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Mode of delivery at birth and the metabolic syndrome in mid-life: the role of the birth environment.

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Running title: Caesarean delivery and the metabolic syndrome

Key words: caesarean delivery, metabolic syndrome, emergency caesarean, lifecourse, 1958 birth cohort,

Abbreviations;

MS: Metabolic syndrome

PROM: Premature rupture of the membranes

Word count: 2581

ABSTRACT

Background: The aim of this study is to examine the hypothesis that mode of delivery at birth may be associated with metabolic disorders in adult mid-life. **Methods**: The National Child Development Study consists of individuals born during one week in 1958 in Great Britain. Respondents with biomedical data on the metabolic syndrome at age 45 were included. The metabolic syndrome was defined based on the NCEP-ATP III classification. **Results**: 7156 were born naturally, among the caesarean births 106 were non elective and 85 were elective caesareans. The metabolic syndrome is present in 37.7% of those born by non elective caesareans, 25.9% of those born by elective caesarean and 27.5% of those born by vaginal delivery. In a multivariate logistic regression model adjusted for antenatal factors, birth history, mother's characteristics and the socioeconomic environment at birth, only birth by non elective caesarean remained associated with the metabolic syndrome in adulthood compared to vaginal (OR 1.51, 95% CI 1.00-2.30). Mother's obesity (OR 1.61, 95% CI 1.12-2.34) and low maternal education level (OR 1.47, 95% CI 1.30-1.67) were also independently associated with mid-life metabolic syndrome. **Conclusion**: Birth by non elective caesarean in 1958 may be associated with metabolic syndrome in adulthood after adjusting for prior confounding factors. We suggest that the birth context of non-elective caesareans in 1958 is suggestive of a 'foetal stress' mechanism affecting health across the lifecourse.

Strengths and limitations

-Being born by non-elective caesarean in 1958 may be associated with the metabolic syndrome in mid-life.

-Mode of delivery may be a proxy for the birth environment and contextually variable clinical practices.

-Given the possible context of non-elective caesareans in 1958, a 'foetal stress' hypothesis is suggested for the subsequent association with the metabolic syndrome.

-It is possible that an unknown confounding factor during early life was omitted from the analyses, which might explain differences observed in MS outcome between the mode-of-delivery groups

INTRODUCTION

In recent years a number of studies have suggested that the mode of delivery at birth may be associated with obesity and metabolic disruption across the lifecourse. This stems from epidemiological research showing associations between birth by caesarean section and obesity in childhood(1, 2). Such associations deserve further investigation given, on the one hand, the dramatic increases in caesarean sections in recent decades, from 21% in 1996 to 32% in 2007(3), and on the other hand, the burden of morbidity due to metabolic diseases(4). The hypothesised mechanism for this association involves the colonisation of the gut microbiota(5).

Animal models have shown that modifications to rat gut microbiota have lead to metabolic disruptions and ultimately obesity in affected animals(6). The gut microbiota is a potential source of inflammatory molecules that may contribute to metabolic diseases(7, 8). This possible link between gut microbiota and metabolic disruptions is relevant to mode of delivery at birth due to the colonisation of the gut flora that occurs when the baby ingests maternal vaginal flora as s/he passes along the birth canal. If a caesarean section is carried out to deliver the baby, this phase of birth is skipped, and the baby is not exposed to the vaginal flora. The colonisation of their digestive tract therefore occurs differently to a baby who was delivered naturally(9, 10, 11). Recent reports have linked differences in infant gut microbiota with subsequent obesity(12).

To explore the hypothesis that mode of delivery may be associated with metabolic disruptions, it is important to consider the context surrounding the pregnancy, birth, and where possible, variations in the mode of delivery. Caesarean sections have become part of routine practice in maternity wards, often planned well in advance in the case of at-risk

pregnancies(13). However, the practice of caesareans was not so commonplace up to three decades ago, and individuals born under rather different practices and clinical conditions are now in their forties and fifties. In this paper we use an historical birth cohort study to explore the possible association between mode of delivery at birth and the occurrence of the metabolic syndrome in mid-life (45 years) under different contextual circumstances surrounding birth. Study participants delivered naturally, born via planned caesarean and via unplanned caesarean will be compared in terms of their metabolic syndrome profile at the age of 45 years using available biomedical data from a birth cohort study.

METHODS

Sample and participants

This study used data from the 1958 National Child Development Study (NCDS) which included all births during one week in 1958 (n= 18558) in Great Britain. Subsequent data collections were carried out on cohort members aged 7, 11, 16, 23, 33, 42, 46 and 50. The NCDS has been described in detail elsewhere(14). A biomedical survey (9377 cohort members participating) was conducted when participants were aged 44-46 years. (Figure 1):

Ethics

Written informed consent was obtained from the cohort member's parents for childhood measurements and ethical approval for the adult data collection was obtained from the National Research Ethics Advisory Panel. NCDS data are open access datasets available to non-profit research organisations.

Outcome measure

The MS was defined using NCEP-ATP III (National Cholesterol Education Program Adult Treatment Panel III) clinical criteria except for plasma glucose which was not recorded and replaced by glycated haemoglobin (HbA1c) ≥ 6.5%(15).

Exposure variable

Mode of delivery was categorized into three groups: non-elective caesarean, elective caesarean or vaginal delivery.

Covariates

The variables taken into account covered four areas:

i) Mother's socioeconomic and health characteristics before the current pregnancy: Mother's educational level (left school before/ after minimum leaving age); household overcrowding (people per room); mother's pre-pregnancy weight and her height measured after the birth were used to construct the mother's pre-pregnancy BMI (weight in kg/(height in m)²). Since some mothers were younger than 18 years of age, age-specific BMI cut-offs were used in order to categorize BMI into 4 groups: thinness, normal, overweight and obese (corresponding to the cut-offs of <18.5 kg/m², 18.5-24.9 kg/m², 25.0-29.9 kg/m² and ≥ 30.0 kg/m² for adults respectively). Mother's parity in 1958, including miscarriages after 28 weeks, was also extracted.

ii) Previous pregnancy complications: previous pregnancy problems (yes/no), constructed based on whether the mother had previously had: an abortion or ectopic pregnancy; previous stillbirths; a previous neonatal death; or other previous pregnancy complications.

iii) Information on the current pregnancy: maternal age at birth; whether the mother smoked during pregnancy beyond the fourth month (yes/ no); abnormality during pregnancy (none/ at least one abnormality including: Antepartum haemorrhage, placenta praevia, vaginal bleeding, and other abnormalities); hypertensive pathology (none/ hypertension/ toxemia/ proteinuria/ eclampsia); and total number of antenatal visits (<5 visits, 5-9 visits, >9 visits).

iv) Details of the labour and birth: time elapsed since rupture of membranes (≥12 hours before delivery ie. premature rupture of the membranes (PROM)/ <12hours before delivery): whether labour was induced (yes/no); birth weight for gestation (<10th percentile, 10-90th percentile, >90th percentile); gestational age was calculated as the duration between the first day of the mother's last menstrual period and childbirth, and categorized into groups (<38 weeks, 38 weeks, 39-41 weeks, >41 weeks).

Statistical analyses

We first determined the prevalence of MS, and used the chi-squared test to assess whether this prevalence differed by mode of delivery. The covariates were summarized as frequencies and percentages for categorical variables, means and standard deviations (SD) for continuous variables. Chi-square or Fisher's exact tests were performed in order to compare the sample characteristics according to the exposure or the outcome. Comparisons of means by mode of delivery category were computed using variance analysis (ANOVA), whereas the comparisons of means by MS status were carried out using the Student's t-test, after validating assumptions of normality and homoscedasticity.

Unadjusted and adjusted logistic regression models were carried out to explore the relationship between MS and mode of delivery. Both complete case and multiple imputation analyses were conducted.

To control for possible bias due to missing data, we imputed data for covariates with missing data using the multiple imputation program ICE in STATA v11(16). For more details see the supplementary data.

RESULTS

Among 7347 observations, the prevalence of the metabolic syndrome was 27.6 % (36.6% for males, 18.6% for females p<0.001). In total, 191 cohort members (2.6% of the sample) were delivered by caesarean section (106 non-elective, 85 elective caesarean sections). The prevalence of the MS in the non-elective and in the elective caesarean were 37.7% (95% CI: 28.5-47.0%) and 25.9% (95%CI: 16.5% to 35.3%) respectively. The estimated prevalence of MS was 27.5% (95%CI: 26.5% to 28.5%) in the vaginal delivery group (p-value comparing the prevalence in the three groups=0.061).

Sample characteristics according to mode of delivery are reported in Table 1. Several maternal characteristics, parity, problems during previous pregnancies, abnormalities during the current pregnancy, induced labour, premature rupture of membranes (PROM) and gestational age were highly associated with the cohort member's mode of delivery at birth (p<0.001). Specifically, older maternal age at birth, nulliparous mothers, induced labour, PROM and overdue birth (> 41 weeks) were more frequent in the non-elective caesarean section group. On the other hand, problems during previous pregnancies (past stillbirth and neonatal deaths and past complications of pregnancy), abnormality during pregnancy and premature birth (<38 weeks) were more frequent in the elective caesarean delivery group. Previous caesarean was also a strong predictor of elective caesarean delivery (data not shown).

Table 2 shows the relationships between mode of delivery, the covariates and MS. A low maternal level of education, smoking after the 4th month of pregnancy and maternal obesity were associated with a higher prevalence of MS. We also found significant links between MS and the following: hypertensive pathology, induced labour, and PROM.

In Table 3 we report unadjusted (model 1.) and adjusted (model 2.) odds ratios (OR) resulting from the final logistic regression model. Model 2. is adjusted for the effects of cohort member's gender, maternal age, mother educational level, smoking habits during pregnancy, BMI, parity, problems with previous pregnancies, hypertensive pathology during pregnancy, birth weight, gestational age, induction of the labour and PROM, (Table 3 - model 2). The results show that non-elective caesarean delivery was associated with an increased proportion of MS compared to vaginal delivery (OR=1.51, p=0.05 results from imputed data).

An increased probability of having MS in mid-life was also associated with: being male, a lower maternal education level, maternal smoking in pregnancy, maternal pre-pregnancy obesity, and maternal hypertensive pathology during pregnancy. Respondents whose mothers were younger at the time of their birth were less likely to have MS in mid-adulthood.

DISCUSSION

These analyses show that differences in the prevalence of the MS at 45 years may be associated with the mode of delivery at birth, after controlling for possible confounders. However, the association observed is not for respondents born by caesarean section overall versus those born vaginally. Rather, non-elective caesarean delivery remained associated with an increased risk of having MS in mid-life compared to individuals born vaginally. These findings differ from studies carried out on cohorts of individuals born more recently showing that caesarean section *per se* is a risk for metabolic disorders via the gut microbiota hypothesis(1, 11). The results from our study using data from births in 1958 suggest that mode of delivery may be a proxy variable for qualities in the birth environment that may have had long term implications for cohort members' health. We suggest that in 1958 caesarean sections were a rare phenomenon (2.6% prevalence), carried out electively in the case of high-

risk pregnancies. When caesareans were non-elective we hypothesise that the birth context was most likely stressful, resulting in an emergency caesarean section. The stressful nature of the birth may play an important part in the observed association between non-elective caesarean births and the metabolic syndrome in mid-life.

Factors occurring prior to, and at the time of birth may contribute to the association between non-elective caesareans and the subsequent development of MS. Based on the information from the NCDS, the women who had non elective caesareans in 1958 were more likely to be overdue, to have had an induced labour and to have broken their waters more than 12 hours before the birth. Such births seem to have gradually become emergency situations presumably after a long labour, with 97% of babies born thus described as having experienced "foetal distress" by the duty midwife (data not shown). Abnormalities occurring at birth as indicated by an induced labour or a late delivery were more frequent in the non-elective caesarean section group. Historically, the main reasons given by clinicians for carrying out caesareans, other than having previously had a caesarean, are the relatively undefined concepts of "foetal distress", "failure to progress" during labour, and breech presentations(17). Given the rare occurrence of caesareans in the late 1950s, we can only speculate that the conditions surrounding a labour ending in a non-elective caesarean were likely to have been fraught and stressful for those involved, not least for the baby. We put forward a foetal stress hypothesis, whereby babies born by non-elective caesarean were subject to physiological stress, and possibly their mother's psychological stress and its consequences in the post-natal period. Such a context of stress may have affected the baby's physiological and psychological stress responses thereafter. Early life stress has been associated with physiological alterations leading individuals along negative health

trajectories(18, 19). Furthermore, in later life a stressful environment, such as job stress, has been associated with a greater prevalence of MS(20).

The colonisation of the gut microbiota during vaginal births, and the lack thereof during caesarean births, has been put forward as a hypothesis for links observed in previous studies on caesarean delivery and metabolic disorders in childhood or adulthood(1). Some authors have reported that mode of delivery is associated with a differential colonisation of the gut flora. Higher proportions of bacteria from the the *Firmicutes* group, and a lower frequency of members of the *Bacteroidetes* group have been observed in children delivered by caesarean section compared to those born vaginally(11). Moreover, infants born by caesarean delivery were significantly less often colonized with bacteria of the *Bacteroides fragilis* group than vaginally delivered infants and these sub-groups represent the majority of the microbiota found in the adult gut(5, 21).

To support this hypothesis we would have observed differences in the prevalence of MS between caesareans per se and vaginal birth, however this was not the case. No association was observed between overall caesarean section in 1958 and MS in mid life. Differences between the gut microbiota of individuals born by non-elective caesarean section versus those delivered vaginally but not in those born by elective caesarean section have been reported. A lower frequency of *Escherichia-Shigella* has been observed in other studies for those born by non-elective caesarean(22). We cannot exclude that babies born by non-elective caesarean were more likely to experience a prolonged exposure to vaginal bacteria and possibly to infectious pathogens, due to the PROM (44% of non-elective caesareans exposed to PROM, versus 15% vaginal delivery and 0% elective caesareans). Mode of delivery has previously been associated with childhood obesity at 3 years of age and the authors postulated

that a longer exposure to bacterial flora could be a mechanism involved in childhood obesity(1).

We reported that maternal pre-pregnancy obesity was associated with an increased probability of having the metabolic syndrome at the modal age of 45 years. Previous work has shown that children exposed to maternal obesity in early life had a twofold increased risk of developing MS, with a trend toward a higher incidence of insulin resistance(23). Offspring exposed to maternal hyperglycaemia during their intrauterine development were also more prone to metabolic disorders in young adulthood leading to insulin resistance(24, 25). Different paths of childhood growth with smaller gains in BMI during infancy could precede the development of metabolic syndrome or hypertension(26, 27).

There are a number of limitations to our study. The definition of MS proposed by different organisations has varied over the past decade. The prevalence of MS is lower when using definitions other than ATPIII, however, the risk of cardiovascular events, diabetes mellitus and hypertension are similar for ATPIII and AHA or IDF definitions(28). Glycaemia was not recorded in the cohort study biomedical survey; therefore we used the HbA1c value with a cut-off above 6.5% to define hyperglycaemia. HbA1c has been defined as a marker to identify diabetes status(29). The reliability of glucose measurements varies widely across laboratories and may result in misclassification of >12% of patients (30). By contrast, HbA1c values are relatively stable after collection(31). The NCDS cohort provides a rare opportunity to study conditions and characteristics at birth and in early life collected prospectively, in relation to good quality biological data sampled in mid-life. It was therefore possible to include a large number of potential confounding variables in the statistical models, however it

is possible that a key unknown confounding factor during early life was omitted, which might explain differences observed in MS outcome between the mode-of-delivery groups.

CONCLUSION

These findings suggest that mode-of-delivery at birth may be an important variable to take into account to understand the aetiology of metabolic disorders. It is likely to represent factors occurring in the environment proximal to the birth which may have an impact on the baby's health across the lifecourse. Our findings show that in 1958, non-elective caesarean sections may be associated with an increased prevalence of the MS in mid-life. We suggest that given the maternity practices of the time, physiological stress experienced by the baby during delivery may be an important mechanism in the subsequent development of metabolic disorders.

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Author contributions

BDB, VE and MKI were involved in the conception and design of the study, analysing and interpreting the data, drafted the manuscript and made modifications. BDB and VE contributed equally to the work. TL, CD and BC analysed and interpreted the analyses, and revised the manuscript. All authors approved the current version.

REFERENCES

1- **Huh SY**, Rifas-Shiman SL, Zera CA, *et al.* Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. *Arch Dis Child* 2012;**97**:610-16.doi: 10.1136/archdischild-2011-301141. Epub 2012 May 23.

2- **Zhou L**, He G, Zhang J, *et al*. Risk factors of obesity in preschool children in an urban area in China. *Eur J Pediatr* 2011;**170**:1401-6.doi: 10.1007/s00431-011-1416-7. Epub 2011 Mar 2.

3- **Menacker F**, Hamilton BE. Recent trends in cesarean delivery in the United States. *NCHS Data Brief* 2010;**35:**1-8.

4- **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*. 2002;**287**:356-9.

5- **Ley RE.** Turnbaugh PJ, Klein S, *et al*. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**:1022-3.

6- **Bäckhed F**, Ding, Wang T, Hooper LV, *et al*.. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;**101:**15718-23. Epub 2004 Oct 25.

7- **Cani PD**, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;**56:**1761-72.

8- **Karlsson FH**, Tremaroli V, Nookaew I, *et al*. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*.2013;**498**:99-103.doi: 10.1038/nature12198. Epub 2013 May 29.

9- **Biasucci G,** Benedati B, Morelli L, Bessi E, Boehm G. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr* 2008;**138**: 1796S-1800S

10- **Salminen S,** Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 2004; **53**:1388-9

11- **Grönlund MM**, Lehtonen OP, Eerola E *et al*. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after caesarean delivery. *J Pediatr Gastroenterol Nutr*1999;**28**:19-25.

12- **Luoto R**, Kalliomäki M, Laitinen K, *et al.* Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastroenterol Nutr* 2011;**52**:90-5.doi: 10.1097/MPG.0b013e3181f3457f.

13- **Zhang J**, Troendle J, Reddy UM, *et al*. Contemporary cesarean delivery practice in the United States. *Am J Obstet Gynecol* 2010;**203**:326.e1-326.e10.doi: 10.1016/j.ajog.2010.06.058. Epub 2010 Aug 12.

14- **Power C**, Elliott J. Cohort profile: 1958 British Birth Cohort (National Child Development Study). *Int J Epidemiol* 2006;**35**:34-41.

15- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486-97.

16- **Royston P**. Multiple imputation of missing values: Further update of ice, with an emphasis on categorical variables. *Stata Journal* 2009;**9**:466-77.

17- **[No authors listed]** Holding back the tide of caesareans. *BMJ.* 1988;**297**:852

18- **Alastalo H**, von Bonsdorff MB, Räikkönen K, *et al*. Early Life Stress and Physical and Psychosocial Functioning in Late Adulthood. *PLoS ONE*. 2013;8:e69011 doi: 10.1371/journal.pone.0069011

19- **Kelly-Irving M**, Lepage B, Dedieu D, *et al*. Adverse childhood experiences and premature all-cause mortality. *Eur J Epidemiol*. 2013:1-14 doi: 10.1007/s10654-013-9832-9

20- **Chandola T**, Brunner E, Marmot M. Chronic stress at work and the metabolic syndrome: prospective study. *BMJ* 2006;**332**:521-5

21- **Bennet R,** Nord CE. Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. *Infection* 1987;**15**:332-6.

22- Azad MB, Konya T, Maughan H, *et al*. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013;**185**:385-94. doi: 10.1503/cmaj.121189. Epub 2013 Feb 11.

23- **Boney CM**, Verma A, Tucker R, *et al*. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;**115**: e290-6.

24- **Dabelea D**, Mayer-Davis EJ, Lamichhane AP, *et al*. Association of Intrauterine Exposure to Maternal Diabetes and Obesity with Type 2 Diabetes in Youth: the SEARCH Case-Control Study. *Diabetes Care* 2008;**31**:1422-6.

25- **Hillier TA**, Pedula KL, Schmidt MM, *et al*. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007;**30**:2287-92.

26- **Salonen MK,** Kajantie E, Osmond C, *et al*. Childhood growth and future risk of the metabolic syndrome in normal-weight men and women. *Diabetes Metab* 2009;**35**:143-50.

27- **Eriksson JG**, Forsen TJ, Kajantie E, *et al*. Childhood growth and hypertension in later life. *Hypertension* 2007;**49:**1415-21.

28- **Mancia G**, Bombelli M, Facchetti R, *et al.* Impact of different definitions of the metabolic syndrome on the prevalence of organ damage, cardiometabolic risk and cardiovascular events. *J Hypertens* 2010;**28**:999-1006.doi: 10.1097/HJH.0b013e328337a9e3.

29- **International Expert committee**. International Expert committee report on the role of the AIC assay in the diagnosis of diabetes. *Diabetes Care* 2009;**32**:1327-34.

30- **Miller WG**, Myers GL, Ashwood ER, *et al*. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. *Arch Pathol Lab Med* 2008;**132**:838-46. doi: 10.1043/1543-2165(2008)132[838:SOTAIT]2.0.CO;2.

31- **Little RR**, Rohlfing CL, Tennill AL, *et al*. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. *Diabetes Technol Ther* 2007; **9**:36-42.

Figure 1. Flow chart showing the sample selection

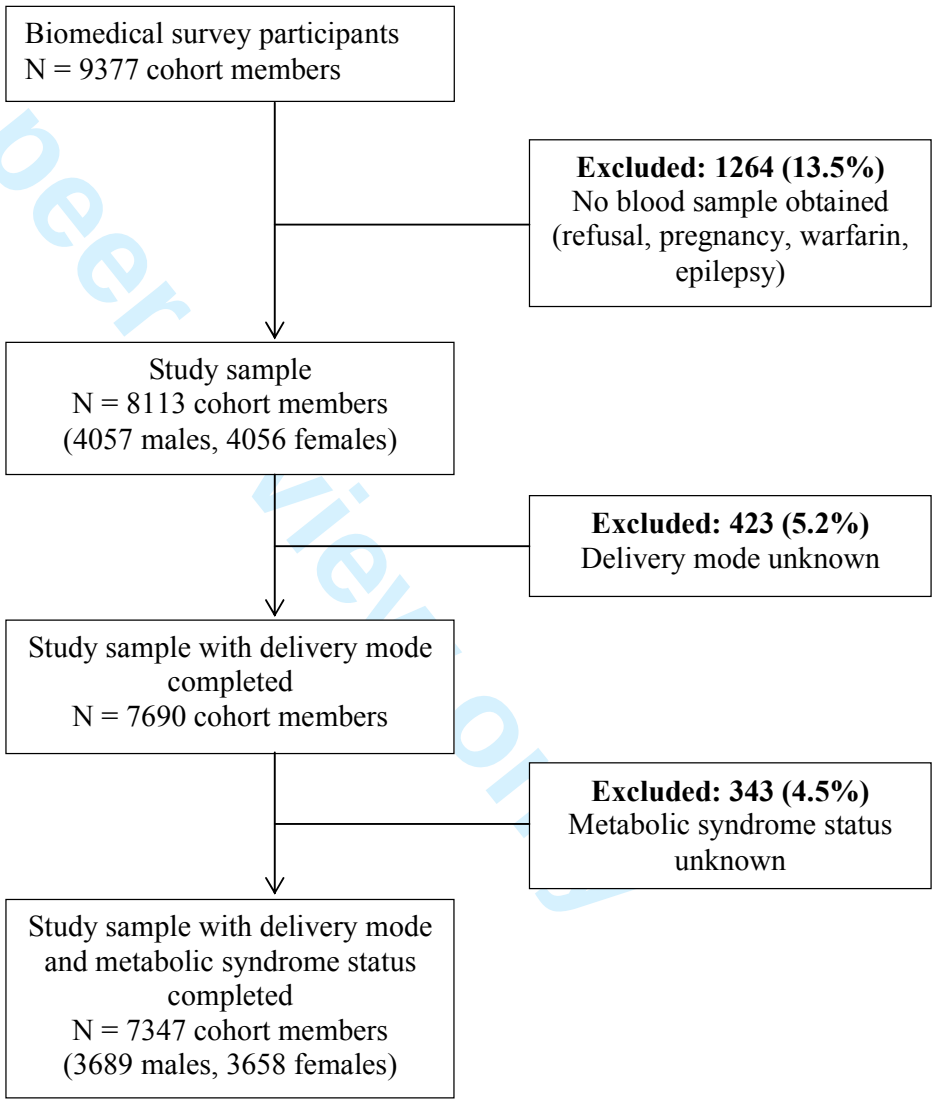


Table 1. Description of covariates in terms of mode of delivery

Variable	Vaginal delivery (N=7156) n (%)	Non-elective caesarean (N=106) n (%)	Elective caesarean (N=85) n (%)	P-value Chi-square
Cohort member's gender				0.156
male	3580 (50.0)	61 (57.6)	48 (56.5)	
female	3576 (50.0)	45 (42.4)	37 (43.5)	
Maternal age				< 0.001 F
<18 years	69 (1.0)	0 (0.0)	0 (0.0)	
18-35 years	6414 (89.7)	87 (82.1)	63 (74.1)	
>35 years	668 (9.3)	19 (17.9)	22 (25.9)	
Mother educational level				0.045
Low	5224 (73.2)	69 (65.7)	54 (64.3)	
High	1913 (26.8)	36 (34.2)	30 (35.7)	
Overcrowding				0.049
1 to 1.5 people per room	6140 (88.1)	96 (96.0)	73 (89.0)	
≥1.5 people per room	832 (11.9)	4 (4.0)	9 (11.0)	
Smoking after the 4th month of pregnancy				0.589
No	4766 (67.4)	74 (71.8)	55 (65.5)	
Yes	2303 (32.6)	29 (28.2)	29 (34.5)	
Maternal BMI before pregnancy				0.075 F
Underweight	305 (4.5)	3 (3.1)	4 (5.3)	
Normal weight	4915 (72.6)	69 (71.1)	48 (63.2)	
Overweight	1293 (19.1)	18 (18.6)	16 (21.0)	
Obesity	262 (3.8)	7 (7.2)	8 (10.5)	
Parity				< 0.001
Nulliparous	2636 (36.9)	67 (63.2)	20 (23.5)	
1 previous pregnancy	2269 (31.7)	20 (18.9)	32 (37.7)	
≥2 previous pregnancies	2250 (31.4)	19 (17.9)	33 (38.8)	
Problems with previous pregnancies				< 0.001
No	5638 (78.9)	73 (68.9)	36 (42.4)	
Yes	1504 (21.1)	33 (31.1)	49 (57.6)	
Hypertensive pathology during pregnancy				0.002
No	4654 (68.3)	59 (62.1)	41 (51.3)	
Yes	2164 (31.7)	36 (37.9)	39 (48.7)	
Abnormality during pregnancy				< 0.001
No	5358 (74.9)	50 (47.2)	23 (27.1)	
Yes	1794 (25.1)	56 (52.8)	62 (72.9)	
Total number of antenatal visits				0.240 F
< 5	295 (4.2)	2 (1.9)	4 (4.8)	
5-9 visits	1912 (27.1)	25 (24.3)	30 (36.1)	
> 9	4859 (68.7)	76 (73.8)	49 (59.2)	
Birthweight				0.392
<10th percentile	525 (8.4)	4 (4.8)	6 (8.5)	
10-90th percentile	5094 (81.7)	67 (79.8)	59 (83.1)	

>90th percentile	619 (9.9)	13 (15.4)	6 (8.4)	
Gestational age				< 0.001
<38 weeks	770 (9.8)	14 (12.1)	25 (25.5)	
38 weeks	610 (7.8)	15 (12.9)	23 (23.5)	
39-41 weeks	5504 (70.4)	59 (50.9)	45 (45.9)	
>41 weeks	935 (12.0)	28 (24.1)	5 (5.1)	
Whether labour induced				< 0.001
No	6242 (87.2)	68 (64.2)	81 (95.3)	
Yes	914 (12.8)	38 (35.8)	4 (4.7)	
PROM> 12h				< 0.001
No	5599 (85.0)	55 (55.6)	83 (100.0)	
Yes	985 (15.0)	44 (44.4)	0 (0.0)	

F: Fisher's exact test; PROM: premature rupture of the membranes

Table 2. Characteristics of mothers and cohort members before pregnancy, during pregnancy and labour, and at birth in terms of the metabolic syndrome at age 44-46y (n=7347)

Variable	Metabolic syndrome No (N=5317) n (%)	Metabolic syndrome Yes (N=2030) n (%)	P-value Chi-square
Mode of delivery			0.061
Vaginal	5188 (72.5)	1968 (27.5)	
Non-elective caesarean	66 (62.3)	40 (37.7)	
Elective caesarean	63 (74.1)	22 (25.9)	
Cohort member's gender			< 0.001
male	2339 (63.4)	1350 (36.6)	
female	2978 (81.4)	680 (18.6)	
Maternal age			0.166
<18 years	44 (63.8)	25 (36.2)	
18-35 years	4743 (72.3)	1821 (27.7)	
>35 years	525 (74.0)	184 (26.0)	
Mother educational level			< 0.001
Low	3751 (70.2)	1596 (29.8)	
High	1549 (78.3)	430 (21.7)	
Overcrowding			0.330
1- 1.5 people per room	4573 (72.5)	1736 (27.5)	
≥1.5 people per room	599 (70.9)	246 (29.1)	
Smoking after the 4th month of pregnancy			< 0.001
No	3606 (73.7)	1289 (26.3)	
Yes	1643 (69.6)	718 (30.4)	
Maternal BMI before pregnancy			< 0.001
Underweight	230 (73.7)	82 (26.3)	
Normal weight	3738 (74.3)	1294 (25.7)	
Overweight	902 (68.0)	425 (32.0)	
Obesity	171 (61.7)	106 (38.3)	
Parity			0.089
Nulliparous	1938 (71.2)	785 (28.8)	
1 previous pregnancy	1716 (73.9)	605 (26.1)	
≥2 previous pregnancies	1662 (72.2)	640 (27.8)	
Problems with previous pregnancies			0.136
No	4886 (72.6)	1843 (27.4)	
Yes	398 (69.7)	173 (30.3)	
Hypertensive pathology during pregnancy			< 0.001
No	3498 (73.6)	1256 (26.4)	
Yes	1555 (69.5)	684 (30.5)	
Abnormality during pregnancy			0.554
No	3941 (72.6)	1490 (27.4)	
Yes	1374 (71.9)	538 (28.1)	
Total number of antenatal visits			0.163
< 5	230 (76.4)	71 (23.6)	
5-9 visits	1436 (73.0)	531 (27.0)	
> 9	3579 (71.8)	1405 (28.2)	
Birthweight			0.252
<10th percentile	374 (69.9)	161 (30.1)	

10-90th percentile	3807 (72.9)	1413 (27.1)	
>90th percentile	472 (74.0)	166 (26.0)	
Gestational age			0.268
<38 weeks	463 (70.3)	196 (29.7)	
38 weeks	383 (70.9)	157 (29.1)	
39-41 weeks	3414 (73.3)	1246 (26.7)	
>41 weeks	567 (71.7)	224 (28.3)	
Whether labour induced			0.002
No	4666 (73.0)	1725 (27.0)	
Yes	651 (68.1)	305 (31.9)	
PROM			0.003
No	4203 (73.3)	1534 (26.7)	
Yes	708 (68.8)	321 (31.2)	

F: Fisher's exact test; PROM: premature rupture of the membranes

Table 3. Unadjusted and adjusted logistic regression models showing relationship between mode of delivery and the metabolic syndrome in mid-life: complete case analyses and analyses using multiply imputed data

Model 1: Unadjusted	Complete case OR [95% IC]	Multiple imputations OR [95% IC]
Mode of delivery		
Vaginal (ref)		
Non-elective caesarean	1.60 (1.42-1.80)***	1.60 (1.08-2.37)*
Elective caesarean	0.92 (0.74-1.07)	0.92 (0.57-1.50)
Model 2: Adjusted [a]		
Mode of delivery		
Vaginal (ref)		
Non-elective caesarean	2.18 (1.28-3.71)**	1.51 (1.00-2.30)*
Elective caesarean	1.00 (0.53-1.91)	0.93 (0.56-1.56)
Cohort member's gender		
Female (ref)		
Male	2.48 (2.18-2.82)***	2.58 (2.31-2.87)***
Maternal age		
Years	0.98 (0.97-0.99)**	0.98 (0.97-0.99)***
Mother educational level		
High (ref)		
Low	1.48 (1.27-1.73)***	1.47 (1.30-1.67)***
Smoking after the 4th month of pregnancy		
No (ref)		
Yes	1.28 (1.11-1.46)**	1.24 (1.10-1.39)***
Maternal BMI before pregnancy		
Normal weight (ref)		
Underweight	0.87 (0.64-1.20)	0.97 (0.74-1.27)
Overweight	1.19 (0.85-1.67)	1.33 (0.99-1.78)
Obesity	1.45 (0.94-2.24)	1.61 (1.12-2.34)**
Hypertensive pathology during pregnancy		
No (ref)		
Yes	1.24 (1.08-1.43)**	1.18 (1.05-1.33)**

*p≤0.05 **p≤0.01 ***p≤0.001

[a] also adjusted for: overcrowding, parity, previous pregnancy problems, total number of antenatal visits, birthweight, gestational age, induced labour, PROM: premature rupture of the membranes.

Supplementary material

Definition of the metabolic syndrome

The MS was defined using NCEP-ATP III (National Cholesterol Education Program Adult Treatment Panel III) clinical criteria except for including: abdominal obesity (increased waist circumference: >102 cm for men; >88 cm for women), raised triglycerides (≥1.50g/L (≥1.69 mmol/L), reduced HDL cholesterol (<40 mg/dL for (men) (<1.04 mmol/L), <50 mg/dL for women (<1.29 mmol/L), elevated blood pressure (BP ≥130 and/or ≥85 mm Hg), and raised plasma glucose. If three out of the five listed characteristics were present, a diagnosis of metabolic syndrome was made. In our analyses, BP was calculated as the average of three readings. Waist circumference was considered only if the measurement was noted by the cohort team as being "reliable". Plasma glucose which was not recorded was replaced by Glycated haemoglobin (HbA1c) ≥ 6.5%.

Statistical analyses

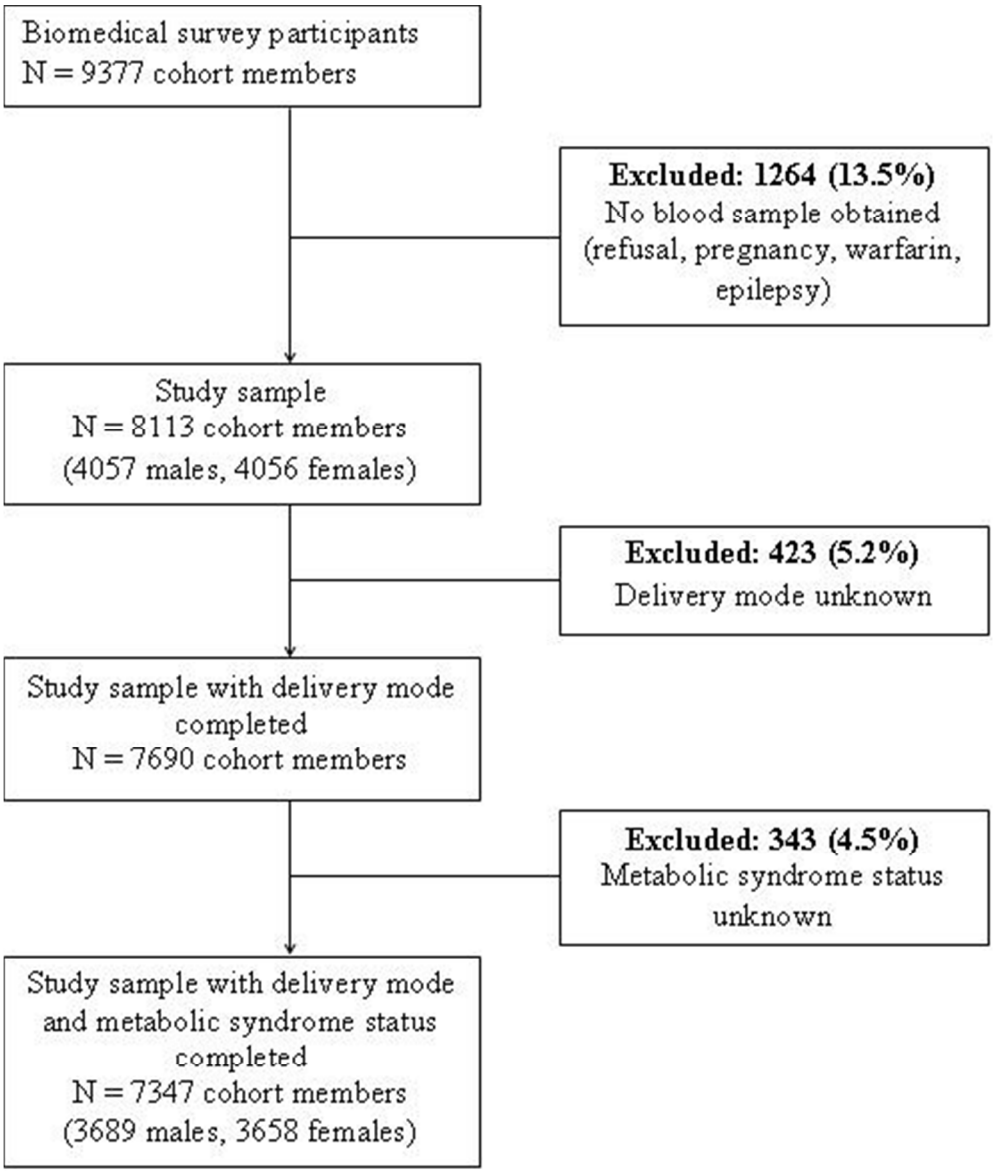
The imputation model included the exposure variables and covariates used in the logistic regression models, as well as other correlated variables which were likely to improve the imputation model but that were not used in analyses: marital status of the mother, socio-economic group of the mother's father, multiple pregnancy. The outcome variable was omitted from the imputation model. Ten dataset imputations were run. The logistic regression models were carried out on the imputed data in order to decrease the potential bias of our estimated ORs and increase analytical power. Standard errors were calculated using Rubin's rules (1). P-values ≤0.05 were considered as statistically significant. Maternal age of birth was treated as a continuous variable, as a fractional polynomial model showed there was no evidence that the change in incidence over time was not linear (2).

Analyses of missing data

Having missing data or not for the covariates was analysed in terms of mode of delivery and in terms of the outcome measure. Missing data for mother's BMI before pregnancy was associated with being born by planned caesarean (10.8% vs 8.7% and 5.4% p=0.03); Missing data for toxaemia was associated with being born by unplanned caesarean (10.6% vs 6.0% and 4.8% p=0.029). Missing data for birthweight was associated with unplanned caesarean birth (20.19% versus 16.9% and 12.8% p=0.05) and with having the MS (14.4% vs 12.5% p=0.029). Missing data for PROM was associated with a vaginal birth (5.9% versus 4.8% and

0% p=0.035) and with having the MS (6.6% s 5.4 p=0.049). No clear pattern emerges from these analyses of missing data that suggests a systematic bias, except perhaps for persons with data missing for birthweight which was associated with both unplanned caesarean birth and the metabolic syndrome. However, the analyses using multiple imputations would adjust for this possible bias.

1. Rubin D. Multiple imputation for nonresponse in surveys. New York: Wiley, 1987 j'ai trouvé ça sur pubmed: Mehrotra DV, Li X, Liu J, Lu K. Analysis of longitudinal clinical trials with missing data using multiple imputation in conjunction with robust regression. Biometrics 2012 Dec;68(4):1250-9.
2. Royston P, Altman DG. Using fractional polynomials to model curved relationships, Stata Technical Bulletin 1994; 21:11-24



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Mode of delivery at birth and the metabolic syndrome in mid-life: the role of the birth environment in a prospective birth cohort study.

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Abbreviations;

MS: Metabolic syndrome

PROM: Premature rupture of the membranes

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ABSTRACT

Objectives: The aim of this study is to examine the hypothesis that mode of delivery at birth may be associated with metabolic disorders in adult mid-life.

Setting: Population cohort study

Participants: The National Child Development Study consists of individuals born during one week in 1958 in Great Britain. Respondents with biomedical data on the metabolic syndrome at age 45 were included.

Outcome measure: The metabolic syndrome was defined based on the NCEP-ATP III classification.

Results: 7156 were born naturally, among the caesarean births 106 were non elective and 85 were elective caesareans. The metabolic syndrome is present in 37.7% of those born by non elective caesareans, 25.9% of those born by elective caesarean and 27.5% of those born by vaginal delivery. In a multivariate logistic regression model adjusted for antenatal factors, birth history, mother's characteristics and the socioeconomic environment at birth, only birth by non elective caesarean remained associated with the metabolic syndrome in adulthood compared to vaginal (OR 1.51, 95% CI 1.00-2.30). Mother's obesity (OR 1.61, 95% CI 1.12-2.34) and low maternal education level (OR 1.47, 95% CI 1.30-1.67) were also independently associated with mid-life metabolic syndrome.

Conclusion: Birth by non elective caesarean in 1958 may be associated with metabolic syndrome in adulthood after adjusting for prior confounding factors. We suggest that the birth context of emergency caesareans in 1958 is suggestive of a 'foetal stress' mechanism affecting health across the lifecourse.

Strengths and limitations

-Being born by emergency caesarean in 1958 may be associated with the metabolic syndrome in mid-life.

-Mode of delivery may be a proxy for the birth environment and contextually variable clinical practices.

-Given the possible context of emergency caesareans in 1958, a 'foetal stress' hypothesis is suggested for the subsequent association with the metabolic syndrome.

-It is possible that an unknown confounding factor during early life was omitted from the analyses, which might explain differences observed in MS outcome between the mode-of-delivery groups

INTRODUCTION

In recent years a number of studies have suggested that the mode of delivery at birth may be associated with obesity and metabolic disruption across the lifecourse. This stems from epidemiological research showing associations between birth by caesarean section and obesity in childhood(1, 2). Such associations deserve further investigation given, on the one hand, the dramatic increases in caesarean sections in recent decades, from 21% in 1996 to 32% in 2007(3), and on the other hand, the burden of morbidity due to metabolic diseases(4). The hypothesised mechanism for this association involves the colonisation of the gut microbiota(5).

Animal models have shown that modifications to rat gut microbiota have lead to metabolic disruptions and ultimately obesity in affected animals(6). The gut microbiota is a potential source of inflammatory molecules that may contribute to metabolic diseases(7, 8). This possible link between gut microbiota and metabolic disruptions is relevant to mode of delivery at birth due to the colonisation of the gut flora that occurs when the baby ingests maternal vaginal flora as s/he passes along the birth canal. If a caesarean section is carried out to deliver the baby, this phase of birth is skipped, and the baby is not exposed to the vaginal flora. The colonisation of their digestive tract therefore occurs differently to a baby who was delivered naturally(9, 10, 11). Recent reports have linked differences in infant gut microbiota with subsequent obesity(12).

To explore the hypothesis that mode of delivery may be associated with metabolic disruptions, it is important to consider the context surrounding the pregnancy, birth, and where possible, variations in the mode of delivery. Caesarean sections have become part of routine practice in maternity wards, often planned well in advance in the case of at-risk

pregnancies(13). However, the practice of caesareans was not so commonplace up to three decades ago, and individuals born under rather different practices and clinical conditions are now in their forties and fifties. In this paper we use aprospective birth cohort study of individuals born in 1958 to explore the possible association between mode of delivery at birth and the occurrence of the metabolic syndrome (MS) in mid-life (45 years) under different contextual circumstances surrounding birth. Study participants delivered naturally, born via planned caesarean and via unplanned caesarean will be compared in terms of their metabolic syndrome profile at the age of 45 years using available biomedical data from a birth cohort study.

METHODS

Sample and participants

This study used data from the 1958 National Child Development Study (NCDS) which included all births during one week in 1958 (n= 18558) in Great Britain. Subsequent data collections were carried out on cohort members aged 7, 11, 16, 23, 33, 42, 46 and 50. The NCDS has been described in detail elsewhere(14). A biomedical survey (9377 cohort members participating) was conducted when participants were aged 44-46 years. (Figure 1):

Ethics

Written informed consent was obtained from the cohort member's parents for childhood measurements and ethical approval for the adult data collection was obtained from the National Research Ethics Advisory Panel. NCDS data are open access datasets available to non-profit research organisations.

Outcome measure

The MS was defined using NCEP-ATP III (National Cholesterol Education Program Adult Treatment Panel III) clinical criteria except for plasma glucose which was not recorded and replaced by glycated haemoglobin (HbA1c) ≥ 6.5%(15).

Exposure variable

Mode of delivery was categorized into three groups: emergency caesarean, elective caesarean or vaginal delivery.

Covariates

The variables taken into account covered four areas:

i) Mother's socioeconomic and health characteristics before the current pregnancy: Mother's educational level (left school before/ after minimum leaving age); household overcrowding (people per room); mother's self-reported pre-pregnancy weight and her height measured after the birth were used to construct the mother's pre-pregnancy BMI (weight in kg/(height in m)^2). Since some mothers were younger than 18 years of age, age-specific BMI cut-offs were used in order to categorize BMI into 4 groups: thinness, normal, overweight and obese (corresponding to the cut-offs of <18.5 kg/m^2, 18.5-24.9 kg/m^2, 25.0-29.9 kg/m^2 and ≥ 30.0 kg/m^2 for adults respectively). Mother's parity in 1958, including miscarriages after 28 weeks, was also extracted.

ii) Previous pregnancy complications: previous pregnancy problems (yes/no), constructed based on whether the mother had previously had: an abortion or ectopic pregnancy; previous stillbirths; a previous neonatal death; or other previous pregnancy complications.

iii) Information on the current pregnancy: maternal age at birth; whether the mother smoked during pregnancy beyond the fourth month (yes/ no); abnormality during pregnancy (none/ at least one abnormality including: Antepartum haemorrhage, placenta praevia, vaginal bleeding, and other abnormalities); hypertensive pathology (none/ hypertension/ toxemia/ proteinuria/ eclampsia); and total number of antenatal visits (<5 visits, 5-9 visits, >9 visits).

iv) Details of the labour and birth: time elapsed since rupture of membranes (≥12 hours before delivery ie. premature rupture of the membranes (PROM)/ <12hours before delivery): whether labour was induced (yes/no); birth weight for gestation (<10th percentile, 10-90th percentile, >90th percentile); gestational age was calculated as the duration between the first day of the mother's last menstrual period and childbirth, and categorized into groups (<38 weeks, 38 weeks, 39-41 weeks, >41 weeks).

Statistical analyses

We first determined the prevalence of MS, and used the chi-squared test to assess whether this prevalence differed by mode of delivery. The covariates were summarized as frequencies and percentages for categorical variables, means and standard deviations (SD) for continuous variables. Chi-square or Fisher's exact tests were performed in order to compare the sample characteristics according to the exposure or the outcome. Comparisons of means by mode of delivery category were computed using variance analysis (ANOVA), whereas the comparisons of means by MS status were carried out using the Student's t-test, after validating assumptions of normality and homoscedasticity.

Unadjusted and adjusted logistic regression models were carried out to explore the relationship between MS and mode of delivery. Both complete case and multiple imputation analyses were conducted.

To control for possible bias due to missing data, we imputed data for covariates with missing data using the multiple imputation program ICE in STATA v11(16). For more details see the supplementary data.

RESULTS

Among 7347 observations, the prevalence of the metabolic syndrome was 27.6 % (36.6% for males, 18.6% for females p<0.001). In total, 191 cohort members (2.6% of the sample) were delivered by caesarean section (106 emergency, 85 elective caesarean sections). The prevalence of the MS in the emergency and in the elective caesarean were 37.7% (95% CI: 28.5-47.0%) and 25.9% (95%CI: 16.5% to 35.3%) respectively. The estimated prevalence of MS was 27.5% (95%CI: 27.2% to 27.8%) within the vaginal delivery group, 37.7% (95%CI: 34.9% to 40.5%) within the emergency caesarean group, and 25.9% (95%CI: 23.1-28.7) within the elective caesarean group (global chi-squared test p =0.061).

Sample characteristics according to mode of delivery are reported in Table 1. Several maternal characteristics, parity, problems during previous pregnancies, abnormalities during the current pregnancy, induced labour, premature rupture of membranes (PROM) and gestational age were highly associated with the cohort member's mode of delivery at birth (p<0.001). Specifically, older maternal age at birth, nulliparous mothers, induced labour, PROM and overdue birth (> 41 weeks) were more frequent in the emergency caesarean section group. On the other hand, problems during previous pregnancies (past stillbirth and neonatal deaths and past complications of pregnancy), abnormality during pregnancy and premature birth (<38 weeks) were more frequent in the elective caesarean delivery group. Previous caesarean was also a strong predictor of elective caesarean delivery (data not shown).

Table 2 shows the relationships between mode of delivery, the covariates and MS. A low maternal level of education, smoking after the 4th month of pregnancy and maternal obesity were associated with a higher prevalence of MS. We also found significant links between MS and the following: hypertensive pathology, induced labour, and PROM.

In Table 3 we report unadjusted (model 1.) and adjusted (model 2.) odds ratios (OR) resulting from the final logistic regression model. Model 2. is adjusted for the effects of cohort member's gender, maternal age, mother educational level, smoking habits during pregnancy, BMI, parity, problems with previous pregnancies, hypertensive pathology during pregnancy, birth weight, gestational age, induction of the labour and PROM, (Table 3 - model 2). The results show that emergency caesarean delivery was associated with an increased proportion of MS compared to vaginal delivery (OR=1.51, p=0.05 results from imputed data).

An increased probability of having MS in mid-life was also associated with: being male, a lower maternal education level, maternal smoking in pregnancy, maternal pre-pregnancy obesity, and maternal hypertensive pathology during pregnancy. Respondents whose mothers were younger at the time of their birth were less likely to have MS in mid-adulthood.

DISCUSSION

These analyses show that differences in the prevalence of the MS at 45 years may be associated with the mode of delivery at birth, after controlling for possible confounders. However, the association observed is not for respondents born by caesarean section overall versus those born vaginally. Rather, emergency caesarean delivery remained associated with an increased risk of having MS in mid-life compared to individuals born vaginally. These findings differ from studies carried out on cohorts of individuals born more recently showing that caesarean section *per se* is a risk for metabolic disorders via the gut microbiota hypothesis(1, 11). The results from our study using data from births in 1958 suggest that mode of delivery may be a proxy variable for qualities in the birth environment that may have had long term implications for cohort members' health. We suggest that in 1958 caesarean

sections were a rare phenomenon (2.6% prevalence), carried out electively in the case of high-risk pregnancies. When caesareans were emergency we hypothesise that the birth context was most likely stressful, resulting in an emergency caesarean section. The stressful nature of the birth may play an important part in the observed association between emergency caesarean births and the metabolic syndrome in mid-life.

Factors occurring prior to, and at the time of birth may contribute to the association between emergency caesareans and the subsequent development of MS. Based on the information from the NCDS, the women who had non elective caesareans in 1958 were more likely to be overdue, to have had an induced labour and to have experienced rupture of the membranes more than 12 hours before the birth. Such births seem to have gradually become emergency situations presumably after a long labour, with 97% of babies born thus described as having experienced "foetal distress" by the duty midwife (data not shown). Abnormalities occurring at birth as indicated by an induced labour or a late delivery were more frequent in the emergency caesarean section group. Historically, the main reasons given by clinicians for carrying out caesareans, other than having previously had a caesarean, are the relatively undefined concepts of "foetal distress", "failure to progress" during labour, and breech presentations(17). Given the rare occurrence of caesareans in the late 1950s, we can only speculate that the conditions surrounding a labour ending in a emergency caesarean were likely to have been fraught and stressful for those involved, not least for the baby. We put forward a foetal stress hypothesis, whereby babies born by emergency caesarean were subject to physiological stress, and possibly their mother's psychological stress and its consequences in the post-natal period. Such a context of stress may have affected the baby's physiological and psychological stress responses thereafter. Early life stress has been associated with physiological alterations leading individuals along negative health trajectories(18, 19).

Furthermore, in later life a stressful environment, such as job stress, has been associated with a greater prevalence of MS(20).

The colonisation of the gut microbiota during vaginal births, and the lack thereof during caesarean births, has been put forward as a hypothesis for links observed in previous studies on caesarean delivery and metabolic disorders in childhood or adulthood(1). Some authors have reported that mode of delivery is associated with a differential colonisation of the gut flora. Higher proportions of bacteria from the the *Firmicutes* group, and a lower frequency of members of the *Bacteroidetes* group have been observed in children delivered by caesarean section compared to those born vaginally(11). Moreover, infants born by caesarean delivery were significantly less often colonized with bacteria of the *Bacteroides fragilis* group than vaginally delivered infants and these sub-groups represent the majority of the microbiota found in the adult gut(5, 21).

To support this hypothesis we would have observed differences in the prevalence of MS between caesareans per se and vaginal birth, however this was not the case. No association was observed between overall caesarean section in 1958 and MS in mid life. Differences between the gut microbiota of individuals born by emergency caesarean section versus those delivered vaginally but not in those born by elective caesarean section have been reported. A lower frequency of *Escherichia-Shigella* has been observed in other studies for those born by emergency caesarean(22). We cannot exclude that babies born by emergency caesarean were more likely to experience a prolonged exposure to vaginal bacteria and possibly to infectious pathogens, due to the PROM (44% of emergency caesareans exposed to PROM, versus 15% vaginal delivery and 0% elective caesareans). Mode of delivery has previously been associated with childhood obesity at 3 years of age and the authors postulated

that a longer exposure to bacterial flora could be a mechanism involved in childhood obesity(1).

We reported that maternal pre-pregnancy obesity was associated with an increased probability of having the metabolic syndrome at the modal age of 45 years. Previous work has shown that children exposed to maternal obesity in early life had a twofold increased risk of developing MS, with a trend toward a higher incidence of insulin resistance(23). Offspring exposed to maternal hyperglycaemia during their intrauterine development were also more prone to metabolic disorders in young adulthood leading to insulin resistance(24, 25). Different paths of childhood growth with smaller gains in BMI during infancy could precede the development of metabolic syndrome or hypertension(26, 27).

There are a number of limitations to our study. The definition of MS proposed by different organisations has varied over the past decade. The prevalence of MS is lower when using definitions other than ATPIII, however, the risk of cardiovascular events, diabetes mellitus and hypertension are similar for ATPIII and AHA or IDF definitions(28). Glycaemia was not recorded in the cohort study biomedical survey; therefore we used the HbA1c value with a cut-off above 6.5% to define hyperglycaemia. HbA1c has been defined as a marker to identify diabetes status(29). The reliability of glucose measurements varies widely across laboratories and may result in misclassification of >12% of patients (30). By contrast, HbA1c values are relatively stable after collection(31). The NCDS cohort provides a rare opportunity to study conditions and characteristics at birth and in early life collected prospectively, in relation to good quality biological data sampled in mid-life. Unfortunately no information was collected at the time about gestational diabetes, however, we include birthweight and variables on other pregnancy complications which may capture the effect of insulin resistance

during pregnancy. It was possible to include a large number of potential confounding variables in the statistical models, however it is possible that a key unknown confounding factor during early life was omitted, which might explain differences observed in MS outcome between the mode-of-delivery groups.

CONCLUSION

These findings suggest that mode-of-delivery at birth may be an important variable to take into account to understand the aetiology of metabolic disorders. It is likely to represent factors occurring in the environment proximal to the birth which may have an impact on the baby's health across the lifecourse. Our findings show that in 1958, emergency caesarean sections may be associated with an increased prevalence of the MS in mid-life. We suggest that given the maternity practices of the time, physiological stress experienced by the baby during delivery may be an important mechanism in the subsequent development of metabolic disorders.

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Author contributions

BDB, VE and MKI were involved in the conception and design of the study, analysing and interpreting the data, drafted the manuscript and made modifications. BDB and VE contributed equally to the work. TL, CD and BC analysed and interpreted the analyses, and revised the manuscript. All authors approved the current version.

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REFERENCES

1- **Huh SY**, Rifas-Shiman SL, Zera CA, *et al.* Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. *Arch Dis Child* 2012;**97**:610-16.doi: 10.1136/archdischild-2011-301141. Epub 2012 May 23.

2- **Zhou L**, He G, Zhang J, *et al*. Risk factors of obesity in preschool children in an urban area in China. *Eur J Pediatr* 2011;**170**:1401-6.doi: 10.1007/s00431-011-1416-7. Epub 2011 Mar 2.

3- **Menacker F**, Hamilton BE. Recent trends in cesarean delivery in the United States. *NCHS Data Brief* 2010;**35:**1-8.

4- **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*. 2002;**287**:356-9.

5- **Ley RE.** Turnbaugh PJ, Klein S, *et al*. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**:1022-3.

6- **Bäckhed F**, Ding, Wang T, Hooper LV, *et al*.. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;**101:**15718-23. Epub 2004 Oct 25.

7- **Cani PD**, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;**56:**1761-72.

8- **Karlsson FH**, Tremaroli V, Nookaew I, *et al*. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*.2013;**498**:99-103.doi: 10.1038/nature12198. Epub 2013 May 29.

9- **Biasucci G,** Benedati B, Morelli L, et al. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr* 2008;**138**: 1796S-1800S

10- **Salminen S,** Gibson GR, McCartney AL, et al. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 2004; **53**:1388-9

11- **Grönlund MM**, Lehtonen OP, Eerola E *et al*. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after caesarean delivery. *J Pediatr Gastroenterol Nutr*1999;**28**:19-25.

12- **Luoto R**, Kalliomäki M, Laitinen K, *et al.* Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastroenterol Nutr* 2011;**52**:90-5.doi: 10.1097/MPG.0b013e3181f3457f.

13- **Zhang J**, Troendle J, Reddy UM, *et al*. Contemporary cesarean delivery practice in the United States. *Am J Obstet Gynecol* 2010;**203**:326.e1-326.e10.doi: 10.1016/j.ajog.2010.06.058. Epub 2010 Aug 12.

14- **Power C**, Elliott J. Cohort profile: 1958 British Birth Cohort (National Child Development Study). *Int J Epidemiol* 2006;**35**:34-41.

15- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486-97.

16- **Royston P**. Multiple imputation of missing values: Further update of ice, with an emphasis on categorical variables. *Stata Journal* 2009;**9**:466-77.

17- **[No authors listed]** Holding back the tide of caesareans. *BMJ.* 1988;**297**:852

18- **Alastalo H**, von Bonsdorff MB, Räikkönen K, *et al*. Early Life Stress and Physical and Psychosocial Functioning in Late Adulthood. *PLoS ONE*. 2013;8:e69011 doi: 10.1371/journal.pone.0069011

19- **Kelly-Irving M**, Lepage B, Dedieu D, *et al*. Adverse childhood experiences and premature all-cause mortality. *Eur J Epidemiol*. 2013:1-14 doi: 10.1007/s10654-013-9832-9

20- **Chandola T**, Brunner E, Marmot M. Chronic stress at work and the metabolic syndrome: prospective study. *BMJ* 2006;**332**:521-5

21- **Bennet R,** Nord CE. Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. *Infection* 1987;**15**:332-6.

22- Azad MB, Konya T, Maughan H, *et al*. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013;**185**:385-94. doi: 10.1503/cmaj.121189. Epub 2013 Feb 11.

23- **Boney CM**, Verma A, Tucker R, *et al*. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;**115**: e290-6.

24- **Dabelea D**, Mayer-Davis EJ, Lamichhane AP, *et al*. Association of Intrauterine Exposure to Maternal Diabetes and Obesity with Type 2 Diabetes in Youth: the SEARCH Case-Control Study. *Diabetes Care* 2008;**31**:1422-6.

25- **Hillier TA**, Pedula KL, Schmidt MM, *et al*. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007;**30**:2287-92.

26- **Salonen MK,** Kajantie E, Osmond C, *et al*. Childhood growth and future risk of the metabolic syndrome in normal-weight men and women. *Diabetes Metab* 2009;**35**:143-50.

27- **Eriksson JG**, Forsen TJ, Kajantie E, *et al*. Childhood growth and hypertension in later life. *Hypertension* 2007;**49:**1415-21.

28- **Mancia G**, Bombelli M, Facchetti R, *et al.* Impact of different definitions of the metabolic syndrome on the prevalence of organ damage, cardiometabolic risk and cardiovascular events. *J Hypertens* 2010;**28**:999-1006.doi: 10.1097/HJH.0b013e328337a9e3.

29- **International Expert committee**. International Expert committee report on the role of the AIC assay in the diagnosis of diabetes. *Diabetes Care* 2009;**32**:1327-34.

30- **Miller WG**, Myers GL, Ashwood ER, *et al*. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. *Arch Pathol Lab Med* 2008;**132**:838-46. doi: 10.1043/1543-2165(2008)132[838:SOTAIT]2.0.CO;2.

31- **Little RR**, Rohlfing CL, Tennill AL, *et al*. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. *Diabetes Technol Ther* 2007; **9**:36-42.

Table 1. Description of covariates in terms of mode of delivery

Variable	Vaginal delivery (N=7156) n (%)	Emergency caesarean (N=106) n (%)	Elective caesarean (N=85) n (%)	P-value Chi-square
Cohort member's gender				0.156
male	3580 (50.0)	61 (57.6)	48 (56.5)	
female	3576 (50.0)	45 (42.4)	37 (43.5)	
Maternal age				< 0.001 F
<18 years	69 (1.0)	0 (0.0)	0 (0.0)	
18-35 years	6414 (89.7)	87 (82.1)	63 (74.1)	
>35 years	668 (9.3)	19 (17.9)	22 (25.9)	
Mother educational level				0.045
Low	5224 (73.2)	69 (65.7)	54 (64.3)	
High	1913 (26.8)	36 (34.2)	30 (35.7)	
Overcrowding				0.049
1 to 1.5 people per room	6140 (88.1)	96 (96.0)	73 (89.0)	
≥1.5 people per room	832 (11.9)	4 (4.0)	9 (11.0)	
Smoking after the 4th month of pregnancy				0.589
No	4766 (67.4)	74 (71.8)	55 (65.5)	
Yes	2303 (32.6)	29 (28.2)	29 (34.5)	
Maternal BMI before pregnancy				0.075 F
Underweight	305 (4.5)	3 (3.1)	4 (5.3)	
Normal weight	4915 (72.6)	69 (71.1)	48 (63.2)	
Overweight	1293 (19.1)	18 (18.6)	16 (21.0)	
Obesity	262 (3.8)	7 (7.2)	8 (10.5)	
Parity				< 0.001
Nulliparous	2636 (36.9)	67 (63.2)	20 (23.5)	
1 previous pregnancy	2269 (31.7)	20 (18.9)	32 (37.7)	
≥2 previous pregnancies	2250 (31.4)	19 (17.9)	33 (38.8)	
Problems with previous pregnancies				< 0.001
No	5638 (78.9)	73 (68.9)	36 (42.4)	
Yes	1504 (21.1)	33 (31.1)	49 (57.6)	
Hypertensive pathology during pregnancy				0.002
No	4654 (68.3)	59 (62.1)	41 (51.3)	
Yes	2164 (31.7)	36 (37.9)	39 (48.7)	
Abnormality during pregnancy				< 0.001
No	5358 (74.9)	50 (47.2)	23 (27.1)	
Yes	1794 (25.1)	56 (52.8)	62 (72.9)	
Total number of antenatal visits				0.240 F
< 5	295 (4.2)	2 (1.9)	4 (4.8)	
5-9 visits	1912 (27.1)	25 (24.3)	30 (36.1)	
> 9	4859 (68.7)	76 (73.8)	49 (59.2)	
Birthweight				0.392
<10th percentile	525 (8.4)	4 (4.8)	6 (8.5)	
10-90th percentile	5094 (81.7)	67 (79.8)	59 (83.1)	

>90th percentile	619 (9.9)	13 (15.4)	6 (8.4)	
Gestational age				< 0.001
<38 weeks	770 (9.8)	14 (12.1)	25 (25.5)	
38 weeks	610 (7.8)	15 (12.9)	23 (23.5)	
39-41 weeks	5504 (70.4)	59 (50.9)	45 (45.9)	
>41 weeks	935 (12.0)	28 (24.1)	5 (5.1)	
Whether labour induced				< 0.001
No	6242 (87.2)	68 (64.2)	81 (95.3)	
Yes	914 (12.8)	38 (35.8)	4 (4.7)	
PROM> 12h				< 0.001
No	5599 (85.0)	55 (55.6)	83 (100.0)	
Yes	985 (15.0)	44 (44.4)	0 (0.0)	

F: Fisher's exact test; PROM: premature rupture of the membranes

% may not add up exactly to 100.0 due to rounding

Table 2. Characteristics of mothers and cohort members before pregnancy, during pregnancy and labour, and at birth in terms of the metabolic syndrome at age 44-46y (n=7347)

Variable	Metabolic syndrome No (N=5317) n (%)	Metabolic syndrome Yes (N=2030) n (%)	P-value Chi-square
Mode of delivery			0.061
Vaginal	5188 (97.6)	1968 (97.0)	
Emergency caesarean	66 (1.2)	40 (2.0)	
Elective caesarean	63 (1.2)	22 (1.1)	
Cohort member's gender			< 0.001
male	2339 (44.0)	1350 (66.5)	
female	2978 (56.0)	680 (33.5)	
Maternal age			0.166
<18 years	44 (0.8)	25 (1.2)	
18-35 years	4743 (89.3)	1821 (89.7)	
>35 years	525 (9.9)	184 (9.1)	
Mother educational level			< 0.001
Low	3751 (70.8)	1596 (78.8)	
High	1549 (29.2)	430 (21.2)	
Overcrowding			0.330
1- 1.5 people per room	4573 (88.4)	1736 (87.6)	
≥1.5 people per room	599 (11.6)	246 (12.4)	
Smoking after the 4th month of pregnancy			< 0.001
No	3606 (3606)	1289 (64.2)	
Yes	1643 (31.3)	718 (35.8)	
Maternal BMI before pregnancy			< 0.001
Underweight	230 (4.6)	82 (4.3)	
Normal weight	3738 (74.2)	1294 (67.9)	
Overweight	902 (17.9)	425 (22.3)	
Obesity	171 (3.4)	106 (5.6)	
Parity			0.089
Nulliparous	1938 (36.5)	785 (38.7)	
1 previous pregnancy	1716 (32.3)	605 (29.8)	
≥2 previous pregnancies	1662 (31.3)	640 (31.5)	
Problems with previous pregnancies			0.771
No	4163 (78.5)	1584 (78.2)	
Yes	1143 (21.5)	443 (21.8)	
Hypertensive pathology during pregnancy			< 0.001
No	3498 (69.2)	1256 (64.7)	
Yes	1555 (30.8)	684 (35.3)	
Abnormality during pregnancy			0.554
No	3941 (74.2)	1490 (73.5)	
Yes	1374 (25.9)	538 (26.5)	
Total number of antenatal visits			0.163
< 5	230 (4.4)	71 (3.5)	
5-9 visits	1436 (27.4)	531 (26.5)	

> 9	3579 (68.2)	1405 (70.0)	
Birthweight			0.252
<10th percentile	374 (8.0)	161 (9.3)	
10-90th percentile	3807 (81.8)	1413 (81.2)	
>90th percentile	472 (10.4)	166 (9.5)	
Gestational age			0.268
<38 weeks	463 (9.6)	196 (10.8)	
38 weeks	383 (7.9)	157 (8.6)	
39-41 weeks	3414 (70.7)	1246 (68.4)	
>41 weeks	567 (11.8)	224 (12.3)	
Whether labour induced			0.002
No	4666 (87.8)	1725 (85.0)	
Yes	651 (12.2)	305 (15.0)	
PROM> 12h			0.003
No	4203 (85.6)	1534 (82.7)	
Yes	708 (14.4)	321 (17.3)	

F: Fisher's exact test; PROM: premature rupture of the membranes

% may not add up exactly to 100.0 due to rounding

Table 3. Unadjusted and adjusted logistic regression models showing relationship between mode of delivery and the metabolic syndrome in mid-life: complete case analyses and analyses using multiply imputed data

Model 1: Unadjusted	Complete case OR [95% IC]	Multiple imputations OR [95% IC]
Mode of delivery		
Vaginal (ref)		
Emergency caesarean	1.60 (1.42-1.80)***	1.60 (1.08-2.37)*
Elective caesarean	0.92 (0.74-1.07)	0.92 (0.57-1.50)
Model 2: Adjusted [a]		
Mode of delivery		
Vaginal (ref)		
Emergency caesarean	2.18 (1.28-3.71)**	1.51 (1.00-2.30)*
Elective caesarean	1.00 (0.53-1.91)	0.93 (0.56-1.56)
Cohort member's gender		
Female (ref)		
Male	2.48 (2.18-2.82)***	2.58 (2.31-2.87)***
Maternal age		
Years	0.98 (0.97-0.99)**	0.98 (0.97-0.99)***
Mother educational level		
High (ref)		
Low	1.48 (1.27-1.73)***	1.47 (1.30-1.67)***
Smoking after the 4th month of pregnancy		
No (ref)		
Yes	1.28 (1.11-1.46)**	1.24 (1.10-1.39)***
Maternal BMI before pregnancy		
Normal weight (ref)		
Underweight	0.87 (0.64-1.20)	0.97 (0.74-1.27)
Overweight	1.19 (0.85-1.67)	1.33 (0.99-1.78)
Obesity	1.45 (0.94-2.24)	1.61 (1.12-2.34)**
Hypertensive pathology during pregnancy		
No (ref)		
Yes	1.24 (1.08-1.43)**	1.18 (1.05-1.33)**
Birthweight		
10-90th percentile (ref)		
<10th percentile	1.05 (0.83-1.34)	1.08 (0.88-1.32)
>90th percentile	0.92 (0.73-1.13)	0.96 (0.78-1.12)

*p≤0.05 **p≤0.01 ***p≤0.001

[a] also adjusted for: overcrowding, parity, previous pregnancy problems, total number of antenatal visits, gestational age, induced labour, PROM: premature rupture of the membranes.

Mode of delivery at birth and the metabolic syndrome in mid-life: the role of the birth environment in a prospective birth cohort study.

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Running title: Caesarean delivery and the metabolic syndrome

Key words: caesarean delivery, metabolic syndrome, emergency caesarean, lifecourse, 1958 birth cohort,

Abbreviations;

MS: Metabolic syndrome

PROM: Premature rupture of the membranes

Word count: 2581

ABSTRACT

~~Background~~**Objectives:** The aim of this study is to examine the hypothesis that mode of delivery at birth may be associated with metabolic disorders in adult mid-life. **Setting**: Population cohort study ~~**Methods**~~**Participants**: The National Child Development Study consists of individuals born during one week in 1958 in Great Britain. Respondents with biomedical data on the metabolic syndrome at age 45 were included. **Outcome measure:** The metabolic syndrome was defined based on the NCEP-ATP III classification. **Results**: 7156 were born naturally, among the caesarean births 106 were non elective and 85 were elective caesareans. The metabolic syndrome is present in 37.7% of those born by non elective caesareans, 25.9% of those born by elective caesarean and 27.5% of those born by vaginal delivery. In a multivariate logistic regression model adjusted for antenatal factors, birth history, mother's characteristics and the socioeconomic environment at birth, only birth by non elective caesarean remained associated with the metabolic syndrome in adulthood compared to vaginal (OR 1.51, 95% CI 1.00-2.30). Mother's obesity (OR 1.61, 95% CI 1.12-2.34) and low maternal education level (OR 1.47, 95% CI 1.30-1.67) were also independently associated with mid-life metabolic syndrome. **Conclusion**: Birth by non elective caesarean in 1958 may be associated with metabolic syndrome in adulthood after adjusting for prior confounding factors. We suggest that the birth context of ~~non elective~~emergency caesareans in 1958 is suggestive of a 'foetal stress' mechanism affecting health across the lifecourse.

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Strengths and limitations

-Being born by ~~non elective~~emergency caesarean in 1958 may be associated with the metabolic syndrome in mid-life.

-Mode of delivery may be a proxy for the birth environment and contextually variable clinical practices.

-Given the possible context of ~~non elective~~emergency caesareans in 1958, a 'foetal stress' hypothesis is suggested for the subsequent association with the metabolic syndrome.

-It is possible that an unknown confounding factor during early life was omitted from the analyses, which might explain differences observed in MS outcome between the mode-of-delivery groups

INTRODUCTION

In recent years a number of studies have suggested that the mode of delivery at birth may be associated with obesity and metabolic disruption across the lifecourse. This stems from epidemiological research showing associations between birth by caesarean section and obesity in childhood(1, 2). Such associations deserve further investigation given, on the one hand, the dramatic increases in caesarean sections in recent decades, from 21% in 1996 to 32% in 2007(3), and on the other hand, the burden of morbidity due to metabolic diseases(4). The hypothesised mechanism for this association involves the colonisation of the gut microbiota(5).

Animal models have shown that modifications to rat gut microbiota have lead to metabolic disruptions and ultimately obesity in affected animals(6). The gut microbiota is a potential source of inflammatory molecules that may contribute to metabolic diseases(7, 8). This possible link between gut microbiota and metabolic disruptions is relevant to mode of delivery at birth due to the colonisation of the gut flora that occurs when the baby ingests maternal vaginal flora as s/he passes along the birth canal. If a caesarean section is carried out to deliver the baby, this phase of birth is skipped, and the baby is not exposed to the vaginal flora. The colonisation of their digestive tract therefore occurs differently to a baby who was delivered naturally(9, 10, 11). Recent reports have linked differences in infant gut microbiota with subsequent obesity(12).

To explore the hypothesis that mode of delivery may be associated with metabolic disruptions, it is important to consider the context surrounding the pregnancy, birth, and where possible, variations in the mode of delivery. Caesarean sections have become part of routine practice in maternity wards, often planned well in advance in the case of at-risk

pregnancies(13). However, the practice of caesareans was not so commonplace up to three decades ago, and individuals born under rather different practices and clinical conditions are now in their forties and fifties. In this paper we use a~~n historical~~prospective birth cohort study of individuals born in 1958 to explore the possible association between mode of delivery at birth and the occurrence of the metabolic syndrome (MS) in mid-life (45 years) under different contextual circumstances surrounding birth. Study participants delivered naturally, born via planned caesarean and via unplanned caesarean will be compared in terms of their metabolic syndrome profile at the age of 45 years using available biomedical data from a birth cohort study.

METHODS

Sample and participants

This study used data from the 1958 National Child Development Study (NCDS) which included all births during one week in 1958 (n= 18558) in Great Britain. Subsequent data collections were carried out on cohort members aged 7, 11, 16, 23, 33, 42, 46 and 50. The NCDS has been described in detail elsewhere(14). A biomedical survey (9377 cohort members participating) was conducted when participants were aged 44-46 years. (Figure 1):

Ethics

Written informed consent was obtained from the cohort member's parents for childhood measurements and ethical approval for the adult data collection was obtained from the National Research Ethics Advisory Panel. NCDS data are open access datasets available to non-profit research organisations.

Outcome measure

The MS was defined using NCEP-ATP III (National Cholesterol Education Program Adult Treatment Panel III) clinical criteria except for plasma glucose which was not recorded and replaced by glycated haemoglobin (HbA1c) ≥ 6.5%(15).

Exposure variable

Mode of delivery was categorized into three groups: ~~non-elective~~emergency caesarean, elective caesarean or vaginal delivery.

Covariates

The variables taken into account covered four areas:

i) Mother's socioeconomic and health characteristics before the current pregnancy: Mother's educational level (left school before/ after minimum leaving age); household overcrowding (people per room); mother's self-reported pre-pregnancy weight and her height measured after the birth were used to construct the mother's pre-pregnancy BMI (weight in kg/(height in m)²). Since some mothers were younger than 18 years of age, age-specific BMI cut-offs were used in order to categorize BMI into 4 groups: thinness, normal, overweight and obese (corresponding to the cut-offs of <18.5 kg/m², 18.5-24.9 kg/m², 25.0-29.9 kg/m² and ≥ 30.0 kg/m² for adults respectively). Mother's parity in 1958, including miscarriages after 28 weeks, was also extracted.

ii) Previous pregnancy complications: previous pregnancy problems (yes/no), constructed based on whether the mother had previously had: an abortion or ectopic pregnancy; previous stillbirths; a previous neonatal death; or other previous pregnancy complications.

iii) Information on the current pregnancy: maternal age at birth; whether the mother smoked during pregnancy beyond the fourth month (yes/ no); abnormality during pregnancy (none/ at least one abnormality including: Antepartum haemorrhage, placenta praevia, vaginal bleeding, and other abnormalities); hypertensive pathology (none/ hypertension/ toxemia/ proteinuria/ eclampsia); and total number of antenatal visits (<5 visits, 5-9 visits, >9 visits).

iv) Details of the labour and birth: time elapsed since rupture of membranes (≥12 hours before delivery ie. premature rupture of the membranes (PROM)/ <12hours before delivery): whether labour was induced (yes/no); birth weight for gestation (<10th percentile, 10-90th percentile, >90th percentile); gestational age was calculated as the duration between the first day of the mother's last menstrual period and childbirth, and categorized into groups (<38 weeks, 38 weeks, 39-41 weeks, >41 weeks).

Statistical analyses

We first determined the prevalence of MS, and used the chi-squared test to assess whether this prevalence differed by mode of delivery. The covariates were summarized as frequencies and percentages for categorical variables, means and standard deviations (SD) for continuous variables. Chi-square or Fisher's exact tests were performed in order to compare the sample characteristics according to the exposure or the outcome. Comparisons of means by mode of delivery category were computed using variance analysis (ANOVA), whereas the comparisons of means by MS status were carried out using the Student's t-test, after validating assumptions of normality and homoscedasticity.

Unadjusted and adjusted logistic regression models were carried out to explore the relationship between MS and mode of delivery. Both complete case and multiple imputation analyses were conducted.

To control for possible bias due to missing data, we imputed data for covariates with missing data using the multiple imputation program ICE in STATA v11(16). For more details see the supplementary data.

RESULTS

Among 7347 observations, the prevalence of the metabolic syndrome was 27.6 % (36.6% for males, 18.6% for females p<0.001). In total, 191 cohort members (2.6% of the sample) were delivered by caesarean section (106 ~~non-elective~~emergency, 85 elective caesarean sections). The prevalence of the MS in the ~~non-elective~~emergency and in the elective caesarean were 37.7% (95% CI: 28.5-47.0%) and 25.9% (95%CI: 16.5% to 35.3%) respectively. The estimated prevalence of MS was 27.5% (95%CI: 2~~6.5~~7.2% to 2~~8.5~~7.8%) within the vaginal delivery group, 37.7% (95%CI: 34.9% to 40.5%) within~~in~~ the emergency caesarean group, and 25.9% (95%CI: 23.1-28.7) within the elective caesarean group -(global chi-squared test p-~~value comparing the prevalence in the three groups~~=0.061).

Sample characteristics according to mode of delivery are reported in Table 1. Several maternal characteristics, parity, problems during previous pregnancies, abnormalities during the current pregnancy, induced labour, premature rupture of membranes (PROM) and gestational age were highly associated with the cohort member's mode of delivery at birth (p<0.001). Specifically, older maternal age at birth, nulliparous mothers, induced labour, PROM and overdue birth (> 41 weeks) were more frequent in the ~~non-elective~~emergency caesarean section group. On the other hand, problems during previous pregnancies (past stillbirth and neonatal deaths and past complications of pregnancy), abnormality during pregnancy and premature birth (<38 weeks) were more frequent in the elective caesarean delivery group. Previous caesarean was also a strong predictor of elective caesarean delivery (data not shown).

Table 2 shows the relationships between mode of delivery, the covariates and MS. A low maternal level of education, smoking after the 4th month of pregnancy and maternal obesity

were associated with a higher prevalence of MS. We also found significant links between MS and the following: hypertensive pathology, induced labour, and PROM.

In Table 3 we report unadjusted (model 1.) and adjusted (model 2.) odds ratios (OR) resulting from the final logistic regression model. Model 2. is adjusted for the effects of cohort member's gender, maternal age, mother educational level, smoking habits during pregnancy, BMI, parity, problems with previous pregnancies, hypertensive pathology during pregnancy, birth weight, gestational age, induction of the labour and PROM, (Table 3 - model 2). The results show that ~~non elective~~emergency caesarean delivery was associated with an increased proportion of MS compared to vaginal delivery (OR=1.51, p=0.05 results from imputed data).

An increased probability of having MS in mid-life was also associated with: being male, a lower maternal education level, maternal smoking in pregnancy, maternal pre-pregnancy obesity, and maternal hypertensive pathology during pregnancy. Respondents whose mothers were younger at the time of their birth were less likely to have MS in mid-adulthood.

DISCUSSION

These analyses show that differences in the prevalence of the MS at 45 years may be associated with the mode of delivery at birth, after controlling for possible confounders. However, the association observed is not for respondents born by caesarean section overall versus those born vaginally. Rather, ~~non elective~~emergency caesarean delivery remained associated with an increased risk of having MS in mid-life compared to individuals born vaginally. These findings differ from studies carried out on cohorts of individuals born more recently showing that caesarean section *per se* is a risk for metabolic disorders via the gut microbiota hypothesis(1, 11). The results from our study using data from births in 1958

suggest that mode of delivery may be a proxy variable for qualities in the birth environment that may have had long term implications for cohort members' health. We suggest that in 1958 caesarean sections were a rare phenomenon (2.6% prevalence), carried out electively in the case of high-risk pregnancies. When caesareans were ~~non elective~~emergency we hypothesise that the birth context was most likely stressful, resulting in an emergency caesarean section. The stressful nature of the birth may play an important part in the observed association between ~~non elective~~emergency caesarean births and the metabolic syndrome in mid-life.

Factors occurring prior to, and at the time of birth may contribute to the association between ~~non elective~~emergency caesareans and the subsequent development of MS. Based on the information from the NCDS, the women who had non elective caesareans in 1958 were more likely to be overdue, to have had an induced labour and to have experienced rupture of the membranes ~~broken their waters~~ more than 12 hours before the birth. Such births seem to have gradually become emergency situations presumably after a long labour, with 97% of babies born thus described as having experienced "foetal distress" by the duty midwife (data not shown). Abnormalities occurring at birth as indicated by an induced labour or a late delivery were more frequent in the ~~non elective~~emergency caesarean section group. Historically, the main reasons given by clinicians for carrying out caesareans, other than having previously had a caesarean, are the relatively undefined concepts of "foetal distress", "failure to progress" during labour, and breech presentations(17). Given the rare occurrence of caesareans in the late 1950s, we can only speculate that the conditions surrounding a labour ending in a ~~non elective~~emergency caesarean were likely to have been fraught and stressful for those involved, not least for the baby. We put forward a foetal stress hypothesis, whereby babies born by ~~non elective~~emergency caesarean were subject to physiological stress, and

possibly their mother's psychological stress and its consequences in the post-natal period. Such a context of stress may have affected the baby's physiological and psychological stress responses thereafter. Early life stress has been associated with physiological alterations leading individuals along negative health trajectories(18, 19). Furthermore, in later life a stressful environment, such as job stress, has been associated with a greater prevalence of MS(20).

The colonisation of the gut microbiota during vaginal births, and the lack thereof during caesarean births, has been put forward as a hypothesis for links observed in previous studies on caesarean delivery and metabolic disorders in childhood or adulthood(1). Some authors have reported that mode of delivery is associated with a differential colonisation of the gut flora. Higher proportions of bacteria from the the *Firmicutes* group, and a lower frequency of members of the *Bacteroidetes* group have been observed in children delivered by caesarean section compared to those born vaginally(11). Moreover, infants born by caesarean delivery were significantly less often colonized with bacteria of the *Bacteroides fragilis* group than vaginally delivered infants and these sub-groups represent the majority of the microbiota found in the adult gut(5, 21).

To support this hypothesis we would have observed differences in the prevalence of MS between caesareans per se and vaginal birth, however this was not the case. No association was observed between overall caesarean section in 1958 and MS in mid life. Differences between the gut microbiota of individuals born by ~~non-elective~~emergency caesarean section versus those delivered vaginally but not in those born by elective caesarean section have been reported. A lower frequency of *Escherichia-Shigella* has been observed in other studies for those born by ~~non-elective~~emergency caesarean(22). We cannot exclude that

babies born by ~~non elective~~emergency caesarean were more likely to experience a prolonged exposure to vaginal bacteria and possibly to infectious pathogens, due to the PROM (44% of ~~non elective~~emergency caesareans exposed to PROM, versus 15% vaginal delivery and 0% elective caesareans). Mode of delivery has previously been associated with childhood obesity at 3 years of age and the authors postulated that a longer exposure to bacterial flora could be a mechanism involved in childhood obesity(1).

We reported that maternal pre-pregnancy obesity was associated with an increased probability of having the metabolic syndrome at the modal age of 45 years. Previous work has shown that children exposed to maternal obesity in early life had a twofold increased risk of developing MS, with a trend toward a higher incidence of insulin resistance(23). Offspring exposed to maternal hyperglycaemia during their intrauterine development were also more prone to metabolic disorders in young adulthood leading to insulin resistance(24, 25). Different paths of childhood growth with smaller gains in BMI during infancy could precede the development of metabolic syndrome or hypertension(26, 27).

There are a number of limitations to our study. The definition of MS proposed by different organisations has varied over the past decade. The prevalence of MS is lower when using definitions other than ATPIII, however, the risk of cardiovascular events, diabetes mellitus and hypertension are similar for ATPIII and AHA or IDF definitions(28). Glycaemia was not recorded in the cohort study biomedical survey; therefore we used the HbA1c value with a cut-off above 6.5% to define hyperglycaemia. HbA1c has been defined as a marker to identify diabetes status(29). The reliability of glucose measurements varies widely across laboratories and may result in misclassification of >12% of patients (30). By contrast, HbA1c values are relatively stable after collection(31). The NCDS cohort provides a rare opportunity

to study conditions and characteristics at birth and in early life collected prospectively, in relation to good quality biological data sampled in mid-life. Unfortunately no information was collected at the time about gestational diabetes, however, we include birthweight and variables on other pregnancy complications which may capture the effect of insulin resistance during pregnancy. It was ~~therefore~~ possible to include a large number of potential confounding variables in the statistical models, however it is possible that a key unknown confounding factor during early life was omitted, which might explain differences observed in MS outcome between the mode-of-delivery groups.

CONCLUSION

These findings suggest that mode-of-delivery at birth may be an important variable to take into account to understand the aetiology of metabolic disorders. It is likely to represent factors occurring in the environment proximal to the birth which may have an impact on the baby's health across the lifecourse. Our findings show that in 1958, ~~non-elective~~emergency caesarean sections may be associated with an increased prevalence of the MS in mid-life. We suggest that given the maternity practices of the time, physiological stress experienced by the baby during delivery may be an important mechanism in the subsequent development of metabolic disorders.

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Author contributions

BDB, VE and MKI were involved in the conception and design of the study, analysing and interpreting the data, drafted the manuscript and made modifications. BDB and VE contributed equally to the work. TL, CD and BC analysed and interpreted the analyses, and revised the manuscript. All authors approved the current version.

REFERENCES

1- **Huh SY**, Rifas-Shiman SL, Zera CA, *et al.* Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. *Arch Dis Child* 2012;**97**:610-16.doi: 10.1136/archdischild-2011-301141. Epub 2012 May 23.

2- **Zhou L**, He G, Zhang J, *et al*. Risk factors of obesity in preschool children in an urban area in China. *Eur J Pediatr* 2011;**170**:1401-6.doi: 10.1007/s00431-011-1416-7. Epub 2011 Mar 2.

3- **Menacker F**, Hamilton BE. Recent trends in cesarean delivery in the United States. *NCHS Data Brief* 2010;**35:**1-8.

4- **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA.* 2002;**287**:356-9.

5- **Ley RE.** Turnbaugh PJ, Klein S, *et al*. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**:1022-3.

6- **Bäckhed F**, Ding, Wang T, Hooper LV, *et al*.. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;**101:**15718-23. Epub 2004 Oct 25.

7- **Cani PD**, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;**56:**1761-72.

8- **Karlsson FH**, Tremaroli V, Nookaew I, *et al*. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*.2013;**498**:99-103.doi: 10.1038/nature12198. Epub 2013 May 29.

9- **Biasucci G,** Benedati B, Morelli L, Bessi E, Boehm G. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr* 2008;**138**: 1796S-1800S

10-**Salminen S,** Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 2004; **53**:1388-9

11-**Grönlund MM**, Lehtonen OP, Eerola E *et al*. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after caesarean delivery. *J Pediatr Gastroenterol Nutr*1999;**28**:19-25.

12-**Luoto R**, Kalliomäki M, Laitinen K, *et al.* Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastroenterol Nutr* 2011;**52**:90-5.doi: 10.1097/MPG.0b013e3181f3457f.

13-**Zhang J**, Troendle J, Reddy UM, *et al*. Contemporary cesarean delivery practice in the United States. *Am J Obstet Gynecol* 2010;**203**:326.e1-326.e10.doi: 10.1016/j.ajog.2010.06.058. Epub 2010 Aug 12.

14-**Power C**, Elliott J. Cohort profile: 1958 British Birth Cohort (National Child Development Study). *Int J Epidemiol* 2006;**35**:34-41.

15-Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486-97.

16-**Royston P**. Multiple imputation of missing values: Further update of ice, with an emphasis on categorical variables. *Stata Journal* 2009;**9**:466-77.

17-**[No authors listed]** Holding back the tide of caesareans. *BMJ.* 1988;**297**:852

18-**Alastalo H**, von Bonsdorff MB, Räikkönen K, *et al*. Early Life Stress and Physical and Psychosocial Functioning in Late Adulthood. *PLoS ONE*. 2013;8:e69011 doi: 10.1371/journal.pone.0069011

19-**Kelly-Irving M**, Lepage B, Dedieu D, *et al*. Adverse childhood experiences and premature all-cause mortality. *Eur J Epidemiol*. 2013:1-14 doi: 10.1007/s10654-013-9832-9

20-**Chandola T**, Brunner E, Marmot M. Chronic stress at work and the metabolic syndrome: prospective study. *BMJ* 2006;**332**:521-5

21-**Bennet R,** Nord CE. Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. *Infection* 1987;**15**:332-6.

22-Azad MB, Konya T, Maughan H, *et al*. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013;**185**:385-94. doi: 10.1503/cmaj.121189. Epub 2013 Feb 11.

23-**Boney CM**, Verma A, Tucker R, *et al*. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;**115**: e290-6.

24-**Dabelea D**, Mayer-Davis EJ, Lamichhane AP, *et al*. Association of Intrauterine Exposure to Maternal Diabetes and Obesity with Type 2 Diabetes in Youth: the SEARCH Case-Control Study. *Diabetes Care* 2008;**31**:1422-6.

25-**Hillier TA**, Pedula KL, Schmidt MM, *et al*. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007;**30**:2287-92.

26-**Salonen MK,** Kajantie E, Osmond C, *et al*. Childhood growth and future risk of the metabolic syndrome in normal-weight men and women. *Diabetes Metab* 2009;**35**:143-50.

27-**Eriksson JG**, Forsen TJ, Kajantie E, *et al*. Childhood growth and hypertension in later life. *Hypertension* 2007;**49:**1415-21.

28-**Mancia G**, Bombelli M, Facchetti R, *et al.* Impact of different definitions of the metabolic syndrome on the prevalence of organ damage, cardiometabolic risk and cardiovascular events. *J Hypertens* 2010;**28**:999-1006.doi: 10.1097/HJH.0b013e328337a9e3.

29-**International Expert committee**. International Expert committee report on the role of the AIC assay in the diagnosis of diabetes. *Diabetes Care* 2009;**32**:1327-34.

30-**Miller WG**, Myers GL, Ashwood ER, *et al*. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. *Arch Pathol Lab Med* 2008;**132**:838-46. doi: 10.1043/1543-2165(2008)132[838:SOTAIT]2.0.CO;2.

31-**Little RR**, Rohlfing CL, Tennill AL, *et al*. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. *Diabetes Technol Ther* 2007; **9**:36-42.

Figure 1. Flow chart showing the sample selection

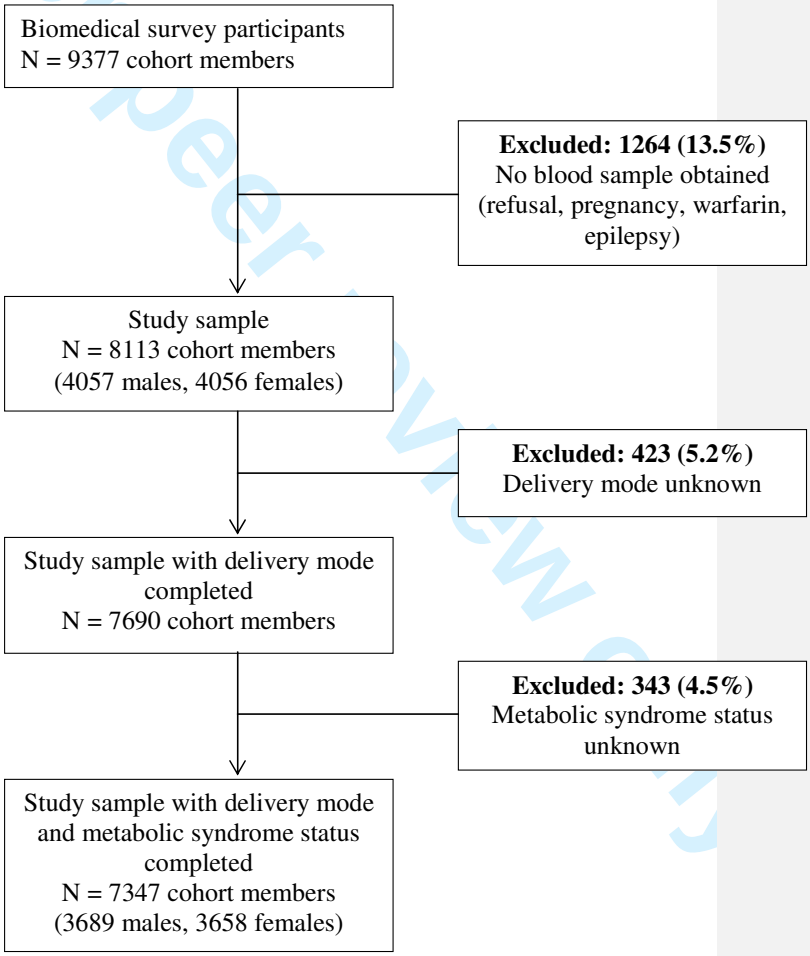

Table 1. Description of covariates in terms of mode of delivery

Variable	Vaginal delivery (N=7156) n (%)	~~Non-electiveEmergency~~ caesarean (N=106) n (%)	Elective caesarean (N=85) n (%)	P-value Chi-square
Cohort member's gender				0.156
male	3580 (50.0)	61 (57.6)	48 (56.5)	
female	3576 (50.0)	45 (42.4)	37 (43.5)	
Maternal age				< 0.001 F
<18 years	69 (1.0)	0 (0.0)	0 (0.0)	
18-35 years	6414 (89.7)	87 (82.1)	63 (74.1)	
>35 years	668 (9.3)	19 (17.9)	22 (25.9)	
Mother educational level				0.045
Low	5224 (73.2)	69 (65.7)	54 (64.3)	
High	1913 (26.8)	36 (34.2)	30 (35.7)	
Overcrowding				0.049
1 to 1.5 people per room	6140 (88.1)	96 (96.0)	73 (89.0)	
≥1.5 people per room	832 (11.9)	4 (4.0)	9 (11.0)	
Smoking after the 4th month of pregnancy				0.589
No	4766 (67.4)	74 (71.8)	55 (65.5)	
Yes	2303 (32.6)	29 (28.2)	29 (34.5)	
Maternal BMI before pregnancy				0.075 F
Underweight	305 (4.5)	3 (3.1)	4 (5.3)	
Normal weight	4915 (72.6)	69 (71.1)	48 (63.2)	
Overweight	1293 (19.1)	18 (18.6)	16 (21.0)	
Obesity	262 (3.8)	7 (7.2)	8 (10.5)	
Parity				< 0.001
Nulliparous	2636 (36.9)	67 (63.2)	20 (23.5)	
1 previous pregnancy	2269 (31.7)	20 (18.9)	32 (37.7)	
≥2 previous pregnancies	2250 (31.4)	19 (17.9)	33 (38.8)	
Problems with previous pregnancies				< 0.001
No	5638 (78.9)	73 (68.9)	36 (42.4)	
Yes	1504 (21.1)	33 (31.1)	49 (57.6)	
Hypertensive pathology during pregnancy				0.002
No	4654 (68.3)	59 (62.1)	41 (51.3)	
Yes	2164 (31.7)	36 (37.9)	39 (48.7)	
Abnormality during pregnancy				< 0.001
No	5358 (74.9)	50 (47.2)	23 (27.1)	
Yes	1794 (25.1)	56 (52.8)	62 (72.9)	
Total number of antenatal visits				0.240 F
< 5	295 (4.2)	2 (1.9)	4 (4.8)	
5-9 visits	1912 (27.1)	25 (24.3)	30 (36.1)	
> 9	4859 (68.7)	76 (73.8)	49 (59.2)	
Birthweight				0.392

<10th percentile	525 (8.4)	4 (4.8)	6 (8.5)	
10-90th percentile	5094 (81.7)	67 (79.8)	59 (83.1)	
>90th percentile	619 (9.9)	13 (15.4)	6 (8.4)	
Gestational age				< 0.001
<38 weeks	770 (9.8)	14 (12.1)	25 (25.5)	
38 weeks	610 (7.8)	15 (12.9)	23 (23.5)	
39-41 weeks	5504 (70.4)	59 (50.9)	45 (45.9)	
>41 weeks	935 (12.0)	28 (24.1)	5 (5.1)	
Whether labour induced				< 0.001
No	6242 (87.2)	68 (64.2)	81 (95.3)	
Yes	914 (12.8)	38 (35.8)	4 (4.7)	
PROM> 12h				< 0.001
No	5599 (85.0)	55 (55.6)	83 (100.0)	
Yes	985 (15.0)	44 (44.4)	0 (0.0)	

F: Fisher's exact test; PROM: premature rupture of the membranes

% may not add up exactly to 100.0 due to rounding

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Table 2. Characteristics of mothers and cohort members before pregnancy, during pregnancy and labour, and at birth in terms of the metabolic syndrome at age 44-46y (n=7347)

	Metabolic syndrome		
	No (N=5317)	Yes (N=2030)	P-value Chi-square
Variable	n (%)	n (%)	
Mode of delivery			0.061
Vaginal	5188 (72.5)	1968 (27.5)	
Non-electiveEmergency caesarean	66 (62.3)	40 (37.7)	
Elective caesarean	63 (74.1)	22 (25.9)	
Cohort member's gender			< 0.001
male	2339 (63.4)	1350 (36.6)	
female	2978 (81.4)	680 (18.6)	
Maternal age			0.166
<18 years	44 (63.8)	25 (36.2)	
18-35 years	4743 (72.3)	1821 (27.7)	
>35 years	525 (74.0)	184 (26.0)	
Mother educational level			< 0.001
Low	3751 (70.2)	1596 (29.8)	
High	1549 (78.3)	430 (21.7)	
Overcrowding			0.330
1- 1.5 people per room	4573 (72.5)	1736 (27.5)	
≥1.5 people per room	599 (70.9)	246 (29.1)	
Smoking after the 4th month of pregnancy			< 0.001
No	3606 (73.7)	1289 (26.3)	
Yes	1643 (69.6)	718 (30.4)	
Maternal BMI before pregnancy			< 0.001
Underweight	230 (73.7)	82 (26.3)	
Normal weight	3738 (74.3)	1294 (25.7)	
Overweight	902 (68.0)	425 (32.0)	
Obesity	171 (61.7)	106 (38.3)	
Parity			0.089
Nulliparous	1938 (71.2)	785 (28.8)	
1 previous pregnancy	1716 (73.9)	605 (26.1)	
≥2 previous pregnancies	1662 (72.2)	640 (27.8)	
Problems with previous pregnancies			0.136
No	4886 (72.6)	1843 (27.4)	
Yes	398 (69.7)	173 (30.3)	
Hypertensive pathology during pregnancy			< 0.001
No	3498 (73.6)	1256 (26.4)	
Yes	1555 (69.5)	684 (30.5)	
Abnormality during pregnancy			0.554
No	3941 (72.6)	1490 (27.4)	
Yes	1374 (71.9)	538 (28.1)	
Total number of antenatal visits			0.163
< 5	230 (76.4)	71 (23.6)	
5-9 visits	1436 (73.0)	531 (27.0)	
> 9	3579 (71.8)	1405 (28.2)	
Birthweight			0.252
<10th percentile	374 (69.9)	161 (30.1)	

10-90th percentile	3807 (72.9)	1413 (27.1)	
>90th percentile	472 (74.0)	166 (26.0)	
Gestational age			0.268
<38 weeks	463 (70.3)	196 (29.7)	
38 weeks	383 (70.9)	157 (29.1)	
39-41 weeks	3414 (73.3)	1246 (26.7)	
>41 weeks	567 (71.7)	224 (28.3)	
Whether labour induced			0.002
No	4666 (73.0)	1725 (27.0)	
Yes	651 (68.1)	305 (31.9)	
PROM> 12hPROM			0.003
No	4203 (73.3)	1534 (26.7)	
Yes	708 (68.8)	321 (31.2)	

F: Fisher's exact test; PROM: premature rupture of the membranes

Variable	Metabolic syndrome No (N=5317) n (%)	Yes (N=2030) n (%)	P-value Chi-square
Mode of delivery			0.061
Vaginal	5188 (97.6)	1968 (97.0)	
Emergency caesarean	66 (1.2)	40 (2.0)	
Elective caesarean	63 (1.2)	22 (1.1)	
Cohort member's gender			< 0.001
male	2339 (44.0)	1350 (66.5)	
female	2978 (56.0)	680 (33.5)	
Maternal age			0.166
<18 years	44 (0.8)	25 (1.2)	
18-35 years	4743 (89.3)	1821 (89.7)	
>35 years	525 (9.9)	184 (9.1)	
Mother educational level			< 0.001
Low	3751 (70.8)	1596 (78.8)	
High	1549 (29.2)	430 (21.2)	
Overcrowding			0.330
1- 1.5 people per room	4573 (88.4)	1736 (87.6)	
≥1.5 people per room	599 (11.6)	246 (12.4)	
Smoking after the 4th month of pregnancy			< 0.001
No	3606 (3606)	1289 (64.2)	
Yes	1643 (31.3)	718 (35.8)	
Maternal BMI before pregnancy			< 0.001
Underweight	230 (4.6)	82 (4.3)	
Normal weight	3738 (74.2)	1294 (67.9)	
Overweight	902 (17.9)	425 (22.3)	
Obesity	171 (3.4)	106 (5.6)	
Parity			0.089
Nulliparous	1938 (36.5)	785 (38.7)	
1 previous pregnancy	1716 (32.3)	605 (29.8)	
≥2 previous pregnancies	1662 (31.3)	640 (31.5)	
Problems with previous pregnancies			0.771
No	4163 (78.5)	1584 (78.2)	
Yes	1143 (21.5)	443 (21.8)	

Hypertensive pathology during pregnancy			< 0.001
No	3498 (69.2)	1256 (64.7)	
Yes	1555 (30.8)	684 (35.3)	
Abnormality during pregnancy			0.554
No	3941 (74.2)	1490 (73.5)	
Yes	1374 (25.9)	538 (26.5)	
Total number of antenatal visits			0.163
< 5	230 (4.4)	71 (3.5)	
5-9 visits	1436 (27.4)	531 (26.5)	
> 9	3579 (68.2)	1405 (70.0)	
Birthweight			0.252
<10th percentile	374 (8.0)	161 (9.3)	
10-90th percentile	3807 (81.8)	1413 (81.2)	
>90th percentile	472 (10.4)	166 (9.5)	
Gestational age			0.268
<38 weeks	463 (9.6)	196 (10.8)	
38 weeks	383 (7.9)	157 (8.6)	
39-41 weeks	3414 (70.7)	1246 (68.4)	
>41 weeks	567 (11.8)	224 (12.3)	
Whether labour induced			0.002
No	4666 (87.8)	1725 (85.0)	
Yes	651 (12.2)	305 (15.0)	
PROM> 12h			0.003
No	4203 (85.6)	1534 (82.7)	
Yes	708 (14.4)	321 (17.3)	

F: Fisher's exact test; PROM: premature rupture of the membranes

% may not add up exactly to 100.0 due to rounding

Table 3. Unadjusted and adjusted logistic regression models showing relationship between mode of delivery and the metabolic syndrome in mid-life: complete case analyses and analyses using multiply imputed data

Model 1: Unadjusted	**Complete case OR [95% IC]**	**Multiple imputations OR [95% IC]**
Mode of delivery		
Vaginal (ref)		
~~Non elective~~Emergency caesarean	1.60 (1.42-1.80)***	1.60 (1.08-2.37)*
Elective caesarean	0.92 (0.74-1.07)	0.92 (0.57-1.50)
Model 2: Adjusted [a]		
Mode of delivery		
Vaginal (ref)		
~~Non elective~~Emergency caesarean	2.18 (1.28-3.71)**	1.51 (1.00-2.30)*
Elective caesarean	1.00 (0.53-1.91)	0.93 (0.56-1.56)
Cohort member's gender		
Female (ref)		
Male	2.48 (2.18-2.82)***	2.58 (2.31-2.87)***
Maternal age		
Years	0.98 (0.97-0.99)**	0.98 (0.97-0.99)***
Mother educational level		
High (ref)		
Low	1.48 (1.27-1.73)***	1.47 (1.30-1.67)***
Smoking after the 4th month of pregnancy		
No (ref)		
Yes	1.28 (1.11-1.46)**	1.24 (1.10-1.39)***
Maternal BMI before pregnancy		
Normal weight (ref)		
Underweight	0.87 (0.64-1.20)	0.97 (0.74-1.27)
Overweight	1.19 (0.85-1.67)	1.33 (0.99-1.78)
Obesity	1.45 (0.94-2.24)	1.61 (1.12-2.34)**
Hypertensive pathology during pregnancy		
No (ref)		
Yes	1.24 (1.08-1.43)**	1.18 (1.05-1.33)**
Birthweight		
10-90th percentile (ref)		
≤10th percentile	1.05 (0.83-1.34)	1.08 (0.88-1.32)
≥90th percentile	0.92 (0.73-1.13)	0.96 (0.78-1.12)

*p≤0.05 **p≤0.01 ***p≤0.001

[a] also adjusted for: overcrowding, parity, previous pregnancy problems, total number of antenatal visits, ~~birthweight,~~ gestational age, induced labour, PROM: premature rupture of the membranes.

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Supplementary material

Definition of the metabolic syndrome

The MS was defined using NCEP-ATP III (National Cholesterol Education Program Adult Treatment Panel III) clinical criteria except for including: abdominal obesity (increased waist circumference: >102 cm for men; >88 cm for women), raised triglycerides (≥1.50g/L (≥1.69 mmol/L), reduced HDL cholesterol (<40 mg/dL for (men) (<1.04 mmol/L), <50 mg/dL for women (<1.29 mmol/L), elevated blood pressure (BP ≥130 and/or ≥85 mm Hg), and raised plasma glucose. If three out of the five listed characteristics were present, a diagnosis of metabolic syndrome was made. In our analyses, BP was calculated as the average of three readings. Waist circumference was considered only if the measurement was noted by the cohort team as being "reliable". Plasma glucose which was not recorded was replaced by Glycated haemoglobin (HbA1c) ≥ 6.5%.

Statistical analyses

The imputation model included the exposure variables and covariates used in the logistic regression models, as well as other correlated variables which were likely to improve the imputation model but that were not used in analyses: marital status of the mother, socio-economic group of the mother's father, multiple pregnancy. The outcome variable was omitted from the imputation model. Ten dataset imputations were run. The logistic regression models were carried out on the imputed data in order to decrease the potential bias of our estimated ORs and increase analytical power. Standard errors were calculated using Rubin's rules (1). P-values ≤0.05 were considered as statistically significant. Maternal age of birth was treated as a continuous variable, as a fractional polynomial model showed there was no evidence that the change in incidence over time was not linear (2).

Analyses of missing data

Having missing data or not for the covariates was analysed in terms of mode of delivery and in terms of the outcome measure. Missing data for mother's BMI before pregnancy was associated with being born by planned caesarean (10.8% vs 8.7% and 5.4% p=0.03); Missing data for toxaemia was associated with being born by unplanned caesarean (10.6% vs 6.0% and 4.8% p=0.029). Missing data for birthweight was associated with unplanned caesarean birth (20.19% versus 16.9% and 12.8% p=0.05) and with having the MS (14.4% vs 12.5% p=0.029). Missing data for PROM was associated with a vaginal birth (5.9% versus 4.8% and

0% p=0.035) and with having the MS (6.6% s 5.4 p=0.049). No clear pattern emerges from these analyses of missing data that suggests a systematic bias, except perhaps for persons with data missing for birthweight which was associated with both unplanned caesarean birth and the metabolic syndrome. However, the analyses using multiple imputations would adjust for this possible bias.

1. Rubin D. Multiple imputation for nonresponse in surveys. New York: Wiley, 1987 j'ai trouvé ça sur pubmed: Mehrotra DV, Li X, Liu J, Lu K. Analysis of longitudinal clinical trials with missing data using multiple imputation in conjunction with robust regression. Biometrics 2012 Dec;68(4):1250-9.
2. Royston P, Altman DG. Using fractional polynomials to model curved relationships, Stata Technical Bulletin 1994; 21:11-24

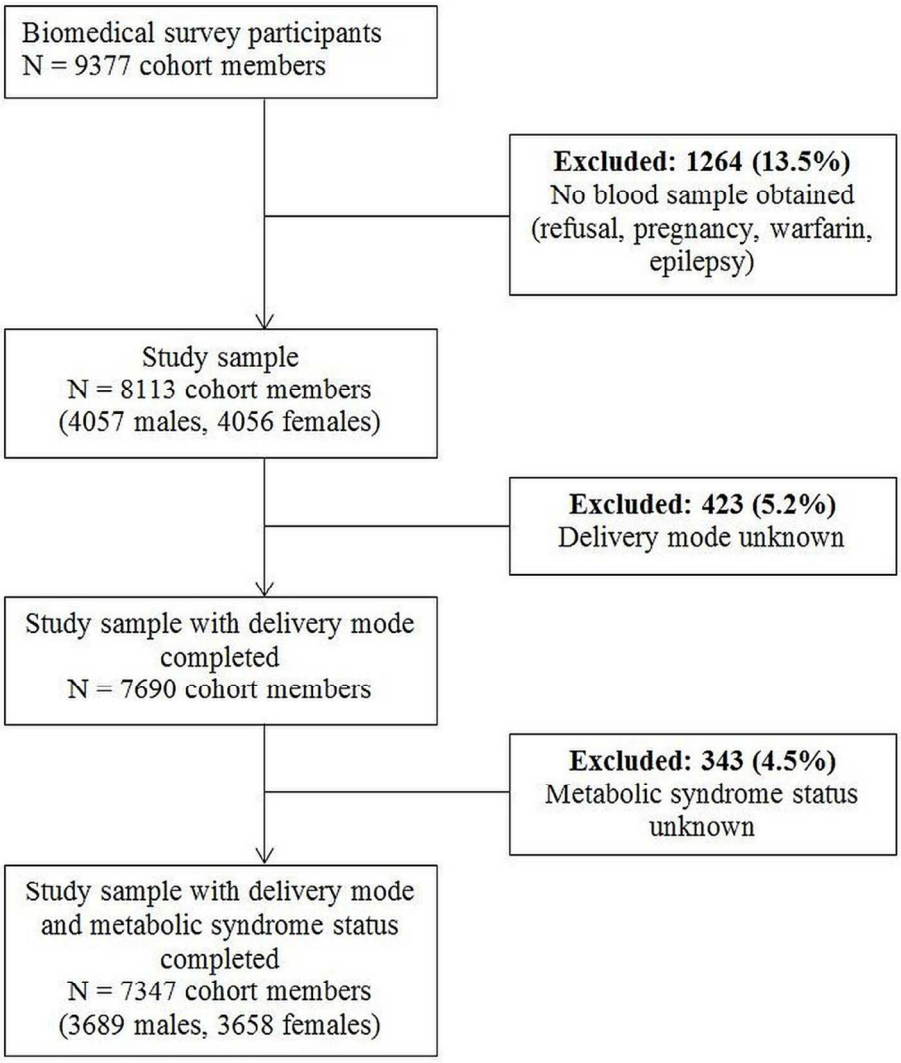

Figure 1. Flow chart showing the sample selection
90x97mm (300 x 300 DPI)