaginal delivery	21	reference		reference	
Assisted vaginal delivery	3	0.74 (0.48-1.04)	0.050	0.95 (0.26-3.44)	0.933
Elective Caesarean	4	0.66 (0.41-0.96)	0.067	1.04 (0.35-3.15)	0.939
Emergency Caesarean	8	1.34 (0.65-1.33)	0.977	1.28 (0.49-3.30)	0.614
Overweight					
Normal vaginal delivery	140	reference		reference	
Assisted vaginal delivery	27	1.00 (0.87-1.24)	0.601	1.01 (0.62-1.66)	0.963
Elective Caesarean	51	1.26 (0.81-1.20)	0.408	1.35 (0.90-2.01)	0.143
Emergency Caesarean	50	1.26 (0.97-1.41)	0.178	1.51 (1.00-2.29)	0.049
Obese					
Normal vaginal delivery	41	reference		reference	
Assisted vaginal delivery	10	1.27 (0.74-1.39)	0.923	1.19 (0.54-	0.666
Elective Caesarean	23	1.94 (0.69-1.37)	0.862	2.62)	0.022
Emergency Caesarean	16	1.37 (1.15-2.08)	0.004	2.01 (1.10-3.67)	0.180
				1.60 (0.80-3.20)	

N for adjusted model = 1028. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes.

Supplementary Table S4-4. Mode of delivery and body mass index at age 3 years, Small for Gestational Age.

BMI category (normal BMI – base outcome)	n	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Thin					
Normal vaginal delivery	56	reference		reference	
Assisted vaginal delivery	21	1.37 (0.80-2.33)	0.250	1.40 (0.79-2.51)	0.251
Elective Caesarean	16	1.72 (0.95-3.12)	0.075	1.36 (0.70-2.64)	0.368
Emergency Caesarean	20	1.43 (0.83-2.46)	0.198	1.25 (0.70-2.26)	0.454
Overweight					
Normal vaginal delivery	109	reference		reference	
Assisted vaginal delivery	18	0.60 (0.35-1.02)	0.060	0.64 (0.37-1.10)	0.108
Elective Caesarean	23	1.27 (0.77-2.09)	0.348	1.51 (0.87-2.60)	0.140
Emergency Caesarean	31	1.14 (0.73-1.76)	0.565	1.31 (0.83-2.07)	0.238
Obese					
Normal vaginal delivery	24	reference		reference	
Assisted vaginal delivery	5	0.76 (0.28-2.02)	0.581	0.90 (0.34-	0.840
Elective Caesarean	6	1.50 (0.60-3.78)	0.384	2.43)	0.053
Emergency Caesarean	5	0.83 (0.31-2.22)	0.716	2.73 (0.99-7.51)	0.755
				1.17 (0.43-3.18)	

N for adjusted model = 1301. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity.

Supplementary Table S4-5. Sensitivity analyses by various variables.

BMI category (normal BMI – base outcome)	**AdjRRR (95% CI)****	**p-value**
Obese		
Normal vaginal delivery	reference	
Elective Caesarean*	1.39 (0.25-	0.707
Elective Caesarean**	7.80)	0.123
Elective Caesarean***	2.70 (0.76-9.52)	0.497
Elective Caesarean****	1.17 (0.74-1.86)	0.237
Elective Caesarean*****	1.28 (0.85-1.91)	0.091
	1.38 (0.95-1.99)	

*pre-term < 37 weeks (N=386), **pre-eclampsia (N=460), *** mothers < 35 years old (N= 4601), ****males (N= 4795), *****females (N= 4671)

Supplementary Table S4-6. Mode of delivery and body mass index at age 5 years, AGA, restricted to non-macrosomic.

BMI category (normal BMI – base outcome)		AdjRRR (95% CI)**	p-value
Thin			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	0.98 (0.71-1.35)	0.899
	Elective Caesarean	0.60 (0.40-0.89)	0.012
	Emergency Caesarean	1.07 (0.76-1.50)	0.693
Overweight			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	1.16 (0.94-1.44)	0.168
	Elective Caesarean	1.04 (0.83-1.31)	0.729
	Emergency Caesarean	1.26 (1.00-1.58)	0.048
Obese			
	Normal vaginal delivery	reference	
	Assisted vaginal delivery	0.90 (0.60-1.35)	0.610
	Elective Caesarean	1.26 (0.86-1.84)	0.231
	Emergency Caesarean	1.56 (1.06-2.29)	0.024

N for adjusted model = 5889. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity.

Supplementary Table S4-7. Mode of delivery and BMI category transition between ages three and five.

Transition (remained normal – base outcome)	Cases n (%)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Remained obese					
Normal vaginal delivery	97 (1.1)	reference		reference	
Assisted vaginal delivery	18 (0.2)	0.74 (0.44-1.22)	0.237	0.79 (0.47-1.34)	0.385
Elective Caesarean	28 (0.3)	1.32 (0.86-2.02)	0.209	1.51 (0.98-2.33)	0.063
Emergency Caesarean	32 (0.4)	1.63 (1.08-2.45)	0.019	1.74 (1.14-2.69)	0.011
Became obese					
Normal vaginal delivery	155 (1.8)	reference		reference	
Assisted vaginal delivery	30 (0.3)	0.77 (0.51-1.14)	0.193	0.86 (0.57-1.29)	0.452
Elective Caesarean	37 (0.4)	1.09 (0.75-1.57)	0.652	1.15 (0.79-1.67)	0.464
Emergency Caesarean	40 (0.5)	1.28 (0.89-1.83)	0.183	1.37 (0.95-1.97)	0.096
Became non obese					
Normal vaginal delivery	138 (1.6)	reference		reference	
Assisted vaginal delivery	40 (0.5)	1.15 (0.80-1.65)	0.450	1.32 (0.91-1.93)	0.147
Elective Caesarean	34 (0.4)	1.12 (0.76-1.65)	0.553	1.20 (0.81-1.78)	0.357
Emergency Caesarean	44 (0.5)	1.58 (1.11-2.24)	0.011	1.74 (1.21-2.49)	0.003
Other transition					
Normal vaginal delivery	1833 (20.8)	reference		reference	
Assisted vaginal delivery	449 (5.1)	0.97 (0.85-1.11)	0.659	1.00 (0.87-1.14)	0.946
Elective Caesarean	383 (4.3)	0.95 (0.83-1.09)	0.494	1.00 (0.86-1.15)	0.946
Emergency Caesarean	431 (4.9)	1.16 (1.01-1.33)	0.031	1.20 (1.04-1.38)	0.013

N for adjusted model = 8819. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted). *p-value<0.05.
** Adjusted for maternal age, education, ethnicity, marital status, region, infant sex, gestational age, pre-eclampsia, gestational diabetes, parity.





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Chapter 6: Caesarean Section Delivery and Childhood Obesity in a British Longitudinal Cohort Study

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(Appendix 1)

6.1 Abstract

Background

Several studies reported an association between Caesarean section (CS) birth and childhood obesity. However, there are several limitations in the current literature. These include an inability to distinguish between planned and emergency CS, small study sample sizes and not adjusting for pre-pregnancy body-mass-index (BMI). We examined the association between CS delivery and childhood obesity using the United Kingdom Millennium Cohort Study (MCS).

Methods

Mother-infant pairs were recruited into the MCS. Use of sampling weights ensured the sample was representative of the population. The exposure was categorised as normal vaginal delivery (VD) [reference], assisted VD, planned CS and emergency CS. Childhood obesity prevalence, at age three, five, seven, eleven and fourteen years was calculated using the International Obesity Taskforce criteria. Mixed-effects linear regression models were fitted with associations adjusted for several potential confounders like maternal age, pre-pregnancy BMI, education and infant macrosomia. Linear regression models were fitted evaluating body fat percentage (BF%), at age seven and fourteen years.

Results

Of the 18,116 infants, 3872 (21.4%) were delivered by CS; 9.2% by planned CS. Obesity prevalence was 5.4%, 5.7%, 6.5%, 7.1% and 7.6% at age three, five, seven, eleven and fourteen years respectively. The mixed-effects linear regression model showed no association between planned (adjusted mean difference=0.00; [95% confidence interval (CI) -0.10; 0.10], p-value = 0.97) or emergency CS (adjusted mean difference=0.08; [95% CI -0.01; 0.17], p-value = 0.09) and child BMI. At age seven years, there was no association between planned

CS and BF% (adjusted mean difference=0.13; [95% CI -0.23; 0.49]); there was no association at age fourteen years.

Conclusions

Infants born by planned CS did not have a significantly higher BMI or BF% compared to those born by normal VD. This may suggest that the association, described in the literature, could be due to the indications/reasons for CS birth or residual confounding.

Key words

Caesarean section; obesity; childhood; United Kingdom

6.3 Introduction

As summarised by several systematic reviews and meta-analyses[15, 27-30], numerous studies have found a consistent association between Caesarean section (CS) birth and subsequent childhood obesity. However, it remains unclear if this association indicates that CS causes obesity in childhood or is indicative of underlying confounding factors. A trial randomising pregnant women to deliver by CS or vaginally (VD) would provide definitive evidence.[140] In the absence of this clinical trial, data from observational studies, albeit limited by the paucity and small sample size of relevant studies, have been leveraged by controlling for major confounding variables, notably from maternal pre-pregnancy body mass index (BMI),[124] by considering obesity in siblings discordant for birth mode,[91, 185] and by comparing those born by elective and emergency CS.[84, 100, 122, 137, 196] Animal[45, 227] and microbial studies[32, 46] have also helped to investigate this question.

Differences in the infant gut microflora, which influence nutrient uptake, is the main hypothesised mechanism by which childhood obesity develops following CS delivery in offspring.[42-44] Differential exposure to the vaginal, perineal and faecal microflora between infants born by CS, particularly elective CS, and those born vaginally is presumed to determine the initial composition of an infant's gut microflora.[228, 229] There is the contentious possibility, however, that the putative placental microbiota influences composition too, regardless of delivery mode.[213, 230] Another potential mechanism relates to differences between infants born by CS and VD in the intrapartum concentration of cortisol, noradrenaline and other inflammatory chemicals,[36, 231] which may result in long-term neuro-immuno-endocrine, epigenetic and other changes which may influence energy metabolism.

Studying the associations underlying the role of CS with childhood obesity is important, given the global increase in CS rates and the epidemic of childhood obesity.[8, 232, 233] We recently performed two studies[137, 138] to address some of the limitations of previous reports, but both studies only followed-up offspring to age five years.

According to the systematic reviews and meta-analyses estimates of the strength of association between birth mode and childhood obesity, albeit with bias favouring positive effects, have been generally less than a relative risk of 1.50.[28, 30]
We aimed to investigate the association between planned/elective CS, a potentially modifiable risk factor, and childhood obesity using a large contemporary prospective longitudinal cohort study. In this study we used a similar approach to our previous work but with a different and larger dataset and much longer follow-up. This included analysis of the link between CS birth and body fat percentage (BF%) as previously performed,[138] on the basis that adiposity may be a more accurate measure of obesity than BMI.[234]

6.3 Materials and methods

The Millennium Cohort Study (MCS) is an ongoing multidisciplinary nationally representative longitudinal cohort study. At approximately nine months of age, children born in the United Kingdom (UK) from September 2000 through to January 2002 were recruited into the study, with over-sampling for ethnic minorities. The overall sample was representative of the population. A total of 18,827 infants were enrolled. To date there have been six major data collection sweeps at nine months, three, five, seven, eleven and fourteen years of age. Data was collected by trained interviewers using validated procedures and

instruments. Further comprehensive details about the MCS are available from its cohort profile.[143]

The exposure, mode of birth, was classified as normal or assisted VD and planned or emergency CS. Assisted VD constituted birth by forceps or vacuum extraction. Planned and emergency CS were mainly pre-labour or in labour respectively.[137]

Height was measured using a Leicester height measure. Weight and BF % were measured using Tanita^{TM} scales; the latter was ascertained by the scale's bioelectric impedance mechanism. BMI in kg/m^2 was classified as thin, normal, overweight or obese according to the standard International Obesity Task Force (IOTF) criteria, which are sex and age specific.[136, 150, 151]. Of the major BMI classification systems, including those from the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC), the IOTF criteria have been the most frequently used for this research topic.[28, 152] Using the 2006 WHO child growth standards, anthropometric z-scores were also calculated.[153]

Statistical analysis

Stata version 14SE (StataCorp LP College Station, TX) was used for statistical analysis. Categorical variables were described using frequencies (n) and percentages (%). Numeric variables were described using the mean (standard deviation-SD) or median (interquartile range-IQR). In the main analysis, to account for the continuous BMI, repeated measures available at age three, five, seven, eleven and fourteen years, crude and adjusted mixed-effects linear regression models were generated. In secondary analysis, to replicate our prior work,[137] multinomial logistic regression models were fitted to investigate the association between birth mode and IOTF BMI category transition between age three and five years; 0 = remained normal (base outcome), 1 = remained obese, 2 = became obese, 3 = became

non-obese and 4 = any other transition. Linear regression models were fitted to investigate the association between birth mode and BF%, available at age seven and fourteen years.

Based on prior literature, potential confounders were defined *a priori*. These included maternal age, ethnicity, education, marital status, couple income, infant sex, birth weight, smoking during pregnancy, gestational age, diabetes mellitus, parity, and pre-pregnancy BMI. We and other researchers found that infant macrosomia explained significant associations,[137, 138] we thus considered it as a potential confounder. Sub-group analysis was performed for infants with mothers aged > 35 years, born pre-term (< 37 weeks) and by their sex. A p-value < 0.05 was considered to be statistically significant.

Missing data

Multiple imputation was performed for maternal pre-pregnancy BMI and childhood BF% which all had substantial amounts of missing data. We assumed this data to be missing at random.[165] Variables in the main analysis were included in the imputation model. Forty-five imputations were done and the results were pooled according to Rubin's rules.[162] Imputed values were checked for plausibility in relation to observed values.

6.4 Results

The final baseline population consisted of 18,116 (96.2%) mother-infant pairs following exclusion of infants with an unknown mode of delivery (143, 0.76%), multiple births (467, 2.48%) and where the main respondent was not the infant's biologic mother because some potentially confounding variables were available only where mothers were the respondents.

Of the 18,116 infants, 3872 (21.4%) were delivered by CS; planned CS (9.2%), emergency CS (12.2%), normal VD 12,567 (69.4%) and assisted VD 1,677 (9.3%) (Table 6-1). At birth, 10.8% of the infants were macrosomic (> 4kg). The IOTF prevalence of obesity at ages three, five, seven, eleven and fourteen years of age was 5.4%, 5.7%, 6.5%, 7.1% and 7.6% respectively (S6-1 Table). According to the WHO criteria overweight and obesity prevalence at age three years was 5.2% and 1.8% respectively (S6-1 Table). At age seven years, the mean (SD) BF% was calculated at 19.1% (±5.1%) and 21.5% (±5.6%) for boys and girls respectively. The respective values at age fourteen years were 14.9% (±8.2%) and 26.6% (±7.0%).

Infants with missing data tended to have mothers that were younger, had General Certificate of Secondary Education grades D-G and an income of 0-10399 UK pounds – S6-2 Table.

The mean BMI by the four birth modes is depicted at each of the five time points, from age three to fourteen years, in S1 Fig. On average, mean BMI was lowest for normal VD and highest for planned CS. The mean BMI reached its nadir, of 16.3 kg/m^2 at age five years. Fig 1 depicts the mean BMI for all VD and CS births; it was highest for the latter. Those born by planned CS had a mean BMI that was similar to those born by normal VD (adjusted mean difference=0.00; [95% confidence interval (CI) -0.10; 0.11], p-value = 0.97) (Table 6-2). For those born by emergency CS the adjusted mean difference was 0.08; [95% CI -0.01; 0.17], p-value = 0.09.

There was no association between planned CS and any BMI category transition, S6-3 Table. The adjusted relative risk ratio of remaining obese from the age of three to five years among those born by emergency CS was 1.34; [95% CI 0.98; 1.82], p-value = 0.07.

At age seven years, there was no association between planned CS and BF% (adjusted BF% mean difference=0.13; [95% CI -0.23; 0.49], p-value = 0.47) and emergency CS (adjusted BF% mean difference=0.21; [95% CI -0.11; 0.54], p-value = 0.20) in comparison to the reference group of children delivered by unassisted VD (Table 6-3). At age fourteen years, there was also no association (Table 6-3). Imputing missing maternal pre-pregnancy BMI and BF% did not alter our results materially (S6-4 Table). The prevalence of being overweight and obese in the observed data was almost identical to that of the pooled data. This meant that the imputation procedure produced results that were not erroneous and could be relied on to draw valid inferences.

Sub-group analysis for infants with mothers > 35 years old, born pre-term or by their sex did not reveal any statistically significant results (S6-5 to S6-8 Tables).

6.5 Discussion

Main findings

From a large contemporary prospective longitudinal cohort study, we found that infants born by planned CS did not have an increased BMI overall, from age three to fourteen years, compared with those born by normal VD. We also found that obesity prevalence increased from age three years onwards. Infants born by planned CS did not have an increased BF% at age seven and fourteen years compared with those born by normal VD.

Interpretation

Our results are identical to those of another study that used MCS data, albeit at age three years.[103] This cross-sectional study, which estimated overweight risk in childhood from

predictors during infancy, found no association between CS birth and being overweight at age three years. One of the few studies to utilise within family analysis, in addition to traditional observational cohort analytic techniques, also found no association between CS birth and childhood obesity.[119] The national representativeness and the generalisability of this MCS study result to the UK population is reinforced by similar CS rates of ~21% in this cohort and in the general population at the turn of the second millennium.[7]

As we previously reported using a different cohort, there was no association between planned/elective CS delivery and obesity or transition into or out of obesity between ages three and five years.[137]

The natural history of BMI across the life course identifies peak BMI during the first two years of life which then reaches the lowest post infancy values at around five years of age.[182] This takes into account that infants born by CS have a higher BMI than those born by VD. We too found this BMI pattern, namely a nadir around age five, and CS infants having a non-significantly higher BMI.[137, 138] Cross sectional analysis of the association between mode of birth and BMI would therefore be influenced by the natural history and the age at which analysis was done. Therefore the first two years of life, during which BMI reaches a peak seems to be when the greatest, statistically significant, divergence in BMI between CS and VD born infants occurs.[122, 138, 182]

The prevalence of childhood obesity, in our study, did not follow a trajectory wherein it declines from age two to fourteen.[212] This may be due to the global childhood obesity epidemic driven by positive caloric intake.[232] In the MCS, family lifestyle may also have been contributory.[235]

That delivery mode is not associated with BF%, in both girls and boys, has been reported from a Brazilian longitudinal cohort study, and also in our previous publication.[138, 184] Disparate findings were reported from a Mexican study (n=256) which also used bioelectric impedance to assess body composition at approximately age seven years.[121] Girls, but not boys, born by CS had a higher fat mass index although no distinction was made between planned and emergency CS. Our main findings are similar to those reported in adolescents, aged fifteen years, where, after adjusting for potential confounders, no association was found between CS birth and obesity - as defined according to WHO Standards.[83] A United States study, albeit with a sample size of less than a thousand, found that delivery type did not predict obesity in adolescence.[95] These aforementioned results would be in keeping with how the infant microbiota undergoes considerable reorganisation in the first six weeks of life which is influenced by body site rather than by delivery mode.[46] Disparate findings have been reported, with obesity rates higher in twenty year olds delivered by CS, although the underlying sample was not nationally representative, thereby reducing external validity.[196] The exposures planned and emergency CS likely have different confounding structures. This is because the indications for planned and emergency CS differ.[60] Although the results were null for both types of exposure, the point estimates were generally greater for emergency CS than for planned CS which is reflective of this underlying dissimilar confounding structure. Around the time of puberty,[236] an acceleration of BMI towards adult values was observed at age eleven and fourteen years, however the association between delivery mode and BMI remained non-significant.

Strengths and limitations

Firstly, the MCS cohort is a large nationally representative prospective study which allows ready generalisation of findings to the population. In contemporary literature, the baseline

sample size of over 18,000 represents one of the largest cohorts and the follow-up to age fourteen years is one of the longest thus far perfomed.[122, 137] Secondly, maternal pre-pregnancy BMI, a key confounder, was available, thus mitigating a key limitation of previous analyses.[28] Thirdly, it was possible to separate CS birth into planned and emergency CS which only a limited number of earlier studies have managed to do.[84, 100, 122, 137] Fourthly, having children born during every month of the year mitigated the effects of seasonality. This was important since birth month can be a proxy for seasonal attributes which may influence future health.[209]

With planned CS, membranes were unlikely to have ruptured as women were not in labour. Since our hypothesis was based on pre-labour CS, the classification of CS[64] into planned and emergency was unlikely to have influenced our results. Although the final mode of birth was obtained from mothers approximately nine months post-partum, maternal recall of delivery mode in the MCS has been demonstrated to be reliable, (approximately 98% of mothers recalled this accurately).[200] Paucity of phenotypic data from fathers represents a constraint because they have been demonstrated to play a significant role in the development of childhood obesity.[237] We did not have data that permitted within family analysis.[91, 185] Due to unavailability of data on antibiotics administered intrapartum, our results were not adjusted for this potentially confounding factor. However, we are confident that this limitation did not alter our results because previous studies that adjusted for intrapartum antibiotic administration did not have their results changed materially.[122, 182] The confounding factor maternal gestational weight gain, which is linked to post-pregnancy weight retention, was not available. This limited our study. It is not possible to determine the change in direction and magnitude, if any, of the outcome risk estimates had maternal gestational weight gain been available. The However because of the high degree of correlation between pre-pregnancy BMI and gestational weight gain we believe our models

had sufficient merit.[238, 239] Using bioelectric impedance, for large studies like the MCS, is advantageous because of its portability, ease of use and low cost; the disadvantage however is that bioelectric impedance underestimates BF%.[240] Using other BMI classification, like the WHO system, would not change the results of the comparisons of the absolute values of BMI.

Most CS births are performed under regional anaesthesia, thus the kind of anaesthesia was unlikely to have contributed to our results.[241] It was not possible to rule out possible confounding due to the underlying reasons for CS because there were no further variables like previous CS available to capture the health of the mother prior to birth and the exact indications for CS birth were unavailable. In addition, as for any observational study, it was not possible to completely exclude residual confounding. Attrition of participants, which was more pronounced at later ages – up to 43.3%, also represents a limitation. Multiple imputation suggested that this missing data did not affect our results. Although there was inherent lack of power for some analyses, particularly at later ages because of loss to follow-up, consistency of the results suggests their merit.

6.6 Conclusion

Infants born by planned CS did not have a significantly higher BMI or BF% compared to those born by normal VD. This may suggest that the association described in the literature could be due to the indications/reasons for CS birth or residual confounding.

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Competing interests

The authors have declared that no competing interests exist.

Ethics approval

Ethical approval for the Millennium Cohort Study surveys was granted by the London Multicentre Research Ethics Committee.

Data sharing statement

The Millennium Cohort Study data is available free of charge to bona fide researchers from the United Kingdom Data Service https://www.ukdataservice.ac.uk/. This was third party data which was not owned or collected by the authors. The datasets can be accessed by others in the same manner as the authors. There were no special access privileges.

Table 6- 1. Characteristics of the study population.

Characteristic	**Overall n (%)**	**Normal vaginal delivery n (%)**	**Assisted vaginal delivery [a] n (%)**	**Planned Caesarean section n (%)**	**Emergency Caesarean section n (%)**
N	18,116 (100)	12,567 (69.4)	1677 (9.3)	1669 (9.2)	2203 (12.2)
Maternal age (years), median IQR	29 (24-33)	28 (23-32)	29 (24-32)	31 (27-34)	30 (25-33)
< 20	1572 (8.7)	1,214 (9.7)	171 (10.2)	42 (2.5)	145 (6.6)
20-24	3491 (19.3)	2,643 (21.0)	291 (17.4)	207 (12.4)	350 (15.9)
25-29	5010 (27.7)	3,491 (27.8)	505 (30.1)	409 (24.5)	605 (27.5)
30-34	5215 (28.8)	3,447 (27.4)	479 (28.6)	605 (36.2)	684 (31.0)
35-39	2443 (13.5)	1,541 (12.3)	210 (12.5)	342 (20.5)	350 (15.9)
≥ 40	382 (2.1)	228 (1.8)	21 (1.3)	64 (3.8)	69 (3.1)
Ethnicity					
European	15,180 (83.3)	10,411 (82.2)	1,525 (90.9)	1,426 (85.4)	1,818 (82.5)
Asian	1,911 (10.5)	1,424 (11.3)	101 (6.0)	163 (9.8)	223 (10.1)
African	664 (3.7)	464 (3.7)	20 (1.2)	51 (3.1)	129 (5.9)
Mixed	186 (1.0)	134 (1.1)	15 (0.9)	17 (1.0)	20 (0.9)
Any other background	146 (0.8)	107 (0.9)	15 (0.9)	11 (0.7)	13 (0.6)
Missing	29 (0.2)	27 (0.2)	1 (0.1)	1 (0.1)	0 (0.0)
Highest education					
GCSE grades D-G	1,944 (10.7)	1,392 (11.1)	158 (9.4)	163 (9.8)	231 (10.5)
O level / GCSE grades A-C	6,047 (33.4)	4,202 (33.4)	567 (33.8)	570 (34.2)	708 (32.1)
A / AS / S levels	1,687 (9.3)	1,153 (9.2)	183 (10.9)	137 (8.2)	214 (9.7)
Diplomas in higher education	1,511 (8.3)	962 (7.7)	179 (10.7)	166 (9.9)	204 (9.3)
First degree	2,229 (12.3)	1,369 (10.9)	302 (18.0)	218 (13.1)	340 (15.4)
Higher degree	604 (3.3)	376 (3.0)	66 (3.9)	72 (4.3)	90 (4.1)
Other academic qualifications (including overseas)	526 (2.9)	382 (3.0)	37 (2.2)	43 (2.6)	64 (2.9)
None of these qualifications	3,521 (19.4)	2,691 (21.4)	184 (11.0)	299 (17.9)	347 (15.8)
Missing	47 (0.3)	40 (0.3)	1 (0.1)	1 (0.1)	5 (0.2)
Total net couple income (UK pounds)					
0-10399	1,858 (10.3)	1,360 (10.8)	136 (8.1)	151 (9.0)	211 (9.6)
10400-15599	2,522 (13.9)	1,837 (14.6)	201 (12.0)	209 (12.5)	275 (12.5)
15600-19799	2,533 (14.0)	1,762 (14.0)	241 (14.4)	226 (13.5)	304 (13.8)

20800-30199	3,185 (17.6)	2,089 (16.6)	336 (20.0)	334 (20.0)	426 (19.3)	
31200-80000+	3,198 (17.7)	1,984 (15.8)	385 (23.0)	371 (22.2)	458 (20.8)	
Not applicable	3,525 (19.5)	2,639 (21.0)	271 (16.2)	227 (13.6)	388 (17.6)	
Don't know	921 (5.1)	652 (5.2)	64 (3.8)	110 (6.6)	95 (4.3)	
Refused	374 (2.1)	244 (1.9)	43 (2.6)	41 (2.5)	46 (2.1)	
Marital status						
Legally separated	516 (2.8)	392 (3.1)	24 (1.4)	39 (2.3)	61 (2.8)	
Married, 1st and only marriage	10016 (55.3)	6,741 (53.6)	958 (57.1)	1,073 (64.3)	1,244 (56.5)	
Remarried, 2nd or later marriage	730 (4.0)	484 (3.9)	46 (2.7)	98 (5.9)	102 (4.6)	
Single never married	6100 (33.7)	4,419 (35.2)	594 (35.4)	370 (22.2)	717 (32.5)	
Divorced	719 (4.0)	507 (4.0)	53 (3.2)	83 (5.0)	76 (3.4)	
Widowed	33 (0.2)	22 (0.2)	2 (0.1)	6 (0.4)	3 (0.1)	
Missing	2 (0.0)	2 (0.0)	0 (0.0)	2 0 (0.0)	0 (0.0)	0
Body mass index (kg/m^2) pre-pregnancy, median IQR	22.7 (20.6-25.7)	22.5 (20.6-25.3)	22.5 (20.7-25.1)	23.7 (21.4-27.1)	23.4 (21.2-26.8)	
Missing	1558 (8.6)	1,110 (8.8)	96 (5.7)	159 (9.5)	193 (8.8)	
Smoking during pregnancy						
Non-smoker	12,927 (71.4)	8,935 (71.1)	1,169 (69.7)	1,244 (74.5)	1,579 (71.7)	
Gave up	2,298 (12.7)	1,526 (12.1)	268 (16.0)	208 (12.5)	296 (13.4)	
Smoker	2,877 (15.9)	2,094 (16.7)	239 (14.3)	216 (12.9)	328 (14.9)	
Missing	14 (0.1)	12 (0.1)14	1 (0.1)	1 (012)	0 (0.0)	1
Diabetes mellitus						
Any kind of diabetes mellitus	313 (1.7)	144 (1.1)	18 (1.1)	79 (4.7)	72 (3.3)	
No diabetes mellitus	17,802 (98.3)	12,422 (98.8)	1,659 (98.9)	1,590 (95.3)	2,131 (96.7)	
Missing	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Number of other children – 'parity'						
1	17,474 (96.5)	12,113 (96.4)	1,663 (99.2)	1,571 (94.1)	2,127 (96.6)	
2	470 (2.6)	320 (2.5)	11 (0.7)	83 (5.0)	56 (2.5)	
3+	168 (0.9)	131 (1.0)	3 (0.2)	15 (0.9)	19 (0.9)	
Missing	4 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	
Sex						
Male	9,322 (51.5)	6,330 (50.4)	930 (55.5)	814 (48.8)	1,248 (56.7)	
Female	8,794 (48.5)	6,237 (49.6)	747 (44.5)	855 (51.2)	955 (43.3)	
Gestational age (weeks)						

Preterm (< 37)	1708 (9.4)	978 (7.8)	100 (6.0)	178 (10.7)	452 (20.5)
Term (37-41)	15,992 (88.3)	11,306 (90.0)	1,535 (91.5)	1,467 (87.9)	1,684 (76.4)
Postterm (> 42)	225 (1.2)	147 (1.2)	28 (1.7)	6 (0.4)	44 (2.0)
Missing	191 (1.1)	136 (1.1)	14 (0.8)	18 (1.1)	23 (1.0)
Birth weight (kg), median IQR	3.37 (3.03-3.71)	3.37 (3.04-3.71)	3.43 (3.15-3.77)	3.35 (3 – 3.69)	3.36 (2.84 - 3.80)
Missing	14 (0.1)	11 (0.1)	0 (0.0)	3 (0.2)	0 (0.0)
Macrosomia (> 4kg)	1,957 (10.8)	1,264 (10.1)	184 (11.0)	177 (10.6)	332 (15.1)

UK (United Kingdom), SD (Standard deviation), IQR (Interquartile range), GCSE (General Certificate of Secondary Education).
Vacuum or forceps [a]

Table 6- 2. Mode of birth and body mass index.

BMI	Coef (95% CI)	p-value	AdjCoef (95% CI)**	p-value
Normal vaginal	reference		reference	
Assisted vaginal	-0.08 (-0.18; 0.02)	0.116	-0.03 (-0.13; 0.07)	0.567
Planned Caesarean	0.18 (0.08; 0.28)	0.000	0.00 (-0.10; 0.10)	0.971
Emergency Caesarean	0.18 (0.09; 0.27)	0.000	0.08 (-0.01; 0.17)	0.091

Time points for adjusted model = 50,917 at ages three, five, seven, eleven and fourteen years. Mixed-effects linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, ethnicity, education, marital status, couple income, infant sex, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

Table 6- 3. Mode of delivery and body fat percent at seven and fourteen years.

Delivery mode (seven years)	**Coef. (95% CI)**	**p-value**	**AdjCoef. (95% CI)****	**p-value**
Normal vaginal delivery	reference		reference	
Assisted vaginal	-0.21 (-0.56; 0.14)	0.248	0.03 (-0.31; 0.37)	0.864
Planned Caesarean	0.43 (0.08; 0.78)	0.016	0.13 (-0.23; 0.49)	0.466
Emergency Caesarean	0.35 (0.03; 0.67)	0.032	0.21 (-0.11; 0.54)	0.199
Delivery mode (fourteen years)	Coef. (95% CI)	p-value	AdjCoef. (95% CI)**	p-value
Normal vaginal delivery	reference		reference	
Assisted vaginal	-1.26 (-1.91; -0.61)	0.000	-0.40 (-0.94; -0.13)	0.139
Planned Caesarean	0.50 (-0.16; 1.15)	0.135	-0.08 (-0.64; 0.47)	0.769
Emergency Caesarean	-0.04 (-0.62; -0.55)	0.904	-0.00 (-0.50 ;0.50)	0.999

N for adjusted model = 10,254 and 8,279 at age seven and fourteen respectively. Linear regression. Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, ethnicity, education, marital status, couple income, infant sex, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy body mass index (Non-macrosomic infants).

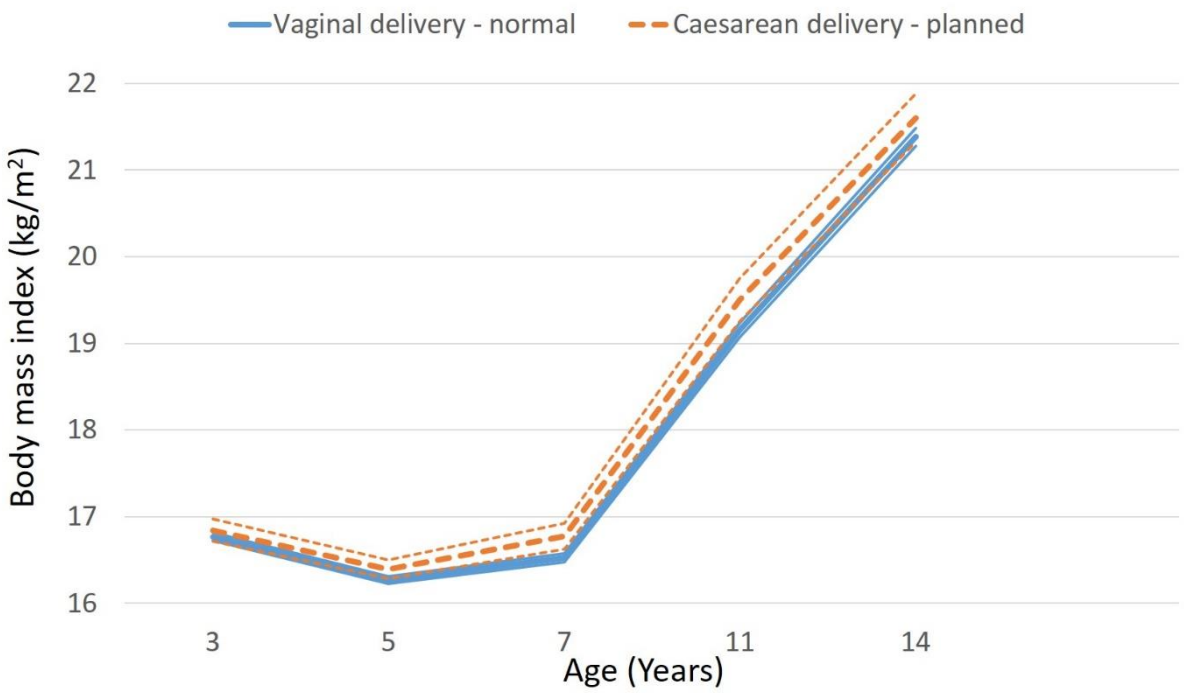


Figure 6- 1. Mean body mass index by birth mode from age three to fourteen years with 95% confidence intervals – thin lines – for non-macrosomic infants born by normal vaginal delivery and by planned Caesarean section.

S6-1 Table. International Obesity Task Force classification of body mass index from age three to fourteen and body fat% at age seven and fourteen.

Characteristic	Overall n (%)	Normal vaginal delivery n (%)	Assisted vaginal delivery [a] n (%)	Planned Caesarean section n (%)	Emergency Caesarean section n (%)
Body mass index (kg/m^2) at 3 years*					
Thin	178 (1.0)	110 (0.9)	17 (1.0)	19 (1.1)	32 (1.5)
Normal	11953 (66.0)	8,303 (66.1)	1114 (66.4)	1111 (66.6)	1,425 (64.7)
Overweight	947 (5.2)	622 (4.9)	100 (6.0)	98 (5.9)	127 (5.8)
Obese	330 (1.8)	217 (1.7)	28 (1.7)	35 (2.1)	50 (2.3)
Missing	4708 (26.0)	3315 (26.4)	418 (24.9)	406 (24.3)	569 (25.8)
Body mass index (kg/m^2) at 3 years					
Thin	706 (3.9)	483 (3.8)	50 (3.0)	67 (4.0)	106 (4.8)
Normal	9568 (52.8)	6,675 (53.1)	890 (53.1)	890 (53.3)	1,113 (50.5)
Overweight	2376 (13.1)	1,590 (12.7)	245 (14.6)	226 (13.5)	315 (14.3)
Obese	758 (4.2)	498 (4.0)	73 (4.4)	84 (5.0)	103 (4.7)
Missing	5,414 (29.9)	3,804 (30.3)	469 (28.0)	469 (28.1)	672 (30.5)
Body mass index (kg/m^2) at 5 years					
Thin	699 (3.9)	476 (3.8)	66 (3.9)	60 (3.6)	97 (4.4)
Normal	10313 (56.9)	7,224 (57.5)	973 (58.0)	921 (55.2)	1,195 (54.2)
Overweight	2266 (12.5)	1,500 (11.9)	228 (13.6)	248 (14.9)	290 (13.2)
Obese	834 (4.6)	557 (4.4)	77 (4.6)	84 (5.0)	116 (5.3)
Missing	3,286 (26.0)	399 (26.1)	416 (23.8)	602 (24.9)	3,286 (27.3)
Body mass index (kg/m^2) at 7 years					
Thin	750 (4.1)	526 (4.2)	63 (3.8)	60 (3.6)	101 (4.6)
Normal	9282 (51.2)	6,475 (51.5)	900 (53.7)	824 (49.4)	1,083 (49.2)
Overweight	1966 (10.9)	1,321 (10.5)	174 (10.4)	217 (13.0)	254 (11.5)
Obese	873 (4.8)	575 (4.6)	77 (4.6)	97 (5.8)	124 (5.6)
Missing	5,995 (33.1)	4,196 (33.4)	526 (31.4)	531 (31.8)	742 (33.7)
Body mass index (kg/m^2) at 11 years					
Thin	722 (4.0)	492 (3.9)	72 (4.3)	69 (4.1)	89 (4.0)
Normal	7946 (43.9)	5,546 (44.1)	789 (47.0)	671 (40.2)	940 (42.7)
Overweight	2607 (14.4)	1,767 (14.1)	220 (13.1)	268 (16.1)	352 (16.0)
Obese	870 (4.8)	596 (4.7)	64 (3.8)	99 (5.9)	111 (5.0)
Missing	6,693 (36.9)	4,658 (37.1)	604 (36.0)	631 (37.8)	800 (36.3)
Body mass index (kg/m^2) at 14 years					
Thin	645 (3.6)	463 (3.7)	61 (3.6)	55 (3.3)	66 (3.0)
Normal	6815 (37.6)	4,728 (37.6)	672 (40.1)	602 (36.1)	813 (36.9)
Overweight	2475 (13.7)	1,411 (11.2)	165 (9.8)	602 (36.1)	297 (13.5)
Obese	797 (4.4)	545 (4.3)	59 (3.5)	81 (4.9)	112 (5.1)
Missing	8,029 (44.3)	5,883 (46.8)	781 (46.6)	384 (23.0)	981 (44.5)
Body fat (%), median IQR at age 7	20.1 (17.5-23.8)	20.1 (17.5-23.7)	20 (17.4-23.4)	20.6 (17.8-24.2)	20.3 (17.5-24.2)
Missing	5435 (30.0)	3801 (30.2)	486 (29.0)	488 (29.2)	660 (30.0)
Body fat (%), median IQR at age 14	21.7 (14.4-28.4)	22 (14.4-28.4)	20 (13.8-26.8)	22.6 (15.3-29.5)	21.1 (14.4-28.8)
Missing	7898 (43.6)	5,510 (43.8)	733 (43.7)	722 (43.3)	933 (42.4)

*World Health Organization z-scores

S6-2 Table. Missing data for body mass index at age two years.

Characteristic	Body mass index missing (n %) n= 5487	Body mass index not missing (n %) n=12772	p-value[a]
Maternal age (years), median IQR[b]	27 (22-32)	29 (25-33)	< 0.001
Ethnicity			< 0.001
European	4325 (28.5)	10,855 (71.5)	
Asian	695 (36.4)	1,216 (63.6)	
African	262 (39.5)	402 (60.5)	
Mixed	79 (42.5)	107 (57.5)	
Any other background	68 (46.6)	78 (53.4)	
Highest education			< 0.001
GCSE grades D-G	671 (34.5)	1,273 (65.5)	
O level / GCSE grades A-C	1,757 (29.1)	4,290 (70.9)	
A / AS / S levels	421 (25.0)	1,266 (75.0)	
Diplomas in higher education	372 (24.6)	1,139 (75.4)	
First degree	411 (18.4)	1,818 (81.6)	
Higher degree	132 (21.9)	472 (78.2)	
Other academic qualifications (including overseas)	202 (38.4)	324 (61.6)	
None of these qualifications	1,441 (40.9)	2,080 (59.1)	
Total net couple income (UK pounds)			< 0.001
0-10399	710 (38.2)	1,148 (61.8)	
10400-15599	837 (33.2)	1,685 (66.8)	
15600-19799	706 (27.9)	1,827 (72.2)	
20800-30199	722 (22.7)	2,463 (77.3)	
31200-80000+	642 (20.1)	2,556 (80.0)	
Not applicable	1,387 (39.4)	2,138 (60.7)	
Don't know	292 (31.7)	629 (68.3)	
Refused	139 (37.2)	235 (62.8)	
Marital status			< 0.001
Legally separated	179 (34.7)	337 (65.3)	
Married, 1st and only marriage	2,675 (26.7)	7,341 (73.3)	
Remarried, 2nd or later marriage	176 (24.1)	554 (75.9)	
Single never married	2,179 (35.7)	3,921 (64.3)	
Divorced	211 (29.4)	508 (70.7)	
Widowed	13 (39.4)	20 (60.6)	
Body mass index (kg/m^2) pre-pregnancy, median IQR[b]	22.5 (20.4-25.4)	22.8 (20.8-25.8)	< 0.001
Smoking during pregnancy			< 0.001
Non-smoker	3,744 (29.0)	9,183 (71.0)	
Gave up	714 (31.1)	1,584 (68.9)	
Smoker	971 (33.8)	1,906 (66.3)	
Diabetes mellitus			0.890
Any kind of diabetes mellitus	95 (30.4)	218 (69.7)	
No diabetes mellitus	5,339 (30.0)	12,463 (70.0)	
Number of other children – 'parity'			

1	5,210 (29.8)	12,264 (70.2)	0.021
2	164 (34.9)	306 (65.1)	
3	59 (35.1)	109 (64.9)	
Sex			< 0.001
Boy	2,922 (31.4)	6,400 (68.7)	
Girl	2,513 (28.6)	6,281 (71.4)	
Gestational age (weeks)			0.001
Preterm (< 37)	562 (32.9)	1,146 (67.1)	
Term (37-41)	4,720 (29.5)	11,272 (70.5)	
Postterm (> 42)	84 (37.3)	141 (62.7)	
Birth weight (kg), median IQR[b]	3.35 (3-3.69)	3.4 (3.03-3.74)	< 0.001

IQR – Interquartile range, BMI – Body mass index, SD – standard deviation, UK – United Kingdom

[a] Pearson's χ^2 test or Fisher's exact

[b] Mann-Whitney test

[c] Two-sample t test

S6-3 Table. Mode of delivery and BMI category transition between ages three and five.

Transition (remained normal – base outcome)	RRR (95% CI)	p-value	AdjRRR (95% CI)**	p-value
Remained obese				
Normal vaginal delivery	reference		reference	
Assisted vaginal delivery	1.09 (0.71; 1.67)	0.695	1.16 (0.74; 1.85)	0.507
Planned Caesarean	1.20 (0.79; 1.81)	0.394	0.94 (0.59; 1.49)	0.780
Emergency Caesarean	1.22 (0.83; 1.79)	0.317	1.09 (0.70; 1.71)	0.698
Became obese				
Normal vaginal delivery	reference		reference	
Assisted vaginal delivery	0.92 (0.65; 1.30)	0.638	1.11 (0.77; 1.59)	0.572
Planned Caesarean	1.13 (0.82; 1.56)	0.444	0.96 (0.67; 1.38)	0.836
Emergency Caesarean	1.35 (1.02; 1.79)	0.035	1.34 (0.98; 1.82)	0.066
Became non obese				
Normal vaginal delivery	reference		reference	
Assisted vaginal delivery	1.18 (0.81; 1.72)	0.387	1.20 (0.81; 1.78)	0.362
Planned Caesarean	1.06 (0.71; 1.57)	0.787	0.81 (0.51; 1.27)	0.350
Emergency Caesarean	1.03 (0.71; 1.50)	0.872	0.92 (0.61; 1.38)	0.688
Any other transition				
Normal vaginal delivery	reference		reference	
Assisted vaginal delivery	1.04 (0.91; 1.18)	0.579	1.10 (0.97; 1.26)	0.143
Planned Caesarean	0.98 (0.86; 1.12)	0.761	1.01 (0.88; 1.16)	0.921
Emergency Caesarean	1.11 (0.99; 1.25)	0.083	1.11 (0.99; 1.27)	0.082

N for adjusted model = 11,421. Multinomial logistic regression. BMI – Body mass index, RRR (Relative Risk Ratio), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

S6-4 Table. Mode of delivery and body fat percent at seven and fourteen years. Imputed pre-pregnancy BMI and childhood body fat percent.

Delivery mode	**Coef. (95% CI)**	**p-value**	**AdjCoef. (95% CI)****	**p-value**
Normal vaginal delivery	reference		reference	
Assisted vaginal	-0.16 (-0.50; 0.19)	0.370	0.05 (-0.29; 0.39)	0.781
Planned Caesarean	0.44 (0.09; 0.79)	0.014	0.15 (-0.21; 0.51)	0.412
Emergency Caesarean	0.31 (-0.00; 0.61)	0.053	0.21 (-0.11; 0.53)	0.199
Delivery mode	Coef. (95% CI)	p-value	AdjCoef. (95% CI)**	p-value
Normal vaginal delivery	reference		reference	
Assisted vaginal	-1.27 (-1.87; -0.66)	0.000	-0.41 (-0.96; 0.13)	0.135
Planned Caesarean	0.49 (-0.13; 1.11)	0.120	0.00 (-0.56; 0.57)	0.988
Emergency Caesarean	-0.05 (-0.62; 0.52)	0.861	0.09 (-0.46; 0.63)	0.755

N for adjusted model = 14,595 and 14,595 at age seven and fourteen respectively. Linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).

**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

S6-5 Table. Mode of birth and body mass index for infants with mothers > 35 years old.

BMI	Coef (95% CI)	p-value	AdjCoef (95% CI)**	p-value
Normal vaginal	reference		reference	
Assisted vaginal	0.03 (-0.27; 0.33)	0.857	0.08 (-0.22; 0.38)	0.606
Planned Caesarean	0.06 (-0.18; 0.29)	0.624	-0.06 (-0.29; 0.18)	0.635
Emergency Caesarean	-0.07 (-0.31; 0.18)	0.589	-0.21 (-0.46; 0.04)	0.105

Time points for adjusted model = 6,195. Mixed-effects linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

S6-6 Table. Mode of birth and body mass index for infants born pre-term.

BMI	Coef (95% CI)	p-value	AdjCoef (95% CI)**	p-value
Normal vaginal	reference		reference	
Assisted vaginal	0.15 (-0.25; 0.55)	0.453	0.12 (-0.28; 0.53)	0.552
Planned Caesarean	0.24 (-0.09; 0.57)	0.147	-0.04 (-0.39; 0.30)	0.816
Emergency Caesarean	-0.33 (-0.55; -0.11)	0.003	-0.12 (-0.36; 0.12)	0.331

Time points for adjusted model = 5,161. Mixed-effects linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex, birth weight, smoking, gestational age – omitted because of collinearity, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

S6-7 Table. Mode of birth and body mass index for male infants.

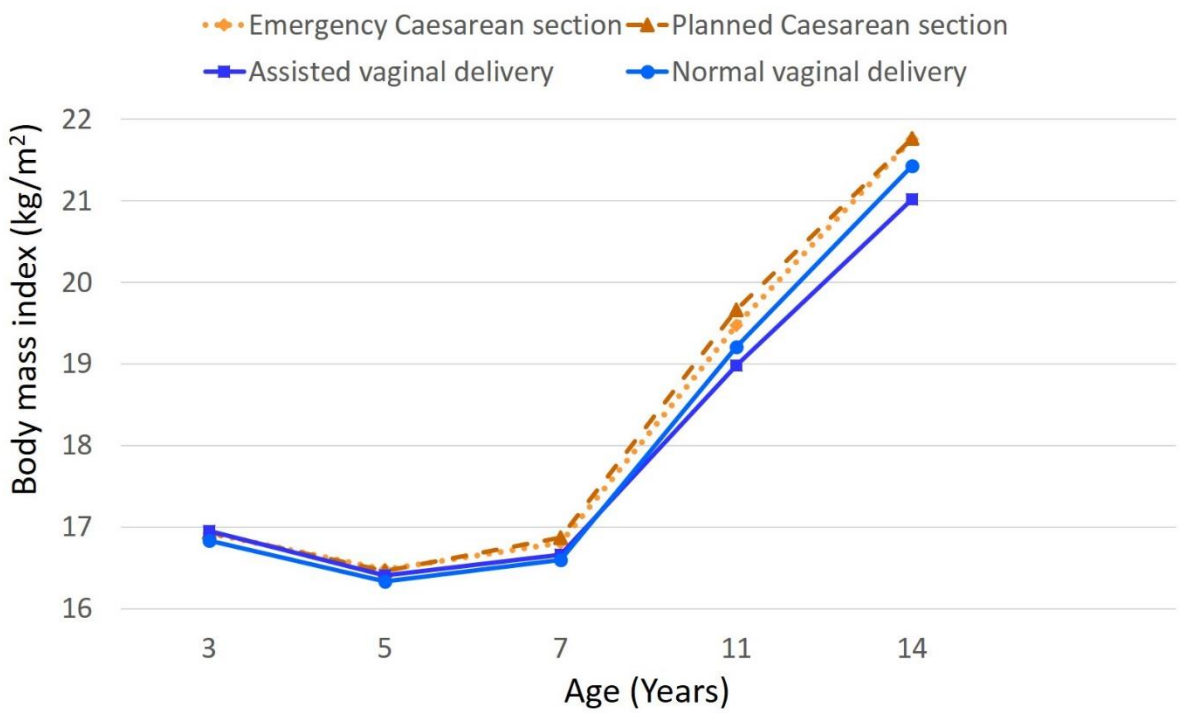
BMI	Coef (95% CI)	p-value	AdjCoef (95% CI)**	p-value
Normal vaginal	reference		reference	
Assisted vaginal	-0.05 (-0.18; 0.08)	0.410	-0.02 (-0.15; 0.11)	0.721
Planned Caesarean	0.29 (0.15; 0.43)	0.000	-0.08 (-0.06; 0.22)	0.282
Emergency Caesarean	0.23 (0.11; 0.35)	0.000	0.10 (-0.03; 0.23)	0.131

Time points for adjusted model = 25,041. Mixed-effects linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex – omitted because of collinearity, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).

S6-8 Table. Mode of birth and body mass index for female infants.

BMI	Coef (95% CI)	p-value	AdjCoef (95% CI)**	p-value
Normal vaginal	reference		reference	
Assisted vaginal	-0.08 (-0.23; 0.07)	0.291	-0.22 (-0.15; 0.11)	0.721
Planned Caesarean	0.09 (-0.06; 0.23)	0.237	-0.09 (-0.06; 0.22)	0.282
Emergency Caesarean	0.16 (0.02; 0.29)	0.024	0.10 (-0.03; 0.23)	0.131

Time points for adjusted model = 25,041. Mixed-effects linear regression. BMI – Body mass index, Coef (Coefficient), CI (Confidence intervals), Adj (Adjusted).
**Adjusted for maternal age, ethnicity education, marital status, couple income, infant sex – omitted because of collinearity, birth weight, smoking, gestational age, diabetes mellitus, parity, pre-pregnancy BMI (Non-macrosomic infants).



S1 Fig. Mean body mass index by birth mode from age three to fourteen years.

Chapter 7: Discussion and Conclusions

7.1 Overall synthesis

This doctoral thesis aimed to investigate the association between CS birth and childhood obesity using data from four longitudinal cohort studies (three large contemporary, nationally representative, prospective longitudinal cohort studies plus one smaller hospital-based cohort). Detailed phenotypic data from these studies was analysed to investigate this potential association. The thesis presents the findings from the analysis of each individual cohort study adding to the body of knowledge concerning the association between CS and childhood obesity and comprises a series of four interlinked papers published in peer-reviewed journals. A fifth published peer-reviewed journal article, albeit on a different topic in perinatal health, emanating from a PhD module enabled the acquisition of transferable skills on the process of conducting, critically evaluating and disseminating systematic reviews and meta-analyses [242].

To recap, the line of inquiry followed for this thesis involved justifying the need for this research. Concomitant increasing rates of CS delivery and childhood obesity merited this research, since correlation does not mean causation. Next was reflecting on potential biologic mechanisms that could link CS birth and childhood obesity. Thereafter identifying gaps in previous literature when answering the question if CS delivery was a determinant of childhood obesity was the logical step. Notable gaps such as failure to distinguish between elective and emergency CS and not adjusting for pre-pregnancy BMI were identified, including the use of disparate statistical analytic techniques. In order to address the research gaps and to harmonise the analyses when answering the research question, data from four contemporary prospective cohorts was used. In addition, this thesis contributed to addressing the problematic issue of publication bias favouring positive effects, as negative studies from some of the cohorts were still published.

The hallmark finding of the thesis was an association between CS birth in general, elective CS in particular, and childhood obesity during the first two years of life. This association had diminished by age three through to fourteen. This may suggest a transient role of the

vaginal microflora or other mechanisms operating in the perinatal and early childhood period related to the genesis of obesity. Whether this association remerges in adulthood or is a risk factor for cardiometabolic disease is an area for future research. The association observed with emergency CS is possibly due to confounding by the underlying reasons for CS, confounding by indication.

7.2 Main findings

Screening for Pregnancy Endpoints (SCOPE) and Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE) cohorts: At two months of age, children born by CS, had a similar BF% to those born vaginally. At age six months, children born by CS had a significantly higher BMI but this did not persist into future childhood, at age five years. There was no evidence to support an association between mode of delivery and long-term risk of obesity in the child.

Growing Up in Ireland (GUI) cohort: We found insufficient evidence to support a relationship between elective CS and childhood obesity at age three and five years. An increased risk of obesity in children born by emergency CS, but not elective, suggests that the influence of vaginal microflora in developing childhood obesity was minimal. The association with emergency CS was likely due to its indications.

Growing Up in New Zealand (GUiNZ) cohort: Planned CS was an independent predictor of obesity in early childhood at age two years but this association was not present by four and a half years. This suggests that differential exposure to vaginal microflora by birth mode may influence postnatal growth, at least in the short term. This association occurred during a critical phase of human development, the first two years of life, and might result in long-term detrimental cardiometabolic changes.

Millennium Cohort Study (MCS) cohort: Infants born by planned CS did not have a significantly higher BMI at ages three, five, seven, eleven and fourteen years or BF% at ages seven and fourteen compared to those born by normal VD. This may suggest that the

association, described in the literature, could be due to the indications/reasons for CS birth or residual confounding.

7.3 Strengths and limitations

The prospective longitudinal cohort design of the studies was a key strength that allowed for a clear temporal chain from birth mode to childhood obesity. Measurement error was minimized because all researchers were trained and used validated instruments and techniques to collect the study data. The nationally representative nature of the three main cohorts helped to safeguard the external validity, or in other words, the degree to which the conclusions of the studies could be applied to the broader population. However, differential attrition of cohort participants posed challenges, particularly with missing outcome data that this engendered. The impact of this missing follow-up data was allayed by a statistically principled approach, multiple imputation [162, 165], where this data was assumed to be missing at random.

Large sample sizes, often exceeding 5000 participants, increased the power of our studies. However some subgroup analyses may have lacked sufficient power, but consistency of the results suggests their value. With the large sample size it was possible to also investigate the association between delivery mode and transition into or out of obesity in early childhood, which very few studies have been able to do [137]. Long follow-up to age fourteen and having multiple time points in early childhood, for some cohorts, permitted greater insight. Another strength of the thesis was that childhood overweight and obesity were classified in the same way across the cohorts, according to the sex and age specific International Obesity Task Force criteria [136, 150, 151]. In prior literature, these were the most utilised criteria [28] due to their suitability for population studies [152]. We also explored the World Health Organization references in two cohorts [153]. The overall results with both BMI classification systems were essentially the same. This was not surprising because BMI has the same inherent characteristics, as an adiposity measure, regardless of which system is used to classify it [154].

Having data on body fat proportion for one hospital-based and for one nationally representative cohort study added value to the analyses that were able to be carried out.

This was because although BMI is a widely used, accepted and practical measure of obesity, its sensitivity in children is about 73% with a high specificity [243]. There was nevertheless concordance between results from BMI and BF% as described in the literature [184], giving further credibility to our results.

A key advantage of the cohorts was the ability to analyse CS subgroups separately, elective and emergency CS, which has been recommended as a way to increase the clarity of results in this research field [122]. Although this subgrouping of CS birth was a strength, it was not possible to determine from the data if a CS was purely on maternal request or if membranes were ruptured. The latter circumstance would have a bearing on the main study hypothesis because extensive exposure of infants to vaginal microbes might have occurred prior to CS birth. Having a sizable suite of potential confounder variables like gestational diabetes and other detailed phenotypic data, which a few previous studies have had [28] bolstered our interrogation.

Unavailability of maternal pre-pregnancy BMI has limited prior studies [28]. Its availability for the New Zealand and UK cohorts bolstered the robustness of our results. However the Irish cohorts did not have this variable [137, 138]. Instead good proxies for pre-pregnancy BMI, namely gestational weight gain and BMI at 15 week's gestation were on hand, allowing our Irish results to have some merit [208]. It is worth mentioning that the cohorts that we used were not specifically designed to address the research question. This meant focal variables like history of previous CS were not available, which may have led to residual confounding.

One's birth month can be consequential for future health [209]. Recruitment of participants during every month of the year, in three of our four cohorts, meant one could assess for potential confounding by birth month. Although participant recruitment occurred only during half of the year for one of our cohorts [137], another cohort from a hospital-based study in the same country recruited participants during the whole year [138]. This helped to account, in part, for seasonal effects in that country.

Although the distinction between CS into elective and emergency was important, this classification system had its limits. It was for example not possible to determine if an elective CS was purely on maternal request; these can be dissimilar from other elective CSs.

It has been shown that women who request CS have a higher burden of psychiatric disease [63]. Improving CS classification is an international effort that is in progress [62, 64].

It would have been worthwhile to determine if the signals of a positive association, that we observed in the SCOPE-BASELINE and GUiNZ cohorts persisted during sibling control analysis. However the primary cohort data, as alluded to earlier, was not designed with our research question in mind – sibling data was not available. Nonetheless one of the largest studies to include a sibling control analysis found no association between elective CS birth and development of obesity, albeit in males aged just above eighteen years [244]. Sibling control studies are based on the fact that siblings share half their genome and a significant proportion of their family context [245]. These shared genetic and environmental factors reduce potential confounding substantially, when compared to the potential confounding in population studies of unrelated individuals. Siblings that are discordant for an exposure of interest, like their mode of birth, are compared and any difference in outcome is more likely to be attributable to the exposure.

Administration of antibiotics and anaesthesia before and during CS birth may be related to development of the outcome, however this has not been supported by evidence [182, 241].

Obstetric studies have, in general, been constrained by limited data available for fathers. The individual papers and consequently the thesis were also limited in this way because fathers are known to contribute to children becoming obese [237]. Thus adjusting for paternal factors, like their BMI prior to their partner's pregnancy would have been apt. The spectre of recall bias hung over birth mode for the MCS and GUI studies. This variable, in these particular cohorts, depended on mothers remembering their birth mode nine months post-partum. It has been demonstrated that such long-term maternal recall is about 98% accurate [200]. This laid the spectre of recall bias, in this instance, to rest.

In light of the SDH framework [65], the lack of full socio economic status variables and adjustment is a potential issue given their association with both perinatal and childhood outcomes. The exact indications for CS delivery, history of previous CS were not available. The possibility of residual confounding remains from, for example, not considering paternal BMI.

In our case, combining the results of our nationally representative studies would not change them materially because, for instance, only one study had data at age two years. Therefore, the positive association observed at this age would persist.

Sub-group analyses were conducted (e.g. maternal age, preterm birth - < 37 weeks, infant sex). There was no material change in our results. There was nevertheless a trend towards infants with mothers older than thirty-five years and were delivered by planned/elective CS being more likely to have childhood obesity at age three years.

Using bioelectric impedance which underestimates BF% [240], Azcorra and colleagues found that CS birth was associated with increased levels of childhood adiposity in girls but not boys [121]. Because their study had a small sample size of 256, was not nationally representative, did not adjustment for pre-pregnancy BMI and did not distinguish between elective and non-elective CS birth their finding may have been spurious. Although not reaching statistical significance, we too reported a tendency towards girls being obese compared to boys [137]. This is consistent with data from CS birth mouse animal models which indicated a stronger weight gain phenotype in female mice [45]. Thus the results reported by Azcorra *et al* signal a continued relevant focus of investigation.

7.4 Public health and clinical implications

The results of this thesis have public health and clinical implications. These results will be of interest to women, their partners, families, public health policy makers and clinicians. The CS rate and prevalence of childhood obesity are increasing. Given the association between them, it is important to characterise it. From a synthesis of our and prior work, the effect size of the association appears to be small, relative risk < 1.50, and concentrated during the first two years of life. In addition to the just mentioned groups, dissemination of these results to those conducting and publishing similar epidemiologic research is warranted. On a broader level, Caesarean section birth remains major abdominal surgery with maternal and neonatal complications. Our study also demonstrates the public health and clinical utility of conducting robust prospective longitudinal cohort studies.

7.5 Future directions

A recent direct microbial study showed that the gut microflora of those born by CS and VD gradually becomes more and more similar as infants grow older [33]. Our studies were consistent with this observation in that BMI was observed to become more and more similar between those born by CS and VD as children grew older. Further triangulation of direct microbial and animal studies with observational epidemiologic studies will be elucidatory [74].

With respect to observational studies, there is potential to improve consistency and robustness in this research field by better and standardised definition particularly of the exposure, CS birth. A consistent signal, during the first two years of life, suggests a greater focus on this time period by future studies, given the developmental origins of health and disease paradigm whereby early life environmental perturbations affect health not only across the life span, but across generations [246]. This raises scope for tracking health outcomes across generations.

A systematic review and meta-analysis, investigating the link between CS birth and childhood obesity, which includes only prospective longitudinal cohort studies that differentiated between elective and emergency CS is warranted. Specific consideration of studies which included a sibling control analysis is also warranted. Furthermore, to the best of our knowledge, meta-regression is yet to be applied to this research topic; it would yield valuable insights [247]. Individual participant data meta-analysis which allows data from various cohorts to be analysed using the same statistical approach would also yield valuable insights [248]. Alternative techniques like use of propensity score matching, to handle confounding, and growth trajectories instead of BMI categories could be explored [249]. By using these alternative approaches, the overall results and interpretation would not be expected to change, however nuances would be revealed.

Bradford Hill criteria, a useful causal inference framework for epidemiologic studies, when applied to our and previous studies suggest a potential causal relationship between CS birth and early childhood obesity [250], however these criteria cannot prove causality. A temporal relationship, biologic plausibility, coherence with laboratory studies, and some degree of consistency among studies are some of the criteria that have motivated for clinical trials. In the absence of trials randomising pregnant women, there are randomised

clinical trials where infants born by CS are swabbed with their mother's vaginal microbes, vaginal seeding, and are then compared with a control group of infants that were not swabbed. These trials are under way in at least four countries [251]. The outcomes of interest in these studies include childhood obesity as well as development of allergic conditions. The results from these clinical trials would more definitively settle the question of causality.

7.5 Conclusion

This thesis adds robust data from three nationally representative and one hospital-based prospective cohort studies on the association between birth mode and childhood obesity. An association between CS birth in general, elective CS in particular, and childhood obesity during the first two years of life was the main finding. This association had dissipated by age three through to fourteen. This supports a potential transient role of the vaginal microflora and/or other mechanisms during early childhood in the genesis of obesity. Whether this association remerges in adulthood or is a risk factor for cardiometabolic disease is an area for future research. The association observed with emergency CS is possibly due to confounding by the underlying reasons for CS, confounding by indication, therefore long-term follow-up for possible sequelae is essential**.** Ongoing and future randomised clinical trials will elucidate the causal nature, if any of the association.

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Appendices

Appendix 1. PhD-related papers

Portable Document Format (PDF) versions of PhD-related papers can be found by using the following Digital Object Identifier's (DOI's):

1. DOI: 10.14712/18059694.2017.94
2. DOI: 10.1038/s41598-018-33482-z
3. DOI: 10.1136/bmjopen-2018-025051
4. DOI: 10.1136/jech-2019-212591
5. DOI: 10.1371/journal.pone.0223856

Appendix 2. A measurement tool to assess systematic reviews

Criterion (Yes, No, Can't answer, not applicable)	Keag OE *et al* [2018] [15]	Kuhle S *et al* [2015] [28]	Sutharsan R *et al* [2015] [30]	Li HT *et al* [2013] [27]
1. Was an 'a priori' design provided? The research question and inclusion criteria should be established before the conduct of the review. Note: Need to refer to a protocol, ethics approval, or pre-determined/a priori published research objectives to score a "yes."	Yes	No	No	No
2. Was there duplicate study selection and data extraction? There should be at least two independent data extractors and a consensus procedure for disagreements should be in place. Note: 2 people do study selection, 2 people do data extraction, consensus process or one person checks the other's work.	Yes	Yes	Yes	Yes
3. Was a comprehensive literature search performed? At least two electronic sources should be searched. The report must include years and databases used (e.g., Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found. Note: If at least 2 sources + one supplementary strategy used, select "yes" (Cochrane register/Central counts as 2 sources; a grey literature search counts as supplementary).	Yes	Yes	Yes	Yes
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	No	No	No	No

The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc. Note: If review indicates that there was a search for “grey literature” or “unpublished literature,” indicate “yes.” SIGLE database, dissertations, conference proceedings, and trial registries are all considered grey for this purpose. If searching a source that contains both grey and non-grey, must specify that they were searching for grey/unpublished lit.				
5. Was a list of studies (included and excluded) provided? A list of included and excluded studies should be provided. Note: Acceptable if the excluded studies are referenced. If there is an electronic link to the list but the link is dead, select “no.”	No	No	No	No
6. Were the characteristics of the included studies provided? In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analyzed e.g., age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported. Note: Acceptable if not in table format as long as they are described as above.	Yes	Yes	Yes	Yes
7. Was the scientific quality of the included studies assessed and documented? 'A priori' methods of assessment should be provided (e.g., for effectiveness studies if the author(s) chose to include only randomized, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant. Note: Can include use of a quality scoring tool or checklist, e.g., Jadad scale, risk of bias, sensitivity analysis, etc., or a description of quality items, with some kind of result for EACH study (“low” or “high” is fine, as long as it is clear which studies scored “low” and which scored “high”; a summary score/range for all studies is not acceptable).	Yes	Yes	Yes	Yes
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	Yes	Yes	Yes

The results of the methodological rigor and scientific quality should be considered in the analysis and the conclusions of the review, and explicitly stated in formulating recommendations. Note: Might say something such as “the results should be interpreted with caution due to poor quality of included studies.” Cannot score “yes” for this question if scored “no” for question 7.				
9. Were the methods used to combine the findings of studies appropriate? For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e., Chi-squared test for homogeneity, I2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e., is it sensible to combine?). Note: Indicate “yes” if they mention or describe heterogeneity, i.e., if they explain that they cannot pool because of heterogeneity/variability between interventions.	Yes	Yes	Yes	Yes
10. Was the likelihood of publication bias assessed? An assessment of publication bias should include a combination of graphical aids (e.g., funnel plot, other available tests) and/or statistical tests (e.g., Egger regression test, Hedges-Olken). Note: If no test values or funnel plot included, score “no”. Score “yes” if mentions that publication bias could not be assessed because there were fewer than 10 included studies.	No	Yes	Yes	Yes
11. Was the conflict of interest included? Potential sources of support should be clearly acknowledged in both the systematic review and the included studies. Note: To get a “yes,” must indicate source of funding or support for the systematic review AND for each of the included studies.	Yes	Yes	Yes	Yes

Appendix 3. Search terms to retrieve systematic reviews and newly published papers

PubMed search terms - (Caesarean* OR Cesarean* OR Abdominal Deliveries OR Abdominal Delivery OR C-Section* OR C Section*) AND (obesity* OR Obesities OR overweight* OR adipose OR adiposity) with Filter – Review. Search date – 7 April 2020.
343 articles retrieved

PubMed search terms - (Caesarean* OR Cesarean* OR Abdominal Deliveries OR Abdominal Delivery OR C-Section* OR C Section*) AND (obesity* OR Obesities OR overweight* OR adipose OR adiposity) with Filter from 1 April to 2017 to 31 December 2020. Search date – 26 March 2020.
708 articles retrieved

Scopus search terms - ALL (Caesarean OR Cesarean OR Abdominal Deliveries OR Abdominal Delivery OR C-Section OR C Section) AND (obesity* OR Obesities OR overweight* OR adipose OR adiposity)

ALL (caesarean OR cesarean OR abdominal AND deliveries OR abdominal AND delivery OR c-section OR c AND section) AND (obesity* OR obesities OR overweight* OR adipose OR adiposity) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017))

Childhood was defined as < 18 years old

Date of viva voce – 26 March 2020