PHARMACOLOGIC SEX HORMONE USE BEFORE AND IN EARLY PREGNANCY IN RELATION TO BIRTH AND EARLY CHILDHOOD ANTHROPOMETRIC OUTCOMES

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ABSTRACT

Elizabeth T. Jensen: Pharmacologic sex hormone use before and in early pregnancy in relation to birth and early childhood anthropometric outcomes
(Under the direction of Julie Daniels)

Objective: Possible adverse effects resulting from use of hormonal contraceptives have been studied extensively, yet few studies have been conducted evaluating the association between hormonal contraceptive use and offspring anthropometric indices and most studies have been unable to explore formulation-specific effects.

Methods: Using data from a large, prospective pregnancy cohort study (n=44,734), with linkage to a national prescription registry, we evaluated the association between use of hormonal contraceptives (defined from dispensed prescription data) prior to and in early pregnancy (characterized by last date of use relative to conception, 12 - >4 months before, 4 - >1 months before, 1 - > 0 months before, and 0-12 weeks after) and preterm birth, small for gestational age, and overweight or obesity at age 3. We characterized use of a hormonal contraception by type (combination oral, progestin-only oral, vaginal ring, transdermal, and injectable) and specific progestin component.

Results: We observed a positive association between use of a combination oral contraceptive and preterm birth for all exposure periods (adjusted OR: 1.21, 95% CI: 1.04, 1.41 for exposure within 12 months before conception). Generally, use of other types of hormonal contraception was not associated with preterm birth. Overall, use of a hormonal contraceptive was unrelated to small for gestational age. Evaluation of the association by progestin type identified variation by progestin formulation. We observed a weak, inverse association between early pregnancy use of a combination oral contraceptive and offspring

overweight or obesity at age 3 (adjusted OR: 0.75, 95% CI: 0.53, 1.08) and a weak, positive association with use of a progestin-only oral contraceptive in early pregnancy (adjusted OR: 1.26, 95% CI: 0.79, 2.02). Observed associations were robust to sensitivity analyses.

Conclusion: Hormonal contraceptive use prior to and in early pregnancy may be associated with preterm birth and offspring overweight. The association appears contingent upon the specific type or progestin component used. The potential for confounding by indication cannot be ruled out. Larger studies using population-based pregnancy cohort data with linkage to prescription registries may offer the potential to explore these questions further.

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LIST OF ABBREVIATIONS

ART	Assisted reproductive technology
BMI	Body mass index
DAG	Directed acyclic graph
DDD	Defined daily doses
DES	Diethylstilbestrol
DNBC	Danish National Birth Cohort
EE	Ethinyl estradiol
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormones
HcG	Human chorionic gonadotropin
IUD	Intrauterine device
LBW	Low birth weight
LGA	Large for gestational age
LH	Luteinizing hormone
LMP	Last menstrual period
MBRN	Medical Birth Registry of Norway
MoBa	Norwegian Mother and Child Cohort Study
NorPD	Norwegian Prescription Database
OC	Oral contraceptive
PTB	Preterm birth
SGA	Small for gestational age
SHBG	Sex hormone binding globulin
IVF	In vitro fertilization

INTRODUCTION

Worldwide, the prevalence of childhood overweight and obesity increased from 4.2 percent in 1990 to 6.7 percent in 2010.(1-3) Children who are overweight or obese are more likely to be overweight or obese into adulthood and to suffer from obesity-precipitated morbidity and mortality. Experiments on animals have shown a positive association between exogenous estrogen exposure *in utero* and metabolic disruption in the offspring, including offspring development of overweight or obesity. These studies generally address the risk of low-dose exposure to environmental sources of estrogen-mimicking compounds, including exposure in early gestation prior to uterine implantation. Animal data have demonstrated that exposure to environmental toxicants with estrogen-like effects result in alterations to both follicular development and embryonic quiescence. Experimental data also suggests that the maternal metabolic environment may alter follicular and embryonic development. These alterations may influence embryonic viability and adiposity in later life. Pharmacologic sex hormones such as hormonal contraceptives have metabolic effects in women. Hormonal contraceptives contain estrogenic and progestogenic compounds and may influence follicular development and embryonic development directly or through altered maternal metabolism.

The potential risks of exogenous sex hormone exposure on offspring overweight or obesity are difficult to assess in human populations. Most cohort studies lack power to assess the influence of exogenous sex hormone exposure during pregnancy on offspring obesity. The Norwegian Mother and Child Cohort Study (MoBa), a prospective, population-based cohort study conducted by the Norwegian Institute of Public Health, offers an unusual

opportunity to assess the influence of *in utero* exposure to exogenous sex hormones on birth outcomes and childhood overweight or obesity. In the present study we assessed the association of exogenous sex hormone exposure during follicular development and early embryonic development on anthropometric measures at birth and at age 36 months. Specifically, we assessed the association between exposures within the discrete periods of 12 months before conception, 4 months before conception, 1 month before conception, and within 12 weeks after conception, on offspring weight for gestational age, gestational length, and Body Mass Index (BMI) at 36 months of age.

Study Aims:

Aim 1: Evaluate the potential association between hormonal contraceptive use and birth outcomes, including weight for gestational age, small for gestational age, gestational length, and preterm birth

Aim 2: Evaluate the potential association between hormonal contraceptive use and offspring body mass index, overweight or obesity at 36 months of age

In the MoBa cohort, questionnaires are self-administered and offspring height and weight are parent-reported. Exposure to pharmaceutical hormones was assessed through linkage to the Norwegian Prescription Database (NorPD). Generalized linear regression models were used to model the association between sex hormone exposure(s) and study outcomes. Sensitivity analyses were conducted to inform the robustness of study results given the potential for confounding and selection bias.

CHAPTER 1: SIGNIFICANCE AND INNOVATION

1.1 Significance

Worldwide, as of 2010, an estimated 43 million children were overweight or obese. The prevalence of childhood overweight and obesity has increased from 4.2 percent in 1990 to 6.7 percent in 2010.(1-3) In Norway, where the present study took place, it is estimated that 17% of school-aged children are overweight or obese.(4) Children who are overweight or obese are more likely to be overweight or obese in adulthood and to suffer from obesity-precipitated morbidity and mortality.(5, 6) Extensive efforts have been made to stem the rising tide of obesity, primarily in the form of interventions targeted to increase physical activity and improve dietary behaviors of populations at risk of or already overweight or obese. These efforts have met with limited success, suggesting that either diet and exercise patterns cannot fully explain the increase in overweight and obese or the interventions themselves are inadequate in modifying individual behaviors. It is likely that the etiology of overweight and obese is extremely complex and no single pathway exists in the development of overweight or obese. There has been increasing interest in developmental origins of disease, including development of overweight and obese. The proposed study explored whether exposure to exogenous sex hormones, specifically use of hormonal contraceptives, was associated with altered birth and early childhood anthropometric indicators.

Biological rationale

The biological mechanism for altered growth of the fetus and development of overweight or obesity as a result of exogenous sex hormone exposure may be rooted in

insults incurred in very early life, in the first few days post-conception or possibly even before conception. The normal hormonal milieu in menstruating women and in early pregnancy serves as a framework for understanding the possible effects of hormonal contraceptives on offspring outcomes. The menstrual cycle and follicular development

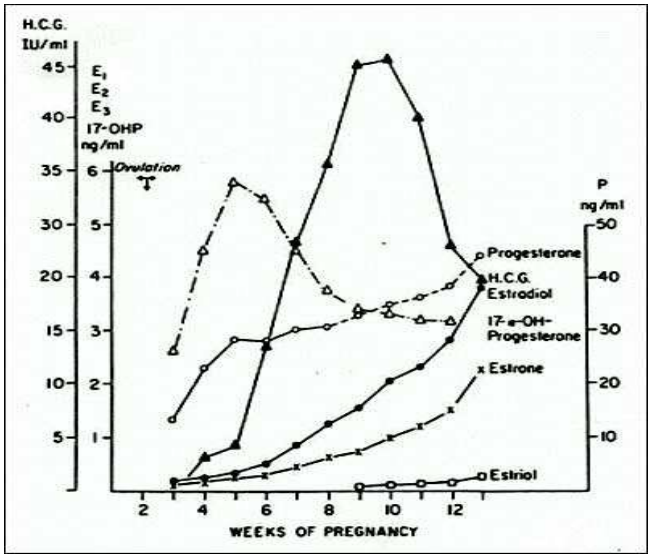
In a typical menstrual cycle, the hypothalamus secretes gonadotropin-releasing hormones (GnRH), which in turn stimulates and modulates pituitary secretion of gonadotropins (follicle stimulating hormone and luteinizing hormone). Ovarian follicles respond to the gonadotropins by synthesizing estradiol and progesterone. Estradiol regulates LH secretion in a complex fashion that leads to the LH surge at midcycle. At the midpoint of the menstrual cycle, LH surges and stimulates ovulation. Following ovulation, the follicle converts to a corpus luteum and begins secreting progesterone. The progesterone level peaks 3-4 days after ovulation and remains elevated until about 14 days post-ovulation. If pregnancy occurs, human chorionic gonadotropin sustains the corpus luteum for 6-7 weeks. If pregnancy does not occur, the corpus luteum decreases in volume and production of progesterone diminishes and estradiol, progesterone, and LH reach their lowest levels in the cycle. In response, FSH production increases and a new menstrual cycle begins. The increase in FSH promotes maturation of ovarian follicles by binding to receptors in the granulosa cells surrounding the oocytes. This in turn promotes cell differentiation and mitosis and an increase in the number of granulosa cells. These granulosa cells then secrete the estradiol that leads to suppression of FSH and secretion of LH in the pituitary gland.(7)

Although selection of a dominant follicle is initiated within the span of the menstrual cycle, follicular and oocyte development begin much earlier. Recruitment of cohorts of primordial follicles into the growing pool of oocytes is an ongoing process that functions independently of gonadotropins. It is estimated that the oocyte destined to ovulate is

recruited 10-12 months prior to ovulation. Intra-ovarian signaling molecules regulate follicle/oocyte growth. Specifically, signaling molecules generated by growing follicles act on non-growing follicles to suppress their growth. This complex milieu is fine-tuned to support a restricted number of follicles to grow and from which the oocyte destined to ovulate will be chosen while the others undergo atresia. These same regulatory signals ensure that most primordial follicles do not grow and are thereby reserved for future recruitment.(8) In animal models, the recruitment of a follicle for selection as a dominant follicle and oocyte maturation has been influenced by exposure to endocrine disrupting chemicals.(9-13)

Endogenous sex hormones in early pregnancy

In a normal pregnancy, the fertilized oocyte implants in the endometrium of the uterus and begins secreting human chorionic gonadotropin (hCG). HCG sustains the corpus luteum for an additional 6-7 weeks which provides sustained release of progesterone.(7) At about 6 weeks gestation the placenta takes over production of progesterone and endogenous levels of both progesterone and estradiol increase dramatically (Figure 1.1).(14)



Source: endotext.org

Figure 1.1 Endogenous sex hormones in early pregnancy

Progesterone is the most abundant and potent progestin in humans. Other, less active forms of progestin include 17α-hydroxyprogesterone and 10α-hydroxyprogesterone. 17β- estradiol is the most abundant and biologically active form of estrogen in humans. Estriol and estrone are less abundant and less active forms of estrogen.(15) Early pregnancy progesterone levels range from 10-35 ng/mL through about 10 weeks of gestation at which point levels rise through the remainder of pregnancy, peaking at a concentration of 100-300 ng/mL. Concentration of 17β-estradiol gradually increases throughout pregnancy, from conception onward, with levels at conception less than 0.4 ng/mL, increasing to a range of 6-30 ng/mL at term.(16)

Hormonal contraception and the menstrual cycle

Hormonal contraceptives are an exogenous, pharmacologic source of sex hormones designed to prevent pregnancy. The primary mechanism by which pregnancy is prevented when using hormonal contraceptives is through suppression of GnRH release from the hypothalamus with resulting suppression of pituitary release of gonadotropins (described above). In preventing release of gonadotropins, the gonadotropin-dependent growth of the follicles from which the dominant follicle would be selected is inhibited and thus ovulation is prevented. Progestin-only contraceptive formulations are less reliable in their suppression of ovulation. About 40% of women will ovulate while taking progestin-only contraceptives. Progestin prevents conception through suppression of LH release, thickening cervical mucus (making it impermeable to sperm), altering fallopian tube peristalsis, and altering the endometrium (making it non-receptive to implantation). The estrogenic component of combination contraceptives, which contain both estrogenic and progestogenic components, suppresses FSH secretion, which prevents selection and development of a dominant follicle. The estrogenic component also increases the effect of the progestin component by

increasing the number of intracellular progesterone receptors, thus increasing the opportunity for progesterone binding and subsequent signaling.(7, 17)

Metabolic effects of hormonal contraceptives

Oral contraceptives can alter lipid profiles, specifically increasing very low-density lipoprotein cholesterol levels and increasing total triglycerides.(18-20) Oral contraceptives also increase blood glucose levels, increase insulin levels, increase plasma cortisol levels (20) and induce a state of insulin resistance.(18, 19) Interestingly, some of the metabolic changes elicited by hormonal contraceptives are similar to the metabolic changes that occur in women who are overweight or obese, specifically increased total cholesterol (21) and increased insulin resistance.(22) These effects have been the source of debate on the safety of oral contraceptives, particularly for women at risk of venous thrombosis and particularly for combination formulation oral contraceptives with an estrogen component. Oral contraceptives are contraindicated for some women. For example, oral contraceptive use is strictly contraindicated in women over the age of 35 who smoke.(7)

Metabolic disruption of early embryo development

Hormonal contraceptive-induced changes in maternal metabolic milieu may alter early embryonic development. In early embryogenesis, alterations in the environment of the embryo, before implantation has occurred, may result in increased metabolic activity of the embryo. This increased metabolic activity may lead to embryonic demise.(23, 24) In more subtle alterations of the environment, it may lead to altered growth and development of the embryo.(25)

The “quiet embryo hypothesis” suggests that metabolically quiet embryos, or embryos with less active mitochondria, are more viable and that a loss of “quietness” can result in reactive oxygen species (oxidative stress) that can damage DNA.(23, 24, 26) There are animal model studies that support this hypothesis. In one study of mouse

embryos, periconception exposure to a high protein diet increased embryonic mitochondrial activity and increased embryonic levels of reactive oxygen species.(27) In another study of the effects of maternal obesity, obese mice, relative to normal weight mice, had an increased number of apoptotic follicles in the ovaries, smaller and fewer oocytes, and smaller pups at birth. By 13 weeks post-birth, these pups were significantly larger than pups born to normal weight mice.(28) Rapid growth, following suppressed growth in early development, may contribute to the subsequent higher weight of offspring born to exposed mice.

Dose and biological activity

Assessing the level of exposure incurred, whether through pharmacologic or environmental sources, is highly complex. Table 1.1 describes exposure by the amount of agent ingested orally.

Table 1.1. Potential daily human exposure to various estrogens

Source	Route	Exposure (µg/d)	Reference
Oral contraceptive, mestranol or ethinyl estradiol	Oral	20-50	Hardman et al. 1996
Hormone replacement therapy, ethinyl estradiol	Oral	50-200	Scott et al. 1991
DES, to prevent spontaneous abortion	Oral	5,000-150,000	Dieckmann et al.1953; Wilcox et al. 1995
Bisphenol A in food cans	Oral	6.3	Howe and Borodinsky 1998; Howe et al. 1998; Wingender et al. 1998
Bisphenol A in beverage containers	Oral	<0.75	Brotons et al. 1995; Howe and Borodinsky 1998; Howe et al. 1998; Wingender et al. 1998
Bisphenol A in dental sealant	Oral	90-931 in first hr	Olea et al. 1996
Nonylphenol in river water	Oral	0.7	Weeks et al. 1996
DDT in total diet	Oral	0.01	Gunderson 1995
PCBs in total diet	Oral	0.002	Gunderson 1995
Phytoestrogen,100 g of wheat	Oral	200	Verdeal and Ryan 1979
Phytoestrogen, total bioflavonoids	Oral	1,000,000	Kuhnau 1976

Source: Commission on Life Sciences. Hormonally active agents in the environment, National Academies Press; 1999.(29)

However, simply evaluating exposure does not take into consideration the biological availability and subsequent action of the substance once it is absorbed. There are many factors that influence the biological effects that these exposures may have on the host. Endogenous steroid hormones bind their receptors and the hormone-receptor complex then either stimulates or represses gene transcription. Hormonally active agents can have similar effects or they can interfere with endogenous hormones, for example, by binding the receptor but not activating gene transcription as efficiently as the endogenous hormone. Different agents have different affinities for binding with sex hormone receptors and their affinity for binding is influenced by their chemical properties and by the environment of the cell, tissue, or organ in which the receptors are located.

Biological activity is also influenced by the half-life of the agent. An agent with a low binding affinity, but longer half-life, may have stronger biological influence than an agent with higher affinity and shorter half-life.(29) The binding affinity of ethinyl estradiol, the synthetic estrogen in most oral contraceptives, is similar to that of 17β-estradiol, the most biologically active form of endogenous estrogen.(30) Environmental sources of estrogen-like compounds have lower relative binding affinities for the estrogen receptor (Table 1.2).(31)

Table 1.2. Relative binding affinities for estradiol competitors*

Chemical name	RBA† (%)	Log RBA	Affinity strength*
Endogenous estrogens			
17β - Estradiol	100.000	2.00	Strong
Estrone	7.309	0.86	Strong
Estriol	9.719	0.99	Strong
Pharmacologic estrogens			
Diethylstilbestrol (DES)	399.556	2.60	Strong
Ethinyl estradiol	190.063	2.28	Strong
Mestranol	2.264	0.35	Strong
Environmental toxicants			
Bisphenol A	0.008	-2.11	weak
Bisphenol B	0.086	-1.07	moderate
o,p-DDT	0.001	-2.85	weak
2,3,4,5 – Tetrachloro-4-biphenylol (a PCB)	0.228	-0.64	moderate

*Adapted from Blair et al., 2000 (31)
†Relative Binding Affinity - defined as IC_{50} for 17β-estradiol ÷ IC_{50} of competitor, where IC_{50} is the molar concentration at which there is 50% inhibition of estradiol binding

Some studies have suggested that there is a non-monotonic association between environmental sources of hormone-like exposures and adverse effects in offspring. These studies generally assess the association between very high levels of exposure.(32) The level of exposure in the present study of sex hormone exposure, while significantly greater than exposures incurred environmentally, are likely low-dose exposures relative to the exposures that demonstrate non-monotonic effects.

The dose incurred through hormonal contraceptives, as compared to environmental agents, is likely higher than environmental sources of estrogen-like compounds, but not as

high as levels encountered through endogenous levels of circulating hormones. The tables below (Tables 1.3 and 1.4) describe the mean serum concentrations of 17β-estradiol and progesterone in pregnant and non-pregnant, menstruating women, and ethinyl estradiol and progestin concentrations in women using oral contraceptives.

Table 1.3. Mean and peak serum concentrations of 17β-estradiol and progesterone over one menstrual cycle and during early pregnancy

	17β-Estradiol (pmol/L)	Progesterone (nmol/L)
Menstrual cycle (no OC)*		
peak†	914.8	40.3
mean daily concentration	352.7	12.0
Early pregnancy‡	1640	49

*Adapted from Stricker et al., 2006 (33)
†Peak estradiol concentration for women spontaneously ovulating is mid-cyle, one day prior to the LH peak, for progesterone the peak concentration is post-ovulation, in the mid-luteal phase
‡At 5 weeks gestation (34)

Table 1.4. Maximum area under the curve (AUC) and mean daily serum concentrations of ethinyl estradiol and progestin concentrations for two oral contraceptives*

Formulation	Ethinyl estradiol (pmol/L)	Progestin (nmol/L)
90 mcg levonorgestrel and 20 mcg ethinyl estradiol		
maximum	251.0	18.2
$AUC_{0\text{-}24\ hours}$	$2491.0_{/24\ hours}$	$236.8_{/24\ hours}$
mean daily concentration	100.8	9.9
1mg norethindrone acetate and 35 mcg ethinyl estradiol		
maximum	381.2	34.7
$AUC_{0\text{-}24\ hours}$	$3876.5_{/24\ hours}$	$297.4_{/24\ hours}$
mean daily concentration	161.5	12.4

*Estimated from drug label data(35)

The estimated mean daily serum concentration of ethinyl estradiol is ~30-45% of the endogenous estradiol concentration but may have stronger binding affinity to the estrogen receptor (Table 1.2) than endogenous estradiol. Other factors that may influence the biological activity of the oral contraceptives are the half-life and drug bioavailability. Unlike 17β-estradiol, ethinyl estradiol does not bind to sex hormone binding globulin (SHBG) but is primarily bound to albumin.(35) These differences further illustrate how exogenous

estrogenic agents of hormonal contraceptives are imperfect in their approximation of endogenous 17β-estradiol.

Summary of existing literature

Experiments on animals have shown a positive association between exogenous perinatal estrogen exposure and metabolic disruption in the offspring, including offspring development of overweight or obesity. These studies generally address the risk of low-dose exposure to environmental sources of estrogen-mimicking compounds.(36-38) For example, neonatal (days 1-5) exposure to 1mg/kg diethylstilbestrol (DES) in mice resulted in an initial period of depressed growth, followed by increasing adiposity through 4 months of age.(39)

Similarly, in another animal model of sows administered varying doses of 17β - estradiol during pregnancy, a period of depressed growth in the offspring, followed by increased growth was observed. At slaughter, ~60 days of age, the proportion of fat mass in piglets from exposed sows was significantly higher than in male piglets from control sows.(40) At least one study, in a mouse model, found an association between exposure in early gestation, prior to uterine implantation, and subsequent offspring obesity.(41)

With respect to use of hormonal contraceptives, there are no published studies to date on long-term effects of hormonal contraceptives on offspring overweight or obesity. Although the long-term effects of oral contraceptives during pregnancy on offspring morbidity or mortality are unknown, there is some evidence that oral contraceptives may increase the risk of some birth defects.(42) Several human studies have explored the association between the use of hormonal contraceptives prior to or after conception on adverse offspring outcomes at birth, with no clear indication as to whether use confers increased risk. A few studies suggest that associations with adverse outcomes were strongest when exposure was near or at the time of conception. Most of these studies

focused on oral contraceptive use before pregnancy. Two studies specifically assessed associations between use in early pregnancy and adverse birth outcomes, but the sample sizes were small.(43, 44) None of the studies have explored differences by progestin-only versus combination formulation contraceptives. A summary of these studies is below (Table 1.5).

Table 1.5. Summary of literature for oral contraceptive (OC) use and selected birth outcomes

Reference	Exposure(s)	Outcome(s)	Study design	Primary result(s)
Rothman K. NEJM 1977(45)	Self-reported OC use before pregnancy (n=~2,000 for non-users and n=~3,000 for former users)	Twinning, birth weight, fetal loss, still birth collected from birth records	Prospective study, self-administered survey	Small reduction in mean birthweight for former versus non-users (p=0.03)
Vessey M. et al. BJOG 1979(43)	Planned versus unplanned pregnancies for OC users (n~1,800 for planned and n=39 for unplanned).	Sex ratio, anomalies, still birth, miscarriages, birth weight, LBW	Prospective study of women recruited through family planning clinics	9.9% LBW in parous unplanned OC user versus 2.0% planned OC user
Alberman E. et al. IJE 1980(46)	Self-reported OC use before pregnancy (n=2,256 for users, n=2,964 for never users)	Congenital anomalies, mean birth weight, stratification by smoking	Cross-sectional, self-administered survey	Mean birthweight 3,398 g vs 3,410 g non-smokers and 3,292 g versus 3,351 g smokers
Polednak A. et al. Teratology 1983(44)	Self-reported OC use in year before (n=665 users and n=716 non-users), around LMP (n=100), after LMP (n=23)	Mean birthweight, proportion LBW	Cross–sectional, telephone-based survey	Trend toward smaller birthweights as exposure approached and surpassed LMP
Pardthaisong T. et al. AJE 1991(47)	Family planning clinic documentation of depo-provera (n=1,573) and OC use (n=601) in pregnancy as compared to former users (n=2,578)	Still birth, termination of pregnancy, miscarriage, ectopic pregnancy, multiples, LBW	Prospective study using medical records, face-to-face interviews, and birth records	1.5 OR (95% CI: 1.2, 1.9) for LBW for depo-provera users versus former users and 1.5 (95% CI: 1.2-2.0) for LBW for OC users versus former users
Mucci L. et al. BJOG 2004(48)	Self-reported OC use before pregnancy (n=205 users and n=55 for non-users, identified at time of birth)	LBW, preeclampsia, birthweight, gestational length, maternal serum levels of estriol and progesterone	Prospective study using maternal interview, birth records, maternal blood draws in pregnancy	Higher birthweight (+200.7 grams) in ever versus never users, no difference in gestational length, increased levels of estriol and progesterone at 27 weeks
Ahn H. et al. Human and Experimental Toxicology 2008(49)	Self-reported use of OC 4 weeks before or after conception (n=120) age and gravidity matched (n=240) to women undergoing risk counseling for use of non-teratogenic drugs	PTB, LBW, LGA, congenital anomalies, birth weight, gestational length	Prospective cohort	No difference in mean birthweight, gestational length, preterm deliveries, LBW, LGA or birth defects
Chen X. et al. EJOG and Reproductive Biology 2009(50)	Prescription drug database account of OCs dispensed at 0-30 days, 31-60, and 61-90 days prior to LMP, individually matched to subjects (1:4 match)	LBW, PTB, Post-term birth as documented in Saskatchewan Health database	Administrative health records study using pharmacy and health registry data	Increased risk of PTB and LBW for users in 0-30 day window -- OR 1.61 (1.01, 2.55) and increased risk of LBW OR 1.93 (1.17, 3.20)

1.2 Innovation

There is evidence to support a mode of action for exogenous sex hormone exposure in causing overweight and obesity in animal models, possibly through metabolic disruption in early embryonic development, but human epidemiologic evidence was nonexistent. Thus, this research took the next step, applying epidemiologic approaches to determine if there is evidence that exogenous sex hormones contribute to the increased prevalence of overweight and obesity. Environmental sources of exogenous hormone exposures are ubiquitous, but confer extremely low-dose exposures. An epidemiologic study of the association resulting from higher dose, pharmaceutical-based use of sex hormones in early pregnancy offered a cost efficient means for studying the potential for harm resulting through lower level exposures.

Few children are exposed to pharmacologic sources of sex hormones in early pregnancy. Although the number of infants conceived through assisted reproductive methods (ART) is increasing, in the United States only 1 percent (2006) of infants born result from use of ART methods.(51) The incidence of oral contraceptive failure varies by country(52) but is estimated to be about 9 percent in the United States.(53, 54) However, of those pregnancies resulting from failed oral contraceptives, many will be intentionally terminated in early pregnancy,(53) further reducing the number of infants whereby effects of exogenous sex hormones can be studied. Consequently, most observational studies are underpowered for assessing the association of pharmacologic sex hormone exposure *in utero* and offspring overweight or obesity. Large cohort studies offer the opportunity to study exposures that are of relatively low incidence, while increasing the potential that there will be sufficient power for adjustment for possible confounders or assessment of possible effect modifiers.

Multiple scientific areas of inquiry may be informed by the findings of this study. Evidence of increased risk for offspring overweight or obesity from early, higher (relative to

environmental sources) dose exposures to sex hormones would lend credence to the hypothesis that low doses, such as those encountered through environmental toxicants, increase risk. This study also yields etiologic clues into the disparity in health outcomes, such as elevated lipid profiles, increased blood pressure, lower birth weight, and shorter gestation,(55, 56) for infants conceived through sex hormone-based assistive reproductive treatments. Most importantly, this epidemiologic study contributes to an improved understanding of one mechanism for the early fetal origins of overweight and obesity upon which future studies and, ultimately, interventions can be built.

CHAPTER 2: APPROACH

2.1 Study design

This study was a secondary analysis of a large, population-based, prospective cohort study, whereby we assessed the association between pharmacologic sex hormone use in early pregnancy and birth outcomes, including small for gestational age, weight for gestational age, preterm birth, and gestational length, and altered weight for height status at 36 months of age, including BMI and overweight or obesity.

2.2 Study population

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective, population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health.(57, 58) The MoBa cohort dataset was the primary source of data for this study and was used to define many of the study variables, either in conjunction with other data sources or in isolation. MoBa study participants were recruited from 1999 through 2008 through mailed invitation and enrolled at the routine ultrasound examination offered to all pregnant women in Norway at 17-19 weeks of gestation. Approximately 42 percent of all pregnant women in Norway, who reached at least 16 weeks of gestation, were invited to participate in the study. Of these, about 40 percent consented to participate. Informed consent was obtained from each MoBa participant upon recruitment. The Regional Committee for Medical Research Ethics in Southeastern Norway approved the study. At enrollment, participants were asked to provide a blood plasma specimen and to complete an initial, self-administered questionnaire to collect demographic characteristics, reproductive health history, disease and medication history, lifestyle factors, and socioeconomic status. Follow-up is conducted

by self-administered questionnaires at regular intervals and by linkage to national health registries. The cohort includes over 108,000 children, over 90,000 mothers, and 71,000 fathers. To date, there are approximately 40,000 children with completed 36-month questionnaires.

The Medical Birth Registry of Norway (MBRN) data was used in this study to define study outcomes (Aim 1) and to estimate the date of conception. MBRN data are available in the MoBa cohort database, through linkage of MoBa data with the MBRN, made possible by the unique personal identifier assigned to all Norwegians. All data in MBRN are collected on a standardized birth notification form completed by the midwife or physician attending the birth. Compared to the source population of all pregnancies in Norway, the MoBa cohort is slightly healthier (Table 2.1).

Table 2.1: Characteristics of 26,777 participants in MoBa and 226,057 births in Norway 2000–2003*

		MoBa cohort	Total population
Parity (%)			
	0	40.3	40.7
	1	36.9	35.7
	2+	22.8	23.6
Maternal age (%)			
	<20	1.2	2.4
	20-24	11.1	14.9
	25-29	34.7	34.2
	30-34	37.5	33.2
	35+	15.5	15.3
Preeclampsia (%)			
	Yes	3.8	3.9
Gestational age (days)			
	Mean (SD)	277.3 (14.7)	276.8 (15.0)
	Median	280	279
Preterm birth (%)			
	Yes	7.2	7.7
Birthweight (grams)			
	Mean (SD)	3587 (626)	3538 (632)
	Median	3630	3575
Low birth weight (%)			
	Yes	4.6	5.1

*Source: Magnus et al. 2006(57)

The Norwegian Prescription Database (NorPD) was linked to the MoBa cohort database to characterize exposure to hormonal contraceptives 12 months before, 4 months

before, 1 month before conception, and within 12 weeks after conception (for women enrolled 2004 or later). By Norwegian law, as of January 1, 2004, all pharmacies must provide electronic data for all prescriptions dispensed. NorPD contains individual-level data on all medications prescribed and dispensed through pharmacies to non-institutionalized individuals in Norway. Classification of drugs is based on the Anatomical Therapeutic Chemical (ATC) classification system.(59) The Norwegian Institute of Public Health has data quality assurance measures for the data collection that include routine evaluation of data completeness and errors. In Norway, between 2004-2006 and based on data collected through the NorPD, an estimated 5.2%, 0.2%, and 0.1% of women during their 1st, 2nd, and 3rd trimesters of pregnancy, respectively, were dispensed pharmacologic sex hormones. During the 1st trimester, 1.5% of women were using sex hormones for contraception and 2.6% were using sex hormones in association with assisted reproductive therapy.(60) In the three months prior to conception, 11.5% of women used pharmacologic sex hormones.

2.3 Study inclusionary/exclusionary criteria

For the purposes of this study, we included all pregnancies conceived after January 1, 2004, resulting in a singleton live birth and without evidence of having received infertility treatment for the index pregnancy. We also excluded pregnancies to women with a history of chronic hypertension and, for assessing the relationship between hormonal contraceptive use and offspring overweight or obesity, infants who died in the first year of life.

2.4 Outcome and exposure assessment

This study examined the association between hormonal contraceptives and fetal growth indicators (weight for gestational age expressed as a continuous measure of birthweight z-score and as a categorical measure of small versus average or large for gestational age) and on body mass index at 36 months of age (expressed as both an age and sex-adjusted continuous measure of weight for age and sex z-score and as a

categorical measure of underweight or normal weight versus overweight or obese). Because childhood obesity status may be influenced by gestational length at birth,(61) we also examined the association between hormonal contraceptive use and preterm birth, both as a categorical measure of preterm birth and as a continuous measure of gestational length. Multiple data sources informed the exposure-outcome analyses (Figure 2.1).

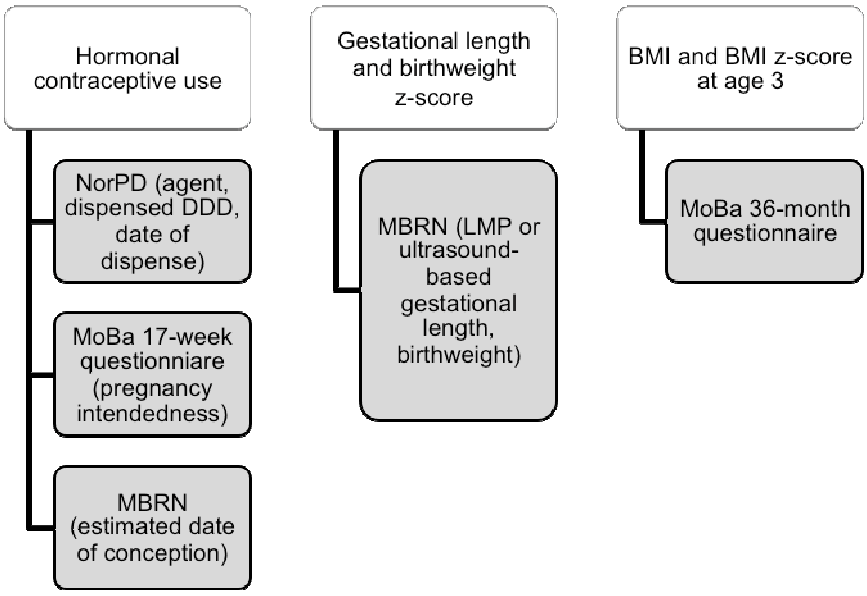


Figure 2.1. Study data sources

Exposure assessment

There were two possible sources of information for hormonal contraceptive use for this study -- self-reported use as reflected on the MoBa questionnaire administered at 17 weeks and the NorPD indicated dispensing of hormonal contraceptives. Ultimately, we chose using the NorPD for assessing exposure to hormonal contraceptives, as there were several limitations to using the self-reported data.

On the MoBa questionnaire administered at about 17 weeks gestation, women report contraceptive use in the 4 months and in the 12 months before conception. Women also report whether they were using contraceptives at the time at which they conceived. In reporting oral contraceptive use in 12 months before conception and at conception, women report whether they were using the progestin-only “mini-pill” or the combination “pill”.

Women had the option of selecting both the combination and progestin-only pill in the year prior to pregnancy. Women did not differentiate between which forms of oral contraceptive they used in the 4 months before pregnancy. Although we could have imputed the type of contraceptive used at 4 months by carrying forward the type of oral contraceptive used in the 12 months before conception, misclassification in characterization of exposure could have occurred in using this approach. It is possible that some women will have been uncomfortable self-reporting oral contraceptive use at the time of pregnancy. Other limitations in using self-reported exposure were that use of some forms of hormonal contraceptives was not collected. Furthermore, there is a potential that some women may not have accurately remembered the type of contraceptive used. Finally, self-reported data would have precluded assessing for potential associations by hormonal contraceptive formulation, including evaluation by progestin type.

Linkage to NorPD provided the date(s) and specific formulation of oral contraceptive(s) prescribed and dispensed to women in MoBa, but is limited to women enrolled in MoBa after 2003. Of the 107,378 pregnancies enrolled in MoBa, there were 32,949 pregnancies enrolled between study inception through 2003.(57) To estimate use in the 12 months preceding conception, we restricted the study population to pregnancies conceived at least 12 months after the date the NorPD registry began. Variables included in the linked file are presented below (Table 2.2).

Table 2.2. Description of variables in linked file

Variable	Description	Valid values
Prescriber		
Prescriber's serial number	Number is generated from prescriber's ID number in health personnel's register	Serial number - unique for every dispensing.
Prescriber's profession		Physician, dentist, veterinary surgeon, nurse, midwife, optician, other profession, profession lacking
Prescriber's specialty		General practitioner, children's illnesses, internal medicine
Patient		
Patient's residential municipality number	Patient's place of residence at time of dispensing of drug. Municipality number contains 4 digits, first two for county, last two for municipality.	0101, 0104, 0211, 0301 etc
Patient's residential municipality name		Halden, Moss, Vestby, Oslo etc.
Patient's residential county number	Patient's place of residence at time of dispensing of drug.	01,02,03, etc
Patient's residential county name		
Dispensed prescriptions		
Dispensing number	Serial number for individual dispensed prescriptions	Numerical, number of digits will vary
Dispensing date		yyyy-mm-dd
Number of packs	Dispensed number of packs	Numerical, with decimals
Number of DDD	Dispensed number of DDD	Numerical, with decimals
Drug		
Drug's description	Name, strength and format	Alphanumerical
Size of packet	Number of tablets, gram, ml, etc	Numerical
Packaging unit		Dosage format
Strength of drug		25mg, 1g, 0.5mg/ml, 2500IU/syringe etc
ATC code	Drug's ATC code 1. - 5	Alphanumerical
DDD	Defined daily dosage (DDD) for ATC code	Numerical, up to 3 decimals
DDD unit	Unit of measurement for DDD	g, mg, IU etc
Dispensing group	Prescription category set by Norwegian Medicine Agency	Norwegian Prescription Category (A, B, C, F(OTC))
Pack's recommended retail price (RRP) at point of sale	Pharmacy retail price at point of sale	Norwegian kroner 00.00

Source: National Institute of Public Health, Oslo, Norway

Date of conception was estimated by subtracting 17 days(62) from the number of days of gestational length at birth (to account for the follicular phase prior to conception) and then subtracting this value from the date of birth. We used the last menstrual period (LMP)-based estimated gestational length unless the LMP-based gestational length was missing or

≥2 weeks from the ultrasound-based estimate of gestational length, in which case we used the ultrasound based measure.(63) We then constructed an exposure window for each hormonal contraceptive prescription filled using the date that the prescription was filled and the number of defined daily doses dispensed (day's supply). We characterized exposure into discrete categories by last date of use relative to conception; specifically last use 12 - >4 months before, 4 - >1 months before, and 1 - > 0 months before, and 0 -12 weeks after. These exposure categories roughly correspond to the periods of primordial follicular growth (12 months before), secondary and primary follicular growth (4 months before), ovulation (1 month before), and early embryo development (up to 12 weeks after conception).

Use of the NorPD to characterize exposure was not without limitations. In using NorPD, we could not know about, or be able to characterize, exposure according to how well women adhered to guidelines for prescription use. Women may have had varying levels of exposure, depending on how closely they followed the prescription regime.

Outcome assessment

Aim 1 (Birth) - For Aim 1, we evaluated the association between hormonal contraceptive use and birth weight for gestational age and length of gestation. These outcomes were characterized as follows:

Birthweight for gestational age z-score:

Birthweight and gestational age at birth were recorded on the MBRN. After identifying and excluding observations with biologically implausible values (gestational age <22 weeks or > 45 weeks or birthweight <1000 grams for infants born at ≥37 weeks), birthweight for gestational age was characterized as both a continuous measure (birthweight for gestational age z-score), and categorically as small versus average or large for gestational age. Small for gestational age (SGA) was characterized as having been born at < 3rd percentile for weight for gestational age.(64) Weight for gestational age percentile was calculated from a population standard as described by Mikolajczyk et al.(65)

Gestational length:

As described previously, we used the last menstrual period (LMP)-based estimated gestational length unless the LMP-based gestational length was missing or ≥2 weeks from the ultrasound-based estimate of gestational length, in which case we used the ultrasound based measure.(63) Last menstrual period (LMP) derived due dates (Naegele's rule) can be inaccurate due to varying menstrual cycle lengths, uncertainty of LMP, and non-menstrual bleeding not associated with menses (from OC use or mid-cycle bleeding). Ultrasound-based due dates can also be inaccurate as reference values are based on expected fetal size values using LMP. For fetuses that are either small or large for gestational age, ultrasound-based due dates will be biased.(66) Preterm birth was defined as delivery before 37 completed weeks of pregnancy,(67) assessed from the gestational length in days.

Aim 2 (36 months of age) - For Aim 2 we evaluated the association between hormonal contraceptive use and BMI z-score at age ~36 months. BMI was characterized as follows:

BMI as a continuous measure:

Variables for defining the outcome at 36 months of age included child age (in days at 36 month questionnaire), child sex, and child height and weight. First, we calculated age and sex adjusted z scores for height, weight and weight for height. The z-scores were generated using the World Health Organization (WHO) Child Growth Standards.(68) BMI was calculated by using the standard definition for BMI, kg/m^2.(69) Next, we identified and excluded observations with biologically implausible values. The WHO Standards include recommendations for excluding biologically implausible values based on calculated z-scores for height, weight and BMI. Extreme (i.e. biologically implausible) z-scores for each indicator were flagged as follows:

- Weight-for-age z-score (ZWEI) ZWEI<-6 or ZWEI >5

- Length/height-for-age z-score (ZLEN) ZLEN<-6 or ZLEN >6
- Weight-for-length/height z-score (ZWFL) ZWFL<-5 or ZWFL>5
- BMI-for-age z-score (ZBMI) ZBMI <-5 or ZBMI >5(68)

We included children with documented height and weight measurements between 30 months and 42 months of age.

Overweight or obese at 36 months:

We characterized children as overweight or obese using BMI cutpoints for overweight or obesity as established by the International Obesity Task Force (IOTF). The IOTF generated standard definitions for defining overweight or obese in children using pooled data collected from nationally representative data from cross sectional surveys on growth in Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the United States.(70) The BMI cutpoints are based on child sex and age and, projected out to adulthood, correspond to adult overweight or obese.

2.5 Data analyses

This was an epidemiologic study of the association between hormonal contraceptive use before and in early pregnancy and offspring anthropometric indicators at birth and at 36 months of age. We hypothesized that hormonal contraceptives would have a negative inverse association with neonatal anthropometric measures and gestational length (Aim 1) and a positive association with offspring BMI, odds of overweight or obese at 36 months of age (Aim 2).

Data preparation

The MoBa cohort data was linked with the MBRN data at the Norwegian Institute of Public Health. For this study, the Norwegian Mother and Child Cohort Study Scientific Committee granted permission to link the MoBa/MBRN data with NorPD for characterization of sex hormone exposure. Once the final data sets were received and linked (by the pseudo

personal identifier assigned for the purposes of this study), we inspected the data for biologically implausible values for study covariates and set to missing those values deemed implausible.

Exposure-outcome analyses

Aim 1 (birth) - We used generalized linear models to assess the relationship between hormonal contraceptive use and continuously measured birth outcomes. Specifically, we fit a linear model (identity link) to assess the association between exogenous sex hormone exposure and birth weight for gestational length z-score and length of gestation. A binomial distribution, logistic model (logit link) was used to estimate the odds for preterm birth as compared to full term birth.

Aim 2 (age 3) - We used generalized linear models to assess the relationship between exogenous sex hormones and offspring obesity. We fit a linear model to assess the association between exogenous sex hormone exposure and body mass index z-score at age 36 months and a logistic model to estimate the odds for overweight or obese versus normal weight.

Aims 1 and 2 - Covariate-outcome relationships were explored non-parametrically first, to determine whether covariates should be characterized continuously, using quadratic terms, through use of an indicator variable, or with splines.

Where data were of sufficient sample size, we assessed for effect modification by maternal pre-pregnancy BMI (underweight or normal weight versus overweight or obese) and by offspring sex through inclusion of an interaction term in the models. We used the Wald-type p-value (p value < 0.20) as an initial screen to assess for evidence of possible effect modification by maternal pre-pregnancy BMI or offspring sex.(71) If there was evidence of possible effect modification, we produced stratum specific estimates and examined confidence intervals for evidence of overlap. Significant overlap suggested insufficient evidence for effect modification. If estimates and confidence intervals were

disparate, we reported results separately across maternal BMI and/or offspring sex categories. All models used Generalized Estimation Equations (GEE) to obtain robust variance estimators and account for multiple children in the study from the same family. All analyses were conducted using SAS 9.3 (SAS Inc, Cary, NC).

2.6 Study biases

Confounding

The association between hormonal contraceptive use and offspring anthropometric indicators is likely confounded by multiple factors that are direct or indirect antecedents of both the exposure and outcome. Women who conceive while taking an oral contraception are unique in that they may either have poor adherence to taking the oral contraceptive and/or they are highly fecund. With perfect use, the 'failure' rate for oral contraceptives is <1%.(54) Fecundity, among women not currently using a hormonal contraceptive, is associated with maternal age, prior oral contraceptive use, irregular or long menstrual cycles,(72, 73) heavy smoking,(72, 74) heavy alcohol use, pre-pregnancy BMI,(72) and some health conditions (polycystic ovarian syndrome, endometriosis, autoimmune disease,(75) and history of cancer treatment).(76)

Studies on hormonal contraceptive adherence cite parity,(77) unawareness of the potential benefits of oral contraception outside of prevention of pregnancy,(78, 79) experiencing side effects,(78, 80) and ability to access prescriptions(77) as factors contributing to prescription adherence.

To estimate the association between hormonal contraceptive use and offspring anthropometric outcomes, we considered which factors could be antecedents of both hormonal contraceptive use and offspring growth. For early pregnancy use, we further considered factors related to hormonal contraceptive adherence. We used directed acyclic graphs (DAGs) to identify the minimally sufficient sets for inclusion of possible study

confounders.(81) Figure 2.2 illustrates a DAG representing the possible relationship between hormonal contraceptives and offspring overweight or obesity.

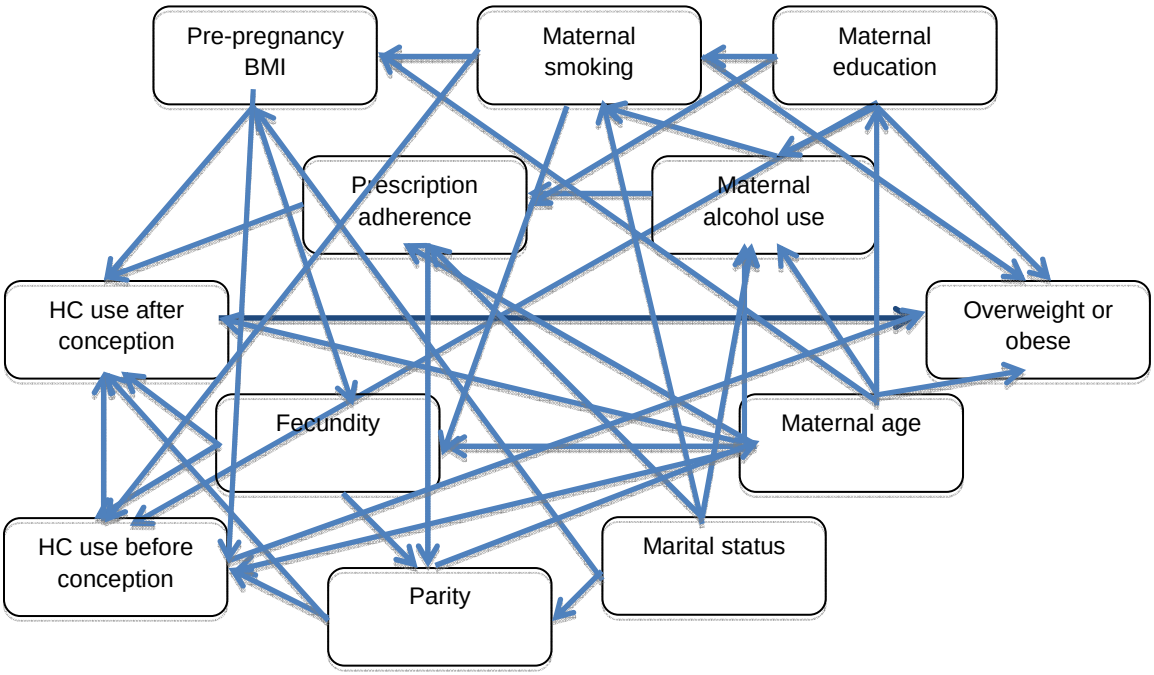


Figure 2.2. DAG for early pregnancy use of hormonal contraceptives and overweight or obesity

In this DAG, there were several minimally sufficient sets that could have been used to minimize the potential for confounding as introduced by these maternal characteristics. These included any of the following:

1. Adherence, Fecundity, Maternal age, Parity, Pre-pregnancy BMI, Maternal smoking

2. Adherence, Fecundity, Hormonal contraceptive use before conception

3. Maternal alcohol use, Fecundity, Maternal Age, Maternal education, Hormonal contraceptive use before conception, Pre-pregnancy BMI

4. Fecundity, Maternal age, Maternal education, Hormonal contraceptive use before conception, Pre-pregnancy BMI, Maternal smoking

5. Maternal education, Parity, Pre-pregnancy BMI, Maternal smoking

To identify which minimally sufficient set to use, we considered which of the measures had the most validity and/or reliability, and which measures had the strongest

association with the exposure and outcome. MoBa does not include a measure of adherence -- this is an unmeasured variable in this DAG. Time to conceive could have served as a proxy for fecundity. However, all women who conceive while taking an oral contraceptive have had a time to conceive of 0. Conditioning on time to conceive might create convergence issues due to non-positivity or insufficient overlap in distribution of covariates. Given these factors, we selected the set including maternal education, parity, pre-pregnancy BMI, and maternal smoking, for our minimally sufficient adjustment set.

Loss to follow up or out-selection bias

In assessing the association between hormonal contraceptive use and offspring overweight or obesity there was the potential for out-selection bias, resulting from loss to follow-up. If out-selection is associated with covariates that are causal of the exposures of interest (or with factors associated with the exposure) and also associated with the outcome, there is a potential for introducing collider stratification bias when limiting a sample population to the risk set remaining after selection has occurred.

Simply adjusting on factors associated with selection may also introduce collider stratification bias if there are unmeasured factors associated with selection. Therefore, we used a multiple imputation approach(82) to impute 10 data sets with imputed follow-up data for offspring BMI. We modeled the association between hormonal contraceptive exposure and offspring overweight or obesity for each of the imputed datasets. The model results for each data set were then synthesized to obtain final estimates for the multiple imputation approach. As an alternative approach to multiple imputation, we also conducted a sensitivity analysis using inverse probability weighting to account for loss to follow-up.(83)

Selection bias due to missing covariate data

We also explored the degree of missing data present among study covariates. If there had been more than 15% missing on any one of the study covariates or combination of study covariates, we would have explored the appropriateness of imputing missing

covariate data by first constructing a predictive model for missingness, using covariates and outcomes selected for inclusion in the study. If missing completely at random, missingness will not bias estimates but could increase variance. If missing at random, whereby missingness is conditional on one or more covariates under observation, both estimates and variance could be biased. Under the assumption that there were not unobserved study variables influencing missingness (in which case multiple imputation would be inappropriate), we could have used multiple imputation to impute the missing data.(82) Analyses would then be carried out using data with and without imputations to assess the sensitivity of the study estimates to imputing missing covariate data.

2.7 Public health relevance

This was an epidemiologic study of the influence of pharmacologic sex hormone exposure, specifically hormonal contraceptives, on offspring birth outcomes and early childhood measures of BMI, overweight or obesity. Although there is considerable concern for the possible risks of exogenous sex hormone exposures conferred through environmental sources, these exposures are small and the effects are difficult to assess in human populations. This study explored the risk of pharmaceutical-based sex hormones in their own right, but also as a proof of principle for understanding the potential for harm from lower dose exposures.

Extensive efforts have been made to stem the rising tide of obesity, primarily in the form of interventions targeted to change individual behavior, specifically increasing physical activity and improving dietary behaviors of populations at risk of or already overweight or obese. These efforts have met with limited success and researchers and public health advocates recognize that addressing individual diet and exercise behaviors may not fully address the growing epidemic of overweight and obesity. Although it is likely that the etiology of overweight and obesity is extremely complex and no single pathway exists in the development of overweight or obesity, there are indications that the origins of overweight

and obesity may be rooted in insults incurred during fetal development. This study evaluated the association between before and in early pregnancy, exogenous sex hormone exposure and altered growth as indicated through offspring anthropometric indicators.

2.8 Study limitations and other considerations

This study had a limitation of exploring pharmacologic sex hormone effects before and during early pregnancy only. Although early pregnancy exposure confers the highest potential for relative increase in sex hormones, in that this is the period of pregnancy when endogenous levels are still relatively low, this study did not assess the risk of relatively low dose exposures aggregated over time throughout pregnancy, such as those experienced through environmental sources. It also did not assess the effects of exposure on later onset (after age 3) overweight or obesity. A proposed mechanism underlying this study, described previously, suggests that early, preimplantation exposure, may lead to downstream effects on fetal development, gestational length, and offspring growth. However there may be other mechanisms, possibly outside this window of exposure that could lead to long-term effects on fetal and postnatal growth. An absence of an association between pharmacologic sources of sex hormones and offspring growth indicators at one point in time does not preclude the possibility that exogenous sex hormone exposures could confer risk for altered growth trajectory of the developing fetus, neonate, or child.

Although use of prescription registry data offers considerable detail in the timing and formulation of the agents prescribed, there is considerable opportunity for misclassification of use. The extent to which women adhered to the guidelines for taking the prescription is unknown. The likelihood for misclassification, likely over-reporting of use, may increase as the date of conception approaches given the likelihood that many women will have discontinued taking hormonal contraceptives to conceive. We minimized the potential for misclassification of exposure in early pregnancy by indicating early pregnancy use only when women also reported the pregnancy was unplanned.

CHAPTER 3: HORMONAL CONTRACEPTIVE USE IN RELATION TO PRETERM BIRTH AND SMALL FOR GESTATIONAL AGE

3.1 Introduction

Outcomes at birth, including preterm birth and small for gestational age at birth, can serve as markers of impaired or altered fetal growth and development. The cascade of events leading to parturition are not well understood but are believed to involve signaling from both the mother and the fetus.(84) Being small for gestational age, particularly for infants born at the extreme lower end of the distribution, can indicate impaired growth during fetal development.(85) Exposure to exogenous sources of hormonally active agents, especially during developmentally sensitive periods, may contribute to altered growth and development.

Animal models provide evidence that developmentally sensitive periods begin even before conception.(9-13) The metabolic activity of preimplantation embryos can be changed as a consequence of alterations in their environment.(23, 24) Even subtle environmental alterations may lead to altered growth and development of the embryo, early parturition and preterm delivery.(25)

Much of the literature concerning exposure to hormonally active agents has been centered on exposure to environmental compounds that may have endocrine effects. However there are pharmacologic sources of hormonally active agents too, the most obvious being hormonal contraceptives.

Hormonal contraceptives contain, most commonly, a synthetic estrogen and progestin component. Some forms of hormonal contraceptives contain a progestin

component only. Possible adverse effects in pregnancy, resulting from use of hormonal contraceptives have been studied. The results of these studies have been largely inconclusive, with no consistent pattern emerging for effects of hormonal contraceptive use on pregnancy outcomes.(43-48) Many of these studies have focused on low birth weight, an imperfect measure of fetal growth and development.(86) Hormonal contraceptives contain, most commonly, a synthetic estrogen and progestin component. Some forms of hormonal contraceptives contain a progestin component only. The potential for exposure to these exogenous hormones to adversely affect pregnancy has been studied, but no consistent patterns of association have emerged.(43-48) Many of these studies have focused on low birth weight, which does not appropriately represent fetal growth and development across all gestational ages, and did not account for different hormones comprising the contraceptives.(86)

To our knowledge, there are no studies of the association between hormonal contraceptive use and birth outcomes whereby exposure to hormonal contraceptives was evaluated according to progestin type. We are also not aware of any studies evaluating birth outcomes for users of the progestin-only oral contraceptive. Given the differences in metabolic effects from hormonal contraceptive formulations with varying progestin components,(18-20, 87) and the relationship between maternal metabolic factors and offspring birth outcomes, evaluating the association between hormonal contraceptive by progestin type is warranted.

We used the Norwegian Mother and Child Cohort Study (MoBa), a prospective, population-based cohort study conducted by the Norwegian Institute of Public Health,(57, 58) to evaluate the association between hormonal contraceptive use before and in early pregnancy, and birth outcomes. The data collected through the MoBa cohort study was linked to the Norwegian Prescription Registry data (NorPD), and to the Medical Birth Registry of Norway (MBRN) data. In linking to the prescription registry, the formulation of

hormonal contraceptive could be characterized with finer detail than has been described previously.

3.2 Methods

Primary analyses

Study population:

MoBa study participants were recruited from 1999 through 2008. Women were identified for eligibility when scheduling the routine prenatal ultrasound offered free of charge to all pregnant women in Norway at 17-20 weeks of gestation. Women were then mailed an invitation to participate before the scheduled ultrasound, with informed consent and enrollment taking place at the ultrasound examination. All hospitals with at least 100 births per year participated in the study recruitment and enrollment. Approximately 42 percent of all pregnant women in Norway were invited to participate in the study. Of these, 39 percent consented to participate. At enrollment, participants were asked to provide a blood sample and to complete an initial, self-administered questionnaire to provide data on demographic characteristics, reproductive health history, disease and medication history, lifestyle factors, and socioeconomic status. Follow-up is ongoing and is conducted through self-administered questionnaires at regular intervals and by linkage to national health registries.

All MBRN birth registry data are collected on a standardized birth notification form completed by the midwife or physician attending the birth. Prescription data from NorPD contains individual-level data on all medications prescribed and dispensed through pharmacies to non-institutionalized individuals in Norway. By Norwegian law, as of January 1, 2004, all pharmacies must provide electronic data for all prescriptions dispensed.

There were 107,308 pregnancies in the MoBa cohort. For the present analysis, we included pregnancies resulting in a singleton live birth and excluded pregnancies with documentation of infertility treatment for the index pregnancy, on either the MoBa 17-week

questionnaire or the MBRN. We additionally excluded pregnancies to women who had documented pre-pregnancy chronic hypertension (n=527). As the NorPD registry was not initiated until January 1, 2004, we further restricted our study population to pregnancies of women enrolled at least 12 months after the date on which the NorPD registry began collection of data on prescription fills (n=48,615). We excluded pregnancies with missing covariate data (n=4,191). The final study population included 44,734 pregnancies to 42,155 women (Figure 3.1).

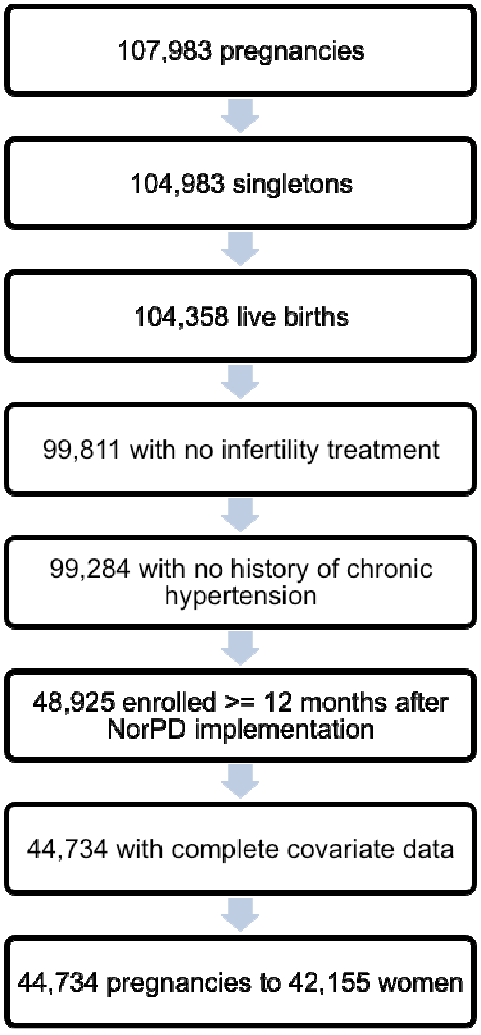


Figure 3.1. Study population selection for Aim 1

All Norwegian residents are assigned a personal identifier number. Linkage of the MoBa questionnaire, MBRN, and NorPD data files were possible through this identifier. The

University of North Carolina at Chapel Hill and the National Institute of Environmental Health Sciences Institutional Review Boards and the Norwegian Southeastern Regional Ethics Committee reviewed and approved this study.

Exposure:

Hormonal contraceptive use in early pregnancy and prior to conception was characterized according to the Anatomical Therapeutic Chemical (ATC) Classification System.(59) We characterized exposure by type and route of administration (combination oral contraceptive, progestin-only oral contraceptive, vaginal ring, transdermal, injectable, implant, and hormonal-based intrauterine device) and by type, route, and progestin component. All hormonal contraceptives with an estrogen component (combination oral contraceptives, vaginal ring, and the transdermal contraceptive) contained ethinyl estradiol, but there were eight different progestin types used solely or in combination with ethinyl estradiol, including desogestrel, drospirenone, levonorgestrel, norelgestromin, norethisterone, lynestronol, medroxyprogesterone, and etonogestrel.

Date of conception was estimated by subtracting 17 days(62) from the total number of days of gestation (to account for the follicular phase prior to conception) and then subtracting this value from the date of birth. We used the last menstrual period (LMP)-based estimated gestational length unless the LMP-based gestational length was missing or ≥2 weeks different from the ultrasound-based estimate of gestational length, in which case we used the ultrasound based measure.(63) We then constructed an exposure window for each hormonal contraceptive prescription filled using the date that the prescription was filled and the number of defined daily doses dispensed (day's supply). We characterized exposure into discrete categories relative to conception; specifically last use 12 - >4 months before, 4 - >1 months before, and 1 - > 0 months before, and 0 -12 weeks after. Women using hormonal contraceptives in early pregnancy were also using hormonal contraceptives before pregnancy. In our analyses, we evaluated last date of use as compared to no

hormonal contraceptive use within any of the exposure periods assessed. For example, women whose last date of use was 1, 4, or 12 months before conception were not included in the analysis evaluating use within 12 weeks after conception.

Most oral contraceptives were dispensed in 3-month supply (82%) or 6-month supply (15%). For pregnancies with more than one type of hormonal contraceptive prescribed, we assigned exposure type according to the type of contraceptive used closest to the estimated date of conception. Because many women may stop taking their hormonal contraceptive in order to achieve conception, we characterized women as exposed in early pregnancy only if they reported on the 17-week questionnaire that the pregnancy was unplanned and they had ≥ 1 day(s) supply of hormonal contraceptive at or after the day of conception as defined above.

Outcome:

Preterm birth was defined as delivery before 37 completed weeks of pregnancy,(67) assessed from the gestational length in days. Small for gestational age (SGA) was characterized as having been born at <3rd percentile for weight for gestational age.(64) Weight for gestational age percentile was calculated from a population standard as described by Mikolajczyk et al.(65) Birthweight z-score was calculated by subtracting the observed birthweight from the expected birthweight based on the population standard distribution and then dividing this value by the standard deviation for each gestational age.

Covariates:

Covariate selection was informed through construction of a directed acyclic graph(81) and included adjustment for factors demonstrated to be causal ancestors of both contraceptive use and adverse pregnancy outcomes, including preterm delivery and fetal growth restriction. These factors included maternal age (14-19, 20-29, 30-39, 40-49 years),(72, 73) maternal pre-pregnancy BMI (kg/m^2)(<18.5, 18.5-24.9, ≥25.0),(65, 72, 88) parity (0, 1, ≥2),(77) maternal smoking (none, former smoker, current smoker),(72, 74) and

maternal education (>4 years of university or technical, 4 year university or technical degree, 3 years of college preparatory high school, 3 years of technical high school, 1-2 years of high school, <9 years of secondary school, other).(78, 79)

Analysis:

Our primary analyses were concerned with assessing the association between hormonal contraceptive use, by type (combination versus progestin-only) and route (oral, vaginal, transdermal, and injectable), and preterm birth or SGA within the discrete categories of within 12 months, 4 months, and 1 month before conception and within 12 weeks after conception. For each exposure period, any contraceptive with < 10 exposed cases were combined into a single "other" exposure category. We used generalized linear models with a logit link, and generalized estimation equations (GEE) with an independent correlation matrix(89) to estimate associations between exposures and outcomes using robust standard errors, accounting for lack of independence between siblings.

We conducted tests of homogeneity to evaluate whether different progestins in the contraceptive formulation were differently associated with preterm birth or SGA. We used generalized linear models to obtain the log likelihood for three, nested models; first modeling any hormonal contraceptive use in each of the exposure periods as compared to no use at any of the exposure periods, second, hormonal contraceptive use by type and route as compared to no use, and third, contraceptive use by type, route and progestin formulation as compared to no use. We conducted likelihood ratio tests to assess whether the model fit improved from characterizing exposure with increasing detail. All analyses were conducted using SAS v9.3 (SAS Inc., Cary, North Carolina).

Sensitivity analyses

Examination of the covariate distribution among pregnancies with hormonal contraceptive use in early pregnancy, as compared to non-users, indicated that women with hormonal contraceptive use were generally older and more parous than non-users

(Appendix, Supplementary Table S3.1). There were also differences in the characteristics of women using different types of hormonal contraceptives. For example, while 71% of combination oral contraceptive users were nulliparous, just 17.2% of progestin-only oral contraceptive users were nulliparous. These differences suggest that prescribing patterns may differ based on individual factors, thus raising the concern for confounding by indication. We explored the robustness of our results through sensitivity analyses designed to mitigate the potential for confounding by indication.

First, because combination oral contraceptive users were much likely to be nulliparous than progestin-only oral contraceptive users, we restricted our analysis to nulliparous pregnancies only. This approach was intended to reduce the potential for confounding based on differences in prescribing patterns for women who were parous. Next, we compared the association comparing combination oral contraceptive use to vaginal ring use (Appendix, Supplementary Table S3.1), because these two groups were socio-demographically most similar. Finally, we conducted a propensity score analysis to reduce residual confounding associated with use of combination oral contraceptives as compared to no use of a hormonal contraceptive.

To construct the propensity scores, we evaluated several models to estimate the predicted probability of obtaining a combination oral contraceptive prescription (propensity for treatment scores). We included in the models those factors believed to be associated with both use of the combination oral contraceptive and preterm birth (parity, maternal pre-pregnancy body mass index, maternal age, maternal education, maternal smoking), but that preceded the use of a combination oral contraceptive. We compared the distribution of propensity scores among those prescribed a combination hormonal contraceptive to those not prescribed any hormonal contraceptive, to evaluate evidence of common support, and trimmed any observation for which there was no corresponding propensity score. We then ranked the scores into deciles and assigned each observation a corresponding rank. We

used GEE models to assess the relationship of combination oral contraceptive use with preterm birth, with inclusion of an indicator term for rank, and obtained a pooled estimate of the association across strata. In addition to the term for rank, these models included all of the potential confounders from the primary analyses models.(90-92) In evaluating the covariate balance within propensity score rank for exposure within 12 months, the distribution of study covariates was similar within rank (Appendix, Supplementary Table S3.2).

3.3 Results

As noted above, there were 44,734 pregnancies that met study inclusionary criteria. Of these, nearly all were to women between the ages of 20-39 (97%), approximately half were first pregnancies (47.1%), and the majority had at least some college education (81.4%). Roughly a third (30.7%) of the pregnancies were in women who were overweight or obese (Table 3.1).

Table 3.1. Study population characteristics among women participating in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

Characteristic		Study population* n=44,734 %
Maternal age (years)		
	14-19	0.9
	20-29	42.1
	30-39	54.9
	40-49	2.1
Maternal BMI (kg/m^2)		
	<18.5	3.2
	18.5-24.9	66.0
	25.0-29.9	21.7
	≥30.0	9.0
Parity		
	0	47.1
	1	35.5
	2	13.7
	3	2.8
	4 or more	0.9
Maternal education		
	More than 4 years of university or technical	27.2
	4 year university degree, regional technical	40.8
	3 years high school, junior college	13.4
	Technical high school	11.2
	1-2 years high school	3.9
	9-year secondary	2.2
	Other	1.4
Maternal smoking (at 17 weeks)		
	None	79.3
	Quit	14.5

*Represents unique pregnancies conceived without infertility treatments, resulting in a singleton live birth, with a date of birth ≥12 months after NorPD registry began (January 1, 2004)

There were 1,969 (4.4%) births before 37 completed weeks of gestation and 1,167 (2.6%) infants born SGA in the study sample. After characterizing hormonal contraceptive use into discrete exposure windows from which estimates could be obtained for modeling the association between use at a given time, there were 7,470 exposed pregnancies within 12 months before, 5,740 exposed pregnancies within 4 months before, 6,465 exposed pregnancies within 1 month before, and 1,638 exposed pregnancies within 12 weeks after conception.

In evaluating hormonal contraceptive use by type and route of administration, we observed a positive association between use of a combination oral contraceptive, as compared to no use of a hormonal contraceptive, and preterm birth across all exposure periods, with the magnitude of the association remaining relatively consistent regardless of the exposure period (Table 3.2).

Table 3.2. Hormonal contraceptive use and gestational length at birth by period of last use, progestin type, and route of administration in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

Exposure	Preterm birth				Gestational length (days)	
	Exposed (n)	Preterm (n)	OR (95% CI)	aOR* (95% CI)	β (95% CI) unadjusted	β (95% CI) adjusted*
None	23,421	964	referent	referent	referent	referent
Within 12 weeks after conception						
Combination OC	1,062	71	1.67 (1.30, 2.14)	1.32 (1.01, 1.73)	-1.19 (-2.08, -0.29)	-0.73 (-1.63, 0.18)
Progestin-only OC	359	18	1.23 (0.76, 1.98)	1.26 (0.78, 2.04)	-0.52 (-1.75, 0.71)	-0.47 (-1.70, 0.76)
Other**	217	5	0.55 (0.23, 1.34)	0.49 (0.20, 1.21)	1.28 (-0.29, 2.85)	1.48 (-0.11, 3.06)
Within 1 month before conception						
Combination OC	4,660	225	1.18 (1.02, 1.37)	1.13 (0.97, 1.31)	0.11 (-0.30, 0.51)	-0.09 (-0.50, 0.33)
Progestin-only OC	1,204	30	0.60 (0.41, 0.86)	0.67 (0.46, 0.97)	0.87 (0.26, 1.48)	0.66 (0.05, 1.27)
Vaginal ring	356	13	0.88 (0.51, 1.54)	0.86 (0.49, 1.51)	0.18 (-1.06, 1.43)	-0.10 (-1.35, 1.16)
Other **	245	7	0.69 (0.32, 1.46)	0.66 (0.31, 1.42)	0.16 (-1.16, 1.48)	0.05 (-1.27, 1.36)
Within 4 months before conception						
Combination OC	3,833	213	1.37 (1.18, 1.60)	1.31 (1.11, 1.53)	-0.49 (-0.93, -0.04)	-0.73 (-1.19, -0.26)
Progestin-only OC	1,284	53	1.00 (0.76, 1.33)	1.15 (0.87, 1.53)	0.39 (-0.27, 1.05)	0.18 (-0.48, 0.85)
Vaginal ring	352	12	0.82 (0.46, 1.47)	0.80 (0.45, 1.43)	0.32 (-0.89, 1.52)	0.04 (-1.16, 1.25)
Other **	271	9	0.80 (0.41, 1.56)	0.76 (0.39, 1.49)	0.39 (-0.83, 1.61)	0.30 (-0.93, 1.52)
Within 12 months before conception						
Combination OC	4,633	241	1.28 (1.11, 1.48)	1.21 (1.04, 1.41)	-0.10 (-0.51, 0.31)	-0.31 (-0.74, 0.11)
Progestin-only OC	1,795	71	0.96 (0.75, 1.23)	1.10 (0.85, 1.40)	-0.38 (-0.96, 0.19)	-0.56 (-1.14, 0.001)
Vaginal ring	424	12	0.68 (0.38, 1.21)	0.68 (0.38, 1.20)	0.83 (-0.32, 1.98)	0.54 (-0.63, 1.70)
Transdermal	295	10	0.82 (0.43, 1.54)	0.78 (0.41, 1.48)	1.12 (-0.11, 2.34)	1.03 (-0.19, 2.25)
Injectable	180	14	1.96 (1.13, 3.40)	1.83 (1.06, 3.18)	-1.58 (-3.49, 0.33)	-1.72 (-3.95, 0.50)
Other **	143	1	0.16 (0.02, 1.17)	0.18 (0.02, 1.27)	2.47 (0.99, 3.96)	2.56 (1.07, 4.05)

*Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth

**Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

For the progestin-only oral contraceptive, we observed an inverse association with preterm birth for use within 1 month of conception (aOR: 0.67, 95% CI: 0.46, 0.97) and a positive, but imprecise association at each of the other exposure periods. No other associations with preterm birth were observed except for use of an injectable contraceptive at 12 months before conception. Use of an injectable contraceptive, at 12 months before conception, was positively associated with preterm birth (aOR: 1.83, 95% CI: 1.06, 3.18). Data were too sparse to evaluate the association between the injectable and preterm birth for any of the exposure periods. The association between use of a hormonal contraceptive and gestational length, in days, was generally consistent with the estimates obtained from modeling the association with preterm birth. For the combination oral contraceptive, the predicted reduction in mean days of gestational length, given exposure within 12 weeks after conception was -0.73 days (95% CI: -1.63, 0.18) (Table 3.2).

Likelihood ratio tests indicated improved model fit ($p<0.05$) when characterizing exposure by type, route, and progestin component for exposure periods of within 4 months and 1 month before conception, and within 12 weeks after conception. For example, for exposure with 12 weeks of conception the magnitude of the association for use of a combination oral contraceptive with norethisterone was much stronger (aOR: 3.33, 95% CI: 1.69, 6.57) than the magnitude of the association for the combination oral contraceptive containing drospirenone (aOR: 1.17, 95% CI: 0.76, 1.80). Similarly, by evaluating the progestin-only oral contraceptive by progestin type, we observed a strong association between use of the norethisterone progestin-only oral contraceptive within 12 weeks after conception and preterm birth (aOR: 2.02, 95% CI: 1.03, 1.79). Data were too sparse to evaluate other forms of the progestin-only oral contraceptive by progestin type for exposure within 12 weeks after conception. Norethisterone in the combination oral contraceptive was consistently positively associated with preterm birth for each exposure period.

The magnitude of the association with norethisterone was strongest for early pregnancy exposure; however evaluation of a trend across all exposure periods did not provide support for any dose response. Norethisterone in the progestin-only oral contraceptive was not associated with preterm birth at any of the other exposure periods (Table 3.3).

Table 3.3. Hormonal contraceptive use and preterm birth by period of last use, progestin type, and route of administration in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

Exposure	Exposed (n)	Preterm (n)	OR (95% CI)	aOR* (95% CI)
None	23,421	964	referent	referent
Within 12 weeks after conception				
Combination OC				
drospirenone and EE	368	22	1.48 (0.96, 2.29)	1.17 (0.76, 1.80)
levonorgestrel and EE	545	34	1.55 (1.09, 2.21)	1.20 (0.83, 1.74)
norethisterone and EE	75	11	4.00 (2.10, 7.62)	3.33 (1.69, 6.57)
Progestin-only OC				
norethisterone	146	11	1.90 (1.02, 3.52)	2.02 (1.09, 3.75)
Other**	504	16	0.76 (0.46, 1.26)	0.69 (0.41, 1.15)
Within 1 month before conception				
Combination OC				
desogestrel and EE	295	25	2.16 (1.42, 3.27)	2.09 (1.38, 3.16)
drospirenone and EE	1,472	61	1.01 (0.77, 1.31)	0.95 (0.73, 1.24)
levonorgestrel and EE	2,521	120	1.16 (0.96, 1.41)	1.11 (0.91, 1.36)
norethisterone and EE	372	19	1.25 (0.79, 2.00)	1.21 (0.76, 1.93)
Progestin-only OC				
desogestrel	690	17	0.59 (0.36, 0.96)	0.65 (0.40, 1.06)
norethisterone	483	13	0.64 (0.37, 1.12)	0.74 (0.43, 1.29)
Vaginal ring				
etonogestrel and EE	356	13	0.88 (0.51, 1.54)	0.86 (0.49, 1.51)
Other**	276	7	0.61 (0.29, 1.29)	0.60 (0.28, 1.28)
Within 4 months before conception				
Combination OC				
desogestrel and EE	197	11	1.38 (0.75, 2.54)	1.27 (0.69, 2.35)
drospirenone and EE	1,227	56	1.11 (0.85, 1.47)	1.07 (0.80, 1.41)
levonorgestrel and EE	2,107	125	1.47 (1.21, 1.78)	1.40 (1.15, 1.70)
norethisterone and EE	302	21	1.74 (1.11, 2.72)	1.67 (1.06, 2.62)
Progestin-only OC				
desogestrel	817	34	1.01 (0.71, 1.43)	1.15 (0.81, 1.63)
norethisterone	417	16	0.93 (0.56, 1.54)	1.09 (0.66, 1.80)
Vaginal ring				
etonogestrel and EE	352	12	0.82 (0.46, 1.47)	0.80 (0.45, 1.43)
Other**	321	12	0.90 (0.51, 1.62)	0.89 (0.50, 1.60)
Within 12 months before conception				
Combination OC				
desogestrel and EE	215	17	2.00 (1.21, 3.30)	1.86 (1.13, 3.09)
drospirenone and EE	1,475	79	1.32 (1.04, 1.67)	1.25 (0.99, 1.60)
levonorgestrel and EE	2,605	122	1.14 (0.94, 1.39)	1.09 (0.89, 1.33)
norethisterone and EE	338	23	1.70 (1.11, 2.61)	1.61 (1.05, 2.49)
Progestin-only OC				
desogestrel	1,031	42	0.99 (0.72, 1.36)	1.13 (0.82, 1.55)
norethisterone	640	22	0.83 (0.54, 1.28)	0.95 (0.62, 1.47)
Vaginal ring				
etonogestrel and EE	424	12	0.68 (0.38, 1.21)	0.67 (0.38, 1.20)
Transdermal patch				
Norelgestromin and EE	295	10	0.82 (0.43, 1.54)	0.78 (0.41, 1.48)
Injectable				
medroxyprogesterone	180	14	1.96 (1.13, 3.40)	1.83 (1.06, 3.18)
Other**	267	8	0.72 (0.36, 1.46)	0.79 (0.39, 1.60)

*Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth
**Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

Levonorgestrel, the most commonly prescribed progestin type in combination oral contraceptives, was weakly associated with preterm birth for exposure within 12 weeks of conception (aOR: 1.20, 95% CI: 0.83, 1.80) and moderately associated with preterm birth for exposure within 4 months of conception (aOR: 1.40, 95% CI: 1.15, 1.70). No association was observed for levonorgestrel at 12 months before or 1 month before conception (Table 3.3).

With the exception of use within 12 months of conception, the combination oral contraceptive containing drospirenone progestin was generally not associated with preterm birth. Etonogestrel, in the vaginal ring, and norelgestromin, in the transdermal hormonal contraceptive, were also not associated with preterm birth at any of the exposure periods (Table 3.3).

Medroxyprogesterone was the only progestin used as an injectable among women in this sample and, as described above, was moderately associated with preterm birth for exposure within 12 months before conception (Table 3.3).

We observed no association between use of a hormonal contraceptive, for any of the exposure periods, and increased odds of SGA or birthweight z-score. We observed an inverse relation with use of a progestin-only oral contraceptive and SGA for exposure within 4 months and 1 month of conception. Sparse data precluded evaluation of the association between early pregnancy use of a progestin-only oral contraceptive and SGA (Appendix, Supplementary Table S3.3), as well as individual progestin types for the progestin-only oral contraceptive (Appendix, Supplementary Table S3.4).

In sensitivity analysis, estimates observed in our primary analyses were robust to restriction of the study sample to nulliparous pregnancies (Appendix, Supplementary Table S3.5). When restricting the comparator population to vaginal ring users within the same exposure period, the magnitude of the association observed for combination oral contraceptive users and preterm birth strengthened (aOR at 12 months: 1.78, 95% CI: 0.99, 3.21), however wider confidence intervals reflect the degree of uncertainty in estimates obtained in the

markedly reduced sample size for these analyses (Appendix, Supplementary Table S3.6). The association between the combination oral contraceptive and preterm birth, at all exposure periods, was similarly robust to approaches employing use of propensity scores (Appendix, Supplementary Table S3.7).

3.4 Discussion

In the present study we observed that some formulations of hormonal contraceptive use, especially combination oral contraceptives, were associated with a higher probability of preterm birth and shorter gestational length. The positive association for the combination oral contraceptive was observed across all exposure periods and was robust to sensitivity analyses. There was little evidence of a dose response effect, or change in estimate as the proximity of the exposure period approached or included time after conception. The consistency in the magnitude of the association, irrespective of exposure period, could be evidence of an association with long-term (>12 months) use, in which case proximity of exposure to conception may be less important. A study evaluating duration of use of oral contraceptives and offspring birthweight identified that long-term use (> 2 years), as compared to short-term use (<12 months), was associated with increased birthweight.(48) This consistency across exposure periods could also be evidence of residual confounding.

Although use of a pharmacy-based registry offers the benefit of studying specific formulations of contraceptives dispensed at specific times, the registry data is only a proxy for actual use of the contraceptives. Classification of exposure in early pregnancy was limited to pregnancies reported to be unplanned to increase the potential that prescribed contraceptives were actually being used, but for exposure in other periods before pregnancy, fewer women may have still been taking a hormonal contraceptive than estimated. The consistent attenuation in adjusted associations at the 1-month before conception exposure interval, as compared to other exposure periods, may reflect the higher potential for misclassification in this exposure period.

The association between the combination oral contraceptive and preterm birth was robust to several sensitivity analyses evaluating potential for uncontrolled confounding in our primary analyses. Although our results were robust to these different approaches, confounding by indication is still possible. There may be factors we did not include in our models -- factors not in our dataset, which lead to differential use of hormonal contraceptives and are associated with pregnancy outcomes. For example, prescribing practices of hormonal contraceptives are often dictated by an individual woman's estrogen, progesterone, androgen sensitivities or excess, as evidenced by her menstrual cycle characteristics(87) and these factors could confound the association between hormonal contraceptives and preterm birth. Still, the propensity score models provide additional support for the associations observed in our primary analyses assuming we have accurately predicted prescribing of a combination oral contraceptive.

Characterizing exposure in finer detail by type, route of administration, and progestin formulation identified differences in association by progestin type. These differences are not surprising. The effects of hormonal contraceptives vary and are largely driven by the progestin component and the pharmacodynamics of progestin and ethinyl estradiol taken in combination. For example, some progestin formulations, such as levonorgestrel, are androgenic, while others, such as drospirenone, have no affinity for androgen receptor binding.(87, 93) The capacity of the progestin to bind to androgen, mineralocorticoid, and glucocorticoid receptors is thought to be a major determinant of the differential actions of progestins in eliciting adverse effects in women,(94) including elevating very low-density lipoprotein cholesterol levels and total triglycerides.(18-20, 87) Hormonal contraceptives also increase blood glucose levels, insulin levels, plasma cortisol levels,(20) and induce a state of insulin resistance.(18, 19, 87) Some of the metabolic changes elicited by hormonal contraceptives are similar to the metabolic changes that occur in women who are overweight or obese.(21, 22) In the animal models, maternal

obesity has led to an increase in the number of apoptotic follicles in the ovaries, smaller and fewer oocytes, and smaller pups at birth.(27, 28)

Medroxyprogesterone, in the injectable hormonal contraceptive, was associated with preterm birth. Medroxyprogesterone readily binds to the glucocorticoid receptor.(93, 94) Excess glucocorticoid hormone, through endogenous or exogenous sources, is associated with low birthweight and is hypothesized to contribute to fetal programming for adult metabolic disease.(95)

We compared associations over exposure periods, assessing for consistency of effect or evidence of possible dose effects according to proximity of exposure to conception. The association with combination oral contraceptives containing norethisterone was consistent across exposure periods. Norethisterone, unlike levonorgestrel, desogestrel, and drospirenone, has estrogenic activity. Norethisterone, in the progestin-only oral contraceptive, was associated only with preterm birth for exposure in early pregnancy. It may be that the association with norethisterone, for use before pregnancy, is observed only in the presence of ethinyl estradiol. The estrogenic component of a hormonal contraceptive increases the effect of the progestin component by increasing the number of intracellular progesterone receptors, thus increasing the opportunity for progesterone binding and subsequent signaling.(7, 17)

In some instances, data were too sparse to evaluate all formulations at every exposure period, limiting our capacity to explore differences across time for some agents. One explanation for differing results across different exposure periods is the inherent differences among women at the different exposure periods. Women who did not conceive for up to 12 months after stopping their hormonal contraceptive could be less fecund or have a different metabolic milieu than women who conceived within 4 months or 1 month of stopping their contraceptive. These underlying differences could result in different effects of exogenous sources of estrogen and progesterone between women.

Exposure to exogenous hormonally active agents may exert adverse agent-specific effects on growth and development. Because pharmacologic sources of exposure are prevalent due to the frequent use of hormonal contraceptives among women of childbearing age, additional research is warranted. We found that the particular progestin component is important when assessing the potential for adverse effects in pregnancy for former users of hormonal contraceptives. The present study provides support for the potential for environmental sources of hormonally active agents to exert developmental effects.

CHAPTER 4: MATERNAL HORMONAL CONTRACEPTIVE USE AND OFFSPRING OVERWEIGHT OR OBESITY

4.1 Introduction

Worldwide, the prevalence of childhood overweight and obesity increased from 4.2 percent in 1990 to 6.7 percent in 2010. (1-3) Children who are overweight or obese are more likely to be overweight in adulthood and to suffer from obesity-related morbidity and mortality.(5, 6) The obesity epidemic has been primarily attributed to changes in dietary and physical activity behaviors in developed countries, but there is evidence that exposure to estrogen-mimicking compounds during developmentally sensitive periods may contribute.

Although estrogenic agents can signal via non-receptor driven pathways, their primary actions are mediated by binding with nuclear receptors that then promote or repress transcription of specific genes.(96) Several studies performed *in vitro* suggest that estrogenic agents can affect adipogenesis. For example, the potent synthetic estrogen diethylstilbestrol (DES) activates expression of both ER and PPAR-γ receptors, both of which are required for adipogenesis(97). Similarly, 17-β estradiol has resulted in increased preadipocyte proliferation, likely though up-regulation of PPAR-γ.(98) Preadipocyte formation can be initiated at any stage of life, but perturbation can occur as early as in the blastocyst stage.(96) More recently, epigenetic effects of obesogens have been described, whereby alterations in gene expression are driven by DNA methylation or histone modifications. These epigenetic effects may perturb priming of multipotent stem cells to promote preadipocyte formation.(36)

Experiments in animal models have shown a positive association between *in utero* and neonatal exogenous estrogen exposure and metabolic disruption in the offspring,

including offspring overweight or obesity.(39, 41) The experimental studies of exposure to hormonally active compounds generally address the risk of low-dose exposure to environmental sources of estrogen-mimicking compounds.(36-39) However, *in utero* exposure to androgens has also been associated with offspring obesity.(99-101) Hormonal contraceptives can be androgenic, depending on the progestin component included. Some studies have suggested that these developmental effects on growth may be sex-dependent, with associations primarily demonstrated in male offspring.(40)

The maternal metabolic milieu is also associated with offspring overweight or obesity.(25, 102) Hormonal contraceptives have, for many women, unintended metabolic effects, including elevating levels of very low-density lipoprotein cholesterol levels and total triglycerides.(18-20, 87) Hormonal contraceptives increase blood glucose levels, increase insulin levels, increase plasma cortisol levels(20) and induce a state of insulin resistance.(18, 19, 87) Some of the metabolic changes elicited by hormonal contraceptives are similar to the metabolic changes that occur in women who are overweight or obese, specifically increased total cholesterol(21) and increased insulin resistance.(22)

Given the existing experimental data suggesting that hormonal compounds directly cause changes in follicular,(9-13) embryonic, and fetal development,(23, 24) and that they may indirectly cause an obesity-like metabolic milieu,(28) studies of hormonal contraceptive exposure and offspring development in humans are needed.

The potential risks of exogenous sex hormone exposure on offspring overweight or obesity are difficult to assess in human populations. Most cohort studies lack the power to evaluate the association between exogenous sex hormone exposures during pregnancy and offspring obesity because use of pharmacologic sex hormones in early pregnancy is relatively uncommon. Hormonal contraceptive failure, even with imperfect use, is only about 3%.(54) With over 40,000 children followed to age 3, the Norwegian Mother and Child Cohort Study (MoBa), a prospective, population-based cohort study conducted by the

Norwegian Institute of Public Health,(57, 58) offers an unusual opportunity to assess the influence of *in utero* exposure to exogenous sex hormones, through hormonal contraceptive use in early pregnancy, on childhood overweight or obesity. In the present study, through linkage of data collected through the MoBa cohort study, the Norwegian Prescription Registry data (NorPD), and the Medical Birth Registry of Norway (MBRN) data, we evaluated the association between hormonal contraceptive use and offspring overweight or obesity at 36 months of age.

4.2 Methods

MoBa study participants were recruited In Norway from 1999 through 2008. Women were identified for eligibility when scheduling the routine prenatal ultrasound offered free of charge to all pregnant women in Norway at 17-20 weeks of gestation. Women were then mailed an invitation to participate before the scheduled ultrasound, with informed consent and enrollment taking place at the ultrasound examination. All hospitals with at least 100 births per year participated in the study recruitment and enrollment. Approximately 42 percent of all pregnant women in Norway were invited to participate in the study. Of these, 39 percent consented to participate. At enrollment, participants were asked to provide a blood specimen and to complete an initial, self-administered questionnaire to collect data on demographic characteristics, reproductive health history, disease and medication history, lifestyle factors, and socioeconomic status. Follow-up is conducted through self-administered questionnaires at regular intervals and by linkage to national health registries.

All MBRN birth registry data are collected on a standardized birth notification form completed by the midwife or physician attending the birth. Prescription data from NorPD contains individual-level data on all medications prescribed and dispensed through pharmacies to non-institutionalized individuals in Norway. By Norwegian law, as of January 1, 2004, all pharmacies must provide electronic data for all prescriptions dispensed.

There were 107,308 pregnancies in the MoBa cohort. For the present analysis, we included pregnancies resulting in a singleton live birth, with no indication of death in the first year of life, and with no documentation, on either the MoBa 17-week questionnaire or the MBRN, of having received infertility treatment for the index pregnancy. We additionally excluded pregnancies to women with documentation of having pre-pregnancy chronic hypertension (n=527). As the NorPD registry was not initiated until January 1, 2004, we further restricted our study population to pregnancies of women enrolled at least 12 months after the date on which the NorPD registry began collection of data on prescription fills (n=48,615). For the primary analyses, we also excluded pregnancies with missing covariate data (n=3,966), and for loss to follow-up at age 3 (n=24,997). The final study population included 19,652 pregnancies to 18,652 women (Figure 4.1).

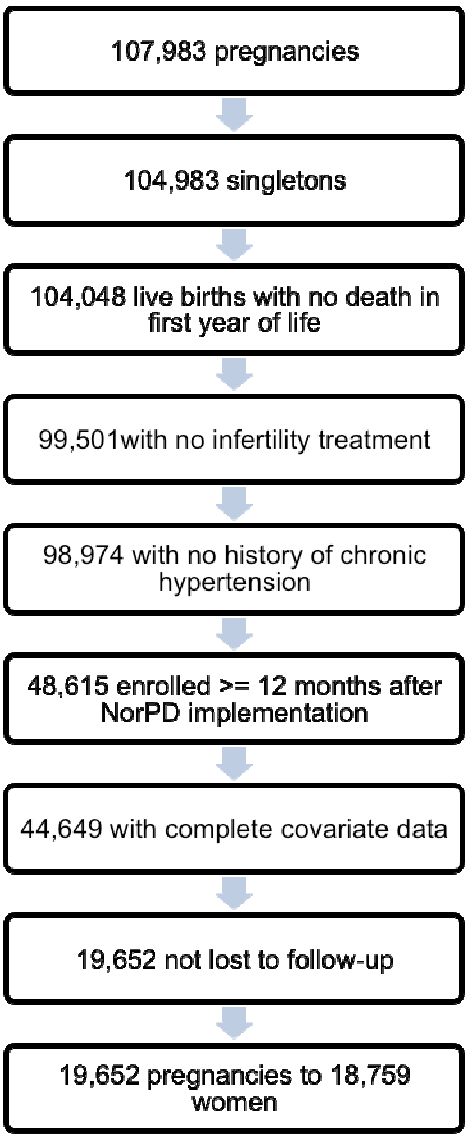


Figure 4.1. Study population selection for Aim 2

All Norwegian residents are assigned a personal identifier number. Linkage of the MoBa questionnaire, MBRN, and NorPD data files were possible through this identifier. The University of North Carolina at Chapel Hill and the National Institute of Environmental Health Sciences Institutional Review Board and the Norwegian Southeastern Regional Ethics Committee reviewed and approved this study.

Hormonal contraceptive use, in early pregnancy and before conception, was characterized according to the Anatomical Therapeutic Chemical (ATC) Classification System.(59) We characterized exposure by type and route of administration (combination

oral contraceptive, progestin-only oral contraceptive, vaginal ring, transdermal, injectable, implant, and hormonal-based intrauterine device). Hormonal contraceptives are a heterogenic group of agents, with varying effects on maternal metabolic markers. Therefore, in addition to characterizing exposure by type, we also characterized exposure by progestin formulation. All hormonal contraceptives with an estrogen component (combination oral contraceptives, vaginal ring, and the transdermal contraceptive) contained ethinyl estradiol, but there were eight different progestin types used solely or in combination with ethinyl estradiol, including desogestrel, drospirenone, levonorgestrel, norelgestromin, norethisterone, lynestronol, medroxyprogesterone, and etonogestrel. Any exposures with fewer than 10 exposed cases we combined into a single "other" category (Appendix, Supplementary Table S4.1).

Although our primary interest was to explore the association between exposure in early pregnancy and offspring overweight or obesity, we also characterized exposure into hormonal contraceptive use occurring within 12 months, 4 months, and 1 month of pregnancy. Exposure periods were characterized into discrete exposure windows, according to last date of use relative to conception, e.g. 12 - >4 months before, 4 - >1 months before, 1 - > 0 months before, and 0-12 weeks after.

Date of conception was estimated by subtracting 17 days(62) from the number of days of gestational length at birth (to account for the follicular phase prior to conception) and then subtracting this value from the date of birth. We used the last menstrual period (LMP)-based estimated gestational length unless the LMP-based gestational length was missing or ≥2 weeks from the ultrasound-based estimate of gestational length, in which case we used the ultrasound based measure.(63) We then constructed an exposure window for each hormonal contraceptive prescription filled using the date that the prescription was filled and the number of defined daily doses dispensed (day's supply). Most oral contraceptives were dispensed in 3 month supply (82%) or a 6 month supply (15%). For pregnancies with more

than one type of hormonal contraceptive prescribed, we assigned exposure type according to the type of contraceptive used closest to the estimated date of conception. Because many women may choose to stop taking their hormonal contraceptive in order to achieve conception, we characterized women as exposed in early pregnancy only if they reported on the 17-week questionnaire that the pregnancy was unplanned and had ≥ 1 day supply of hormonal contraceptive at or after the day of conception as defined above.

Offspring overweight or obesity was defined by first calculating the offspring body mass index (kg/m^2) at age 3 from questionnaire-reported height and weight measures. In Norway, families are provided a health card for tracking health-related data for their children. Directions included on the questionnaire instructed mothers to record the height, weight, and date that the measure was obtained (and documented on the health card) at the 3-year well child exam. Offspring were characterized as overweight or obese using the age- and sex-specific cut points for overweight or obese developed by the International Obesity Taskforce (IOTF).(70)

Covariate selection was informed through construction of a directed acyclic graph(81) and included adjustment for factors demonstrated to be causal ancestors of both contraceptive use and childhood overweight or obesity. These factors included maternal age (14-19, 20-29, 30-39, 40-49),(72, 73) maternal pre-pregnancy BMI (kg/m^2)(<18.5, 18.5-24.9, ≥25.0),(72, 88, 103) parity (0, 1, ≥2),(77, 104) maternal smoking (none, quit during pregnancy, smoker),(72, 74, 105) and maternal education (>4 years of university or technical, 4 year university or technical degree, 3 years of college preparatory high school, 3 years of technical high school, 1-2 years of high school, <9 years of secondary school, other).(78, 79, 106)

Primary analyses

Our primary analyses were concerned with assessing the association between early pregnancy hormonal contraceptive exposure, as compared to no use of a hormonal

contraceptive in early pregnancy or within 12 months of pregnancy, and offspring overweight or obesity. We used generalized linear models with a logit link, and generalized estimation equations (GEE) with an independent correlation matrix(89) to estimate robust standard errors and account for lack of independence between siblings. We also used generalized linear models with GEE to assess the association between hormonal contraceptive use prior to conception (within discrete exposure categories of 12 months, four months, and one month, as described above) and offspring overweight or obesity.

Subgroup analyses

In subgroup analyses, we explored the association between route of administration and type of progestin agent and offspring overweight or obesity. Evaluation of associations was conducted only where there were sufficient numbers (≥10) of exposed pregnancies resulting in an outcome of overweight or obese at age 3 (Appendix, Supplementary Table S4.1).

We also explored whether there was evidence of interaction between hormonal contraceptive use and offspring sex or maternal pre-pregnancy overweight/obese status (Yes/No) using interaction terms. *A priori*, we considered a p value <0.20 as evidence of potential interaction.(71) If there was evidence of possible interaction, we generated stratum-specific estimates of the association between contraceptive use and offspring overweight or obesity and examined confidence intervals for the degree of overlap. All analyses were conducted using SAS v9.3 (SAS Inc., Cary, North Carolina).

Sensitivity analyses

Examination of the covariate distribution among pregnancies to women with hormonal contraceptive use in early pregnancy, as compared to non-users, indicated that the characteristics of women with hormonal contraceptive use were somewhat different from women without contraceptive use (Appendix, Supplementary Table S4.2). We explored the robustness of our primary results using three different comparator groups, selected to more

closely approximate the counterfactual population for exposed pregnancies. These included 1) a comparator group consisting of women without hormonal contraceptive use in early pregnancy but that were reported to be unplanned, to assess for the potential for bias resulting from residual factors associated with having an unplanned pregnancy that are also associated with offspring overweight or obesity, 2) a comparator group of women who were former users of the same contraceptive (within 12 months, but not within 4 months of conception or during early pregnancy, to assess for the potential for confounding by indication bias -- bias resulting from factors associated with both the type of hormonal contraceptive prescribed and with offspring overweight or obesity), and 3) an analysis comparing the two oral contraceptive groups: combination vs progestin-only, to account for factors associated with oral contraceptive use and childhood overweight that are not related to the particular hormone used. Assuming that any factors contributing to contraceptive failure may be similar for different contraceptive types (and that these factors may also be associated with offspring overweight or obesity), comparison of progestin-only oral contraceptive users as compared to combination oral contraceptive users assesses the potential from bias as result of this unmeasured confounding.

In our sensitivity analysis of exposure to hormonal contraceptive use *before* pregnancy, we evaluated use of the vaginal ring as compared to the combination oral contraceptive. The distribution of study covariates among women obtaining a prescription for the vaginal ring was comparable to the distribution of covariates among women obtaining a prescription for a combination oral contraceptive (Appendix, Supplementary Table S4.2). This approach offered the opportunity to assess the association between vaginal ring users and overweight or obesity while minimizing the potential for residual confounding from differences in the characteristics of women who use this form of contraception as compared to non-users.

We conducted additional sensitivity analyses to evaluate the potential for bias through loss to follow-up. Pregnancies with exposure to a hormonal contraceptive within the first 12 weeks after the estimated date of conception were more likely to have been lost to follow-up than pregnancies with no exposure (Appendix, Supplementary Table S4.3). However there was no evidence of any pattern in the missing data whereby a covariate or set of covariates could predict the pattern of missingness. We used a multiple imputation approach(82) (SAS PROC MI - Monte Carlo Multiple Chain) to impute 10 data sets with imputed follow-up data for offspring BMI. Imputation models were richly constructed and included all of the study covariates, study exposures, study outcomes, offspring birthweight and length, offspring sex, pregnancy intendedness, and gestational age at birth. We modeled the association between hormonal contraceptive exposure and offspring overweight or obesity for each of the imputed datasets. The model results for each data set were then synthesized to obtain final estimates for the multiple imputation approach (SAS PROC MIANALYZE).

As an alternative approach to multiple imputation, we also conducted a sensitivity analysis using inverse probability weighting to account for loss to follow-up. To do this, we constructed weights based on the inverse of the predicted probability of staying in the study, i.e., up-weighing those with a high probability of being lost to follow-up. These inverse probability weights were scaled by multiplying the weights by the marginal probability of staying in the study.(83)

4.3 Results

In general, compared to all MoBa pregnancies with baseline data collected in pregnancy and at birth, the pregnancies included in the final study sample were to women who were older, slightly less parous, slightly less likely to have smoked in pregnancy, and slightly more educated (Table 4.1).

Table 4.1: Study and baseline population characteristics among women participating in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

	Baseline population	Study population
	n=44,649	n=19,652
	%	**%**
Maternal age (years)		
14-19	0.9	0.4
20-29	42.1	40.3
30-39	54.9	57.1
40-49	2.1	2.3
Maternal BMI (kg/m^2)		
<18.5	3.2	2.9
18.5-24.9	66.1	67.2
25.0-29.9	21.7	21.8
≥30.0	9.0	8.1
Parity		
0	47.1	50.0
1	35.5	34.3
2	13.7	12.4
3	2.8	2.4
4 or more	0.9	0.8
Maternal education		
More than 4 years of university or technical	27.2	29.9
4 year university degree, regional technical	40.8	43.9
3 years high school, junior college	13.5	12.0
Technical high school	11.2	9.3
1-2 years high school	3.9	2.6
9-year secondary	2.2	1.1
Other	1.4	1.4
Maternal smoking (at 17 weeks)		
None	79.3	82.8
Quit	14.5	12.6
Daily	1.4	1.2
Sometimes	4.8	3.4

*Represents unique pregnancies with no use of IVF treatment, resulting in a singleton live birth and no death in the first year of life, with a date of birth >=12 months after NorPD registry began (January 1, 2004)

At 36 months of age, 2,653 (13.1%) children in the study sample met the IOTF definitions for overweight or obese. Linkage to the NorPD identified 9,675 (48.6%) women with a prescribed hormonal contraceptive within 12 months prior, 6,132 (31.0%) within 4 months prior, 3,469 (17.6%) within 1 month prior, and 567 (2.9%) within 12 weeks after conception. Characterizing hormonal contraceptive use into discrete exposure windows, described according to last use, identified 3,392 exposed pregnancies within 12 months

before, 2,541 exposed pregnancies within 4 months before, 2,997 exposed pregnancies within 1 month before, and 567 exposed pregnancies within 12 weeks after conception.

Primary analyses

In early pregnancy, the combination oral contraceptive was weakly, inversely associated with offspring overweight or obesity at age 3. The progestin-only oral contraceptive was weakly, positively associated with overweight or obesity (Table 4.2).

Table 4.2. Association between hormonal contraceptive use in early pregnancy* and offspring overweight or obese in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

Exposure	Exposed (n)	Overweight or obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None†	9,987	1,342	referent	referent
Combination OC	380	38	0.72 (0.51, 1.01)	0.75 (0.53, 1.08)
Progestin-only OC	127	21	1.28 (0.80, 2.05)	1.26 (0.79, 2.02)
Other‡	60	7	0.85 (0.39, 1.88)	0.88 (0.40, 1.94)

*Use within 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception
**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity
†No use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception
‡Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Use of a hormonal contraceptive prior to pregnancy was generally not associated with overweight or obesity at age 3, with the exception of use of a vaginal ring-type hormonal contraceptive, which was inversely associated with offspring overweight or obesity at age 3, particularly for exposure estimated to have occurred within 1 month of conception (Appendix, Supplementary Tables S4.4-S4.6). Data were too sparse to evaluate the association between early pregnancy use of the vaginal ring and subsequent offspring overweight or obesity.

Subgroup analyses

Among combination oral contraceptive users, the association with overweight or obesity was similar across combination oral contraceptives with differing progestin components (Appendix, Supplementary Tables S4.7-S4.10). In early pregnancy only, the desogestrel progestin-only oral contraceptive was moderately associated with offspring

overweight or obesity (aOR: 1.87, 95% CI: 1.06, 3.32) (Appendix, Supplementary Table S4.7).

There was little evidence of effect modification by offspring sex or maternal pre-pregnancy BMI. However, for exposure to the combination oral contraceptive in early pregnancy, the observed association with overweight or obesity was present only in males (aOR: 0.56, 95% CI: 0.32, 0.97 in males vs aOR: 0.98, 95% CI: 0.63, 1.53 in females).

Sensitivity analyses

In early pregnancy, for the analyses evaluating the use of different comparator groups, the inverse association between the combination oral contraceptive and offspring overweight or obesity was robust to restricting the comparator population to only unplanned pregnancies. This inverse relationship was also qualitatively unchanged when comparing early pregnancy combination oral contraceptive users to former users of the combination oral contraceptive and when comparing the combination oral contraceptive users to the progestin-only oral contraceptive users. Similarly, the relationship between progestin use in early pregnancy and offspring overweight or obesity was robust to choice of comparator groups (Table 4.3).

Table 4.3. Sensitivity analyses for early pregnancy exposure to oral contraceptives and overweight or obesity in the Norwegian Mother Child Prospective Cohort Study (2004-2008)

	n	OR (95% CI)	Adjusted** OR (95% CI)
Approach 1: Compared to former users			
Combination OC			
former* user of Combination OC	6,146	referent	referent
**early pregnancy Combination OC user	380	0.76 (0.54, 1.07)	0.75 (0.52, 1.06)
Progestin-only OC			
former* user of Progestin OC	1,962	referent	referent
*early pregnancy Progestin OC user	127	1.20 (0.74, 1.94)	1.24 (0.75, 2.04)
Approach 2: Compared to unplanned pregnancies			
No hormonal contraception†	2,264	referent	referent
Combination OC	380	0.67 (0.46, 0.96)	0.70 (0.48, 1.02)
Progestin-only OC	127	1.19 (0.73, 1.93)	1.22 (0.75, 1.98)
Other‡	60	0.79 (0.36, 1.76)	0.79 (0.35, 1.78)
Approach 3: Compared to other oral hormonal contraceptive users			
Progestin-only OC	127	referent	referent
Combination OC	380	0.46 (0.23, 0.91)	0.56 (0.32, 1.00)

*Former use defined as within 12 months but not within 4 months or 1 month of conception and early defined as within 12 weeks of conception
**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity
†No use of a hormonal contraceptive within the discrete categories of within 12, 4, and 1 month before conception and within 12 weeks after conception
‡Hormonal contraceptives with < 10 exposed cases were combined into an "other" category

The estimate comparing vaginal ring use to the combination oral contraceptive (within 1 month prior to conception) was aOR 0.59 (95% CI: 0.33, 1.03) and consistent with estimates obtained when comparing vaginal ring users to non-users of a hormonal contraceptive.

Estimates from models employing multiple imputation or inverse probability weighting, to evaluate the potential for bias resulting from loss to follow-up at age 3, were qualitatively similar to those obtained in the primary analyses (Appendix, Supplementary Tables S4.11-S4.12).

4.4 Discussion

In our primary analysis of the association between early pregnancy hormonal contraceptive use and offspring overweight or obesity at age 3, we found that use of a

combination oral contraceptive was weakly, inversely associated with offspring overweight or obesity at age 3. Use of the progestin-only oral contraceptive in early pregnancy was weakly, positively associated with offspring overweight or obesity. A moderate association for early pregnancy progestin-only oral contraceptive use was observed when examining the desogestrel form of the progestin-only oral contraceptive. For exposure to hormonal contraceptives before pregnancy, there was an inverse association between use of the vaginal ring and overweight or obesity. All of the associations were qualitatively unchanged with selection of different comparators groups. Observed associations were robust to sensitivity analyses employing approaches for evaluating the potential for bias as a result of loss to follow-up.

In experimental animal models, *in utero* and neonatal exposure to estrogenic agents (DES and 17β-estradiol) results in an initial period of depressed growth, followed by increasing adiposity at follow-up.(39, 40) The weak, inverse association with the combination pill in the present study may be congruent with observations of an initial period of depressed growth in these experimental studies. Follow-up of the MoBa cohort could allow investigation of associations at later ages to determine whether the growth pattern exhibited in animal models is relevant in humans.

Not surprisingly, we observed differences in association depending on the type of contraceptive and progestin used. The androgen, progestogen, and estrogen receptor binding affinities of the agents in different contraceptives vary. Some exert androgenic properties, others antiandrogenic properties.(87) Variability in pharmacokinetics, for different formulations of hormonal contraceptives, and also between women, impacts activity. Unlike many medications that are titrated to the weight of the individual, each hormonal contraceptive formulation is prescribed at the same dose, irrespective of body weight.

To our knowledge, studies of offspring adiposity or growth following exposure to progestogenic compounds during early fetal development have not been conducted in animals or humans. However, endogenous serum progesterone levels in both early and mid-pregnancy have been positively associated with offspring birthweight(107, 108) and birthweight has been associated with offspring weight at follow-up.(109) Fetal exposure to androgenic agents has resulted in metabolic abnormalities, including a polycystic ovarian syndrome-like phenotype in animal models.(99) When unopposed by ethinyl estradiol, desogestrel has high progestational and moderate androgenic activity relative to other progestin types, but when desogestrel is present in combination with ethinyl estradiol, the progestational and androgenic activities are substantively reduced.(87) Norethisterone is only weakly androgenic. This property may explain, in part, the difference in association observed between desogestrel and norethisterone progestin-only contraceptives in this study.

Given the positive association between the combination oral contraceptive and offspring overweight or obesity, the inverse association for the vaginal ring contraceptive was surprising. The inverse association between use of the vaginal ring and offspring overweight or obesity may be attributable to the pharmacokinetic properties of the vaginal ring. The vaginal ring contains etonogestrel and ethinyl estradiol. Hormonal constituents of the vaginal ring are absorbed through the vaginal epithelium and provide daily, slow release of etonogestrel and ethinyl estradiol for the three week period after the ring is inserted.(87) Pharmacokinetic studies comparing the vaginal ring to the combination oral contraceptive indicate that the dose of ethinyl estradiol, as measured in blood serum and represented by the area under the curve, is lower than that of doses experienced in combination oral contraceptive users.(110) The agents in the vaginal ring do not experience first pass metabolism. The ring provides a consistent release of hormones unaffected by dietary or

gastrointestinal factors and is less subject to fluctuation in delivered dose when compared to oral or transdermal-administered forms of contraception.(111, 112)

Sparse data limited the ability to assess for variation in effects from different progestin types in early pregnancy. With the exception of early pregnancy use of the combination oral contraceptive, we found little evidence of effect modification by offspring sex or maternal pre-pregnancy BMI. Sample size limitations may have precluded the ability to detect effect modification. The effect modification observed for early pregnancy is consistent with animal data suggesting that developmental effects of estrogenic compounds may be sex-specific, with stronger associations observed among male offspring.(40)

The associations observed for the combination oral contraceptive, progestin-only oral contraceptive, or vaginal ring contraceptive could be attributable to residual confounding or confounding by indication. Women may be prescribed different contraceptive formulations based on factors for which we cannot control in our data. Notwithstanding, estimates obtained from comparator group sensitivity analyses, conducted to assess the potential for confounding by indication, were robust to choice of comparator group selection.

Magnitude of estimates was also robust to loss to follow-up, based on two separate strategies to explore the potential bias. However, these strategies are effective only insofar as we have correctly assumed that we were able to successfully impute missing values from the covariates in our imputation models (multiple imputation approach) or correctly predicted the probability of staying in the study from the covariates in our models for generating weights (inverse probability weighting approach).

There is also the potential for misclassification of exposure. We characterized exposure using registry-based data for prescription fills of hormonal contraceptives and, for early pregnancy, indication that the pregnancy was unplanned, but this is an imperfect measure of true exposure. Women may have been inconsistently taking the contraceptive or may have had some other concomitant factor leading to contraceptive failure that

explains the associations observed. Finally, while the registry data provides detailed information on formulation and doses, it cannot provide any account of actual use of the contraceptive. In early pregnancy, women were only characterized as exposed if they reported the pregnancy was unplanned. Our assumption is that women with unplanned pregnancies were more likely to have been using the form of contraception documented in the registry at the time of conception.

Lastly, it is possible that the height and weight data reported on the questionnaire were inaccurate. Although women are instructed to record the height and weight data obtained by clinical staff at the time of the 3-year health exam, most of the study population reported that the measurements obtained were collected from their own measurements of height and weight. However, the overall proportion of overweight or obese children at age 3 in this study (13.1%), as identified by these maternal-reported height and weight measures, is relatively consistent with national prevalence estimates for overweight or obesity at age 3 that were obtained from height and weight data collected by research staff (11.3%).(4)

Finally, we were only able to evaluate associations with children at age 3. Some of the animal model literature suggests that the association between hormonally active agents and offspring adiposity only becomes evident at later ages.(39, 40) It may be that a similar study, in children at older ages, would identify stronger associations than those observed in the present analysis. Furthermore, BMI is less specific for identifying adiposity in children when compared to other measures of assessing childhood adiposity. Higher BMI may reflect increased muscle mass, as opposed to increased fat mass.(113, 114)

This study builds on experimental animal data suggesting that overweight and obesity may be rooted in developmental insults from exogenous sex hormone exposure in early life. These data suggest that pharmacologic sex hormone agents may be associated with offspring overweight or obesity at age 3. The direction of the relationships appears contingent upon hormone formulation. This area of research is in its infancy, but provides

the rationale for additional investigation exploring these associations. Hormonal contraceptive use is prevalent, yet little is known about long-term, formulation-specific effects on offspring.

CHAPTER 5: CONCLUSION

5.1 Summary of results

We assessed the potential for exposure to pharmacologic sex hormones, conferred through use of hormonal contraceptives, to exert effects on offspring growth and development. Specifically, we evaluated whether use of a hormonal contraceptive, up to 12 months before and within 12 weeks after conception, was associated with gestational length at birth, preterm birth, weight for gestational age z-score, small for gestational age, body mass index z-score, and overweight or obesity at age 3.

We characterized use of hormonal contraceptive by type and route of administration, but also by progestin component. In doing so, we advanced the current literature that is either limited to experimental animal data (primarily evaluating the association between estrogenic compounds and overweight or obesity) or to evaluation, in humans, of exposure to hormonal contraceptives characterized more broadly (e.g. oral contraceptives). To our knowledge, there have been no studies exploring the potential for an association between a progestin-only oral contraceptive and indicators of altered growth and development in offspring. Furthermore, there are no published studies examining hormonal contraceptives by specific progestin component.

We identified an association between use of hormonal contraceptives and gestational length at birth and preterm birth and with BMI z-score and overweight or obesity at age 3. The associations observed were specific to some forms of hormonal contraceptive and, in some instances, only to use during early pregnancy. Specifically, use of a combination oral contraceptive, across all exposure periods, was associated with increased

odds for birth. The magnitude of the association differed by progestin component within the combination oral contraceptive. Use of the norethisterone progestin-only oral contraceptive in early pregnancy was associated with preterm birth. Use of medroxyprogesterone, found in the injectable type of hormonal contraceptive, was associated with preterm birth when used prior to pregnancy. Data were too sparse to evaluate medroxyprogesterone use in early pregnancy. There was no association between use of the transdermal hormonal contraceptive, containing norelgestromin, or vaginal ring hormonal contraceptive, containing etonogestrel, with preterm birth.

We observed an inverse association between use of a progestin-only oral contraceptive and SGA, for use before pregnancy; however, sample size limitations precluded assessing the association by type of progestin used in the progestin-only oral contraceptive. Small sample size also precluded evaluation of the association between use of the progestin-only oral contraceptive in early pregnancy. No other associations between hormonal contraceptive use and SGA were observed.

At age 3, we observed a positive association between early pregnancy use of a progestin-only oral contraceptive, specifically use of desogestrel, and overweight or obesity. Early pregnancy use of a combination oral contraceptive was inversely associated with overweight or obesity at age 3. Use of the vaginal ring hormonal contraceptive, particularly in the month prior to conception, was inversely associated with overweight or obesity.

Several different sensitivity analyses were conducted to assess the robustness of the study results given assumptions made in conducting the complete case analyses. These included inverse probability weighting and multiple imputation to account for possible bias introduced through loss to follow-up, and also evaluation of the potential for residual confounding or confounding by indication by using different comparator groups and a propensity score analysis. Generally, the magnitude of associations observed in the primary analyses was robust to these approaches.

To summarize, we observed a potential association between hormonal contraceptives and indicators of offspring growth and development. The associations observed were dependent on the exposure period and formulation of contraceptive used. Overall, the strongest associations were observed for use in early pregnancy. Interestingly, use of the combination oral contraceptive, in early pregnancy, was positively associated with preterm birth and inversely associated with overweight or obesity at age 3, suggesting the possibility that offspring exposed *in utero* may still be catching up in growth at age 3. A longitudinal analysis could assess the growth patterns of exposed offspring to evaluate this question further. Similarly, the progestin-only oral contraceptive, protective for SGA for use prior to pregnancy, was observed to be associated with overweight or obesity at age 3 for use in early pregnancy. Small sample limitations precluded assessment of the progestin-only oral contraceptive with SGA in early pregnancy, therefore a direct comparison, based on timing of exposure, was not possible.

5.2 Limitations and strengths

Limitations

There are several important limitations to this research. These limitations are inherent to pharmacoepidemiology research and observational research in general and include: 1) sparse data – specifically for evaluating specific drug formulations and the relatively rare outcomes of SGA and PTB,, 2) the potential for intractable confounding by indication, and 3) the potential for misclassification of both the exposure and outcome. Details of these limitations follow.

Sample size limitations:

An important limitation in this study was the sparse data for early pregnancy use of a hormonal contraceptive. There was also sparse data for some of the less commonly used contraceptive formulations, such as the intrauterine device, implant and injectable, and for

some contraceptives of any type with certain progestin formulations. Sparse data was most limiting for analyses of the association between hormonal contraceptive use and SGA.

Insufficient sample sizes for some contraceptive formulations precluded comparisons across exposure periods for certain hormonal contraceptive types. The inability to compare, across exposure periods, limited the ability to assess for differences in magnitude of association with respect to proximity to conception, but also differences between use of a particular progestin component between hormonal contraceptives of different types at the same exposure period. Small sample limitations also made evaluation of possible effect measure modification difficult. We assessed for effect modification by offspring sex and maternal pre-pregnancy overweight or obesity status. Models assessing for potential effect measure modification for birth outcomes often failed to converge. Ultimately, given the limitations of the data, we chose to only pursue presentation of effect medication when evaluating the association between hormonal contraceptive use and offspring overweight or obese.

Confounding:

Another important limitation to this study is the potential for intractable confounding, specifically, unmeasured or residual factors that may be antecedents of both hormonal contraceptive use of a particular type and offspring growth and development outcomes. Parity was identified as a significant confounder in the association in our models evaluating hormonal contraceptive use and preterm birth or SGA. Maternal pre-pregnancy BMI was a significant confounder in our models evaluating the association between hormonal contraceptive use and offspring overweight or obesity at age 3. We conducted sensitivity analyses with varying comparator groups to assess for possible residual confounding. Some of these approaches included restricting models to nulliparous pregnancies, comparing early pregnancy users to former users, restricting to unplanned pregnancies for associations observed in early pregnancy, and comparing users of one formulation to users

of a different formulation. Generally, results were robust to these various approaches; however the potential for confounding is still possible. For example, although in one of the sensitivity analyses we restricted the analysis to nulliparous pregnancies, there is the potential that there are factors related to prescription for the combination oral combination, as opposed to selecting a different form of contraceptive, which may be associated with preterm birth. The underlying hormonal milieu of a woman can influence her tolerance for different forms of contraception and thus the type of hormonal contraceptive she is prescribed. It has been hypothesized that these underlying factors potentially contribute to growth and development of the fetus.(25) Women with a history of a previous preterm birth are often provided progestin therapy, starting in the second trimester of pregnancy, to reduce risk for a subsequent preterm delivery.(115) These women are believed to have inadequate or premature signaling of parturition as indicated by declining progesterone levels.(116, 117)

We also conducted a propensity score analysis whereby we modeled the probability of treatment (prescription for combination oral contraceptive use). The propensity score analysis assumes that we have adequately modeled the probability of treatment using the covariates selected, and offers the opportunity to evaluate lack of common support for exposed and unexposed in the data. However, propensity score approaches cannot offer improved control of confounding for potential unobserved factors predictive of treatment and offspring anthropometric indicators. As with analyses employing use of different comparator groups, the assumption is that there is no unmeasured or unaccounted confounding remaining.

We observed a relatively consistent effect estimate when modeling the association between the combination oral contraceptive and preterm birth. Such an observation could be an indication of residual or unaccounted confounding. Alternatively, it could also be indicative of association between combination oral contraceptives used long term and

preterm birth. It is also possible that the hormonal contraceptives are operating independently at each of the exposure periods and that, by chance, the estimates were qualitatively similar. In other words, the combination oral contraceptive could be acting on each of the follicular, oocyte, and embryonic developmental stages in such a way as to disrupt the cascade of events leading to fetal signaling of parturition.

Misclassification:

Another limitation in this study is the potential for misclassification of exposure to hormonal contraceptives. Although linkage to the NorPD registry offered the opportunity to explore exposures in much greater detail than has been described previously in the literature, use of prescription registry data is inherently limited in that it represents prescription data and not actual use. Furthermore, in this study population, most prescriptions for hormonal contraceptives were provided in 3 month's supply (82%). Likely, the more days of supply provided, the greater the potential that a woman will have stopped taking the hormonal contraceptive before the last day supplied. Obviously, the association between the injectable contraceptive and preterm birth would not be subject to this limitation. Once the injectable is administered, the progestin (medroxyprogesterone) remains detectable (>100 pg/mL) for up to 200 days post-injection, although there are some women for whom the progestin levels have remained detachable for as long as 8 months post-injection.(118) Peak values are reached much earlier, between 9 -21 days, depending on the dose administered, and women are advised to return for a repeat dose at 3 month intervals.(87)

Self-reported data was limited in that it did not provide data distinguishing the type of oral contraceptive used within 4 months. It also did not allow for exploring use within 1 month of conception, a period of interest given the potential for developmental effects on the oocyte. An assessment of agreement between self-report and NorPD-indicated use suggested moderate agreement between the two sources of data for any oral contraceptive

use (Kappa values: 0.45 for early pregnancy, 0.60 for exposure within 4 months, and 0.69 for exposure within 12 months of conception). Assessing agreement in more recent time periods, 24 or 36 months after NorPD implementation, had no effect on the degree of agreement between the two data sources.

There was also the potential for misclassification of the outcome. It is possible that the height and weight data reported on the questionnaire were inaccurate. Although women are instructed to record the height and weight data obtained by clinical staff at the time of the 3-year health exam, most of the study population reported that the measurements obtained were collected from their own measurements of height and weight. However, the overall proportion of overweight or obese children at age 3 in this study (13.1%), as identified by these maternal-reported height and weight measures, is relatively consistent with national prevalence estimates for overweight or obesity at age 3 that were obtained from height and weight data collected by research staff (11.3%).(4) Our intention was to conduct a validation study of height and weight, comparing the height and weight data reported on the MoBa questionnaire to data collected in the Bergen Growth Study. Unfortunately, there were numerous delays in obtaining the data for completing this validation study, in part because of insufficient number of children represented in both the Bergen Growth Study(119) and the MoBa cohort. Because the sample size was determined too be too small, we requested height and weight data for children represented in both studies for ages 6 and 18 months also. This validation work is on-going and it has yet to be determined whether there will be a sufficient sample from which a validation study can be completed.

Still, another concern is the lack of specificity in using BMI for identifying clinically relevant adiposity in children when compared to other measures of assessing childhood adiposity.(113, 114) Higher BMI can be indicative of higher muscle mass, as opposed to fat

mass, particularly for children identified as overweight, but not obese. Our sample size limitations precluded assessing associations with obesity only.

Finally, we were only able to evaluate associations with children at age 3. Some of the animal model literature suggests that the association between hormonally active agents and offspring adiposity only becomes evident at later ages.(39, 40) We observed a stronger, and inverse magnitude of an association between hormonal contraceptive use and anthropometric indicators at birth. Associations were attenuated or null at age 3. This pattern may reflect the pattern observed in the animal literature suggestive of an inverse association at birth, followed by a period of catch-up growth whereby the exposed and unexposed are similar in weight, and then, later in life whereby exposed exceed the controls in fat mass.(39)

Selection bias:

The potential for selection bias was an important limitation in our evaluation of the possible association between hormonal contraceptive use and offspring overweight or obesity. Overall, 44% of the study population was lost to follow-up at age 3. Among hormonal contraceptive users, the loss to follow-up was similar, with one exception. When compared to non-users, a slightly higher proportion of women characterized as early pregnancy hormonal contraceptive users were lost to follow-up, when compared to non-users (Appendix, Supplementary Table S4.3).

We used two approaches to evaluate the potential for selection bias; 1) multiple imputation to impute missing measurements at age 3, and 2) an inverse probability weighting approach whereby we assigned weights to those staying in the study, up-weighting those who remained in the study but who had the same characteristics of those who left the study early. The estimates from these approaches were qualitatively similar, and similar to the estimates obtained in the complete case analysis. The comparability of the estimates obtained provides support for the results obtained in the complete case

analysis, but with the caveat that there is the potential that we did not include important covariates in our imputation model or in our model predicting study follow-up. Both of these approaches assume that missing data are missing at random and that factors related to missingness have been accounted for in the modeling approaches. In this study, there is little evidence to suggest otherwise; the covariate distribution of those who remained in the study is similar to the distribution of covariates for women who met study inclusion criteria (Table 4.1).

Strengths

There are several notable strengths to this research. This is a novel study that translates ideas that have been explored in animal data into observational human research, specifically evaluating the potential for pre- and early post-conception exposure to exogenous sex hormones to act as obesogens for offspring.

Although there is some literature assessing the potential for adverse effects for birth outcomes, as a result of hormonal contraceptive use, this literature is limited by relatively small sample sizes and crude estimation of exposure. The present study is the first study to explore associations between hormonal contraceptive use and birth outcomes, defining hormonal contraceptive use by type, route, and progestin component.

The MoBa study cohort is a relatively homogenous population. Although this could have implications for generalizability of study results to other populations, this relative homogeneity provides the opportunity to study exposure outcome associations with a reduced potential for confounding.

Although our sample size was too small to estimate some associations, particularly for less commonly used formulations, we did have sufficient data to explore the use of multiple comparator groups and to employ a variety of methods to assess the robustness of the study results given the potential for threats to study validity. The greatest threat to validity was the potential for residual confounding. Results were generally insensitive to

choice of comparator group or to analyses employing a propensity score analysis approach in the evaluation of combination oral contraceptive use and odds of preterm birth.

5.3 Additional analyses

Characterizing timing of exposure

There were additional analyses conducted in this study that are not reflected in Chapters 3 and 4 of this document. These analyses primarily concerned approaches to characterizing the use of hormonal contraceptives. Initially, we characterized exposure according to any use within 12, 4, or 1 month before conception and any use within 12 weeks after conception. The referent group for this analysis was non-users of a hormonal contraceptive for the exposure period of interest. Each period of exposure was modeled separately. In this approach, the windows of exposure were nested within one another. For example, users of a hormonal contraceptive within 1 month would also be included in the characterization of use within 4 months and 12 months. A limitation in using this approach was the inability to differentiate associations observed in one period of exposure from another period of exposure. Any association observed within 4 months could be an indication of an association between exposure in the 1 month before or in the 12 weeks after conception, or in the period between 4 and 1 month prior to conception. This approach limited our ability to assess for differences of association across time or evaluation of any "dose response" as the exposure period approached and surpassed the estimated date of conception.

Seeking to address the limitations of the approach described above, we re-characterized exposure into discrete exposure periods – discrete in that they represented the window of time in which the last dose of contraceptive would have been taken. Furthermore, we limited our comparator group to non-users of hormonal contraceptives within any of the exposure periods. In doing so, we could assess whether there were differences in association based on the exposure period, relative to the date of conception,

as compared to unexposed pregnancies. By not including users of hormonal contraceptives in other exposure periods, we answered the question of whether or not use of a hormonal contraceptive, in a given exposure period, relative to non-users, confers increased odds for a given outcome. Contrasting use against non-users for that exposure period answered a different question; specifically, does use of a hormonal contraceptive in a given period of time, relative to non-users in that period of time, incur increased odds for a given outcome. While this provided more information about timing of use, this approach offered less information about whether use, in general, confers increased odds for the outcomes of interest.

Last date of use

In characterizing exposure, we based the last date of possible use according to the number of daily doses dispensed, adding this number to the date on which the prescription was dispensed. In doing so, we assumed that the use of the drug began on the day on which it was prescribed. To evaluate this assumption, we calculated the mode, median, and mean for the difference in days between the last dose date and next dispensing date for serial dispensing of the same type of hormonal contraceptive (oral combination, oral progestin-only, vaginal ring, transdermal, and injectable). The mean distribution was not distributed normally as there were some women who had long breaks between dispensing. Based on the median and mode, we determined that the assumption that the last dose date, as estimated by the number of DDDs dispensed, provided a reasonable approximation of the last day on which the drug could have been taken. Specifically, for the vaginal ring, the mode difference was -1 days and the median difference was -2 days, representing that women commonly were dispensed a subsequent prescription for the vaginal ring within 1 to 2 days after the final dose of the preceding dispense. The mode and median difference for all other forms of hormonal contraceptives was 0 days.

Self reported exposure

We also considered using self-reported use of hormonal contraceptives to define our exposure. However, as described previously, this approach had the limitation of not being able to characterize use by formulation and within periods of time closer to conception (only within 12 months and after conception were women asked to differentiate between combination and progestin-only oral contraceptives). There was also the potential that women would not be able to accurately recall or know whether the formulation of hormonal contraceptive they were using was a progestin-only or combination type oral contraceptive. We assessed the distribution of study covariates by self-reported use and saw that the distribution was inconsistent with the empirical data on prescribing patterns for different contraceptive formulations (Table 5.1).(87)

Table 5.1. Distribution of study covariates by self-reported use of hormonal contraceptives

Number exposed (within one year of pregnancy) Covariate		Combination OC %	Progestin-only OC %	Hormonal IUD %	Injectable %
Maternal age					
	14-19	1.0	1.7	1.9	8.2
	20-29	43.7	55.6	57.0	59.8
	30-39	53.3	42.2	40.6	31.3
	40-49	1.9	0.5	0.5	0.7
Maternal BMI (kg/m$^{2)}$					
	<18.5	3.2	3.1	3.1	5.4
	18.5-24.9	64.6	66.5	66.8	61.8
	25.0-29.9	22.6	22.1	22.2	21.0
	≥30	9.6	8.3	7.9	11.7
Parity					
	0	44.5	58.6	57.9	57.4
	1	35.8	29.4	29.1	22.0
	2	15.3	9.8	10.6	14.8
	3	3.3	1.8	1.9	4.4
	4 or more	1.0	0.4	0.5	1.4
Maternal education					
	More than 4 years of university or technical	22.3	21.0	20.3	8.9
	4 year university degree, regional technical	40.1	40.5	40.3	24.1
	3 years high school, junior college	15.6	16.7	17.5	25.4
	Technical high school	12.6	13.1	13.1	19.7
	1-2 years high school	5.0	4.7	4.7	11.0
	9-year secondary	2.8	2.6	2.7	9.0
	Other	1.6	1.5	1.5	1.8
Maternal smoking (at 17 weeks gestation)					
	None	76.1	75.3	74.9	58.7
	Quit	15.6	16.4	16.4	23.3
	Daily	1.8	1.9	2.1	3.3
	Sometimes	6.6	6.5	6.7	14.7

For example, the progestin-only oral contraceptive is more commonly prescribed to older women and women who smoke. The combination oral contraceptive is contraindicated for women over the age of 35 who smoke. The progestin-only oral contraceptive is also more likely to be prescribed to women who are parous, as it is believed that the progestin-only oral contraceptive will not interfere with the milk supply of breastfeeding mothers.(87) The NorPD-characterized hormonal contraceptive was more consistent with the expected prescribing patterns (Appendix, Supplementary Table S3.1).

5.4 Future directions

In the present study we identified a positive association between the combination hormonal contraceptive and preterm birth and an inverse association between the progestin-only oral contraceptive and small for gestational age. At age 3, there was evidence of an inverse association between combination oral contraceptive use and overweight or obesity and a positive association between use of a progestin-only oral contraceptive and overweight or obesity. It may be that the infants born preterm continue to be smaller at age 3 and that the infants at less risk for having been born SGA are larger at age 3. Modeling these associations to assess differences in longitudinal growth patterns in early life could help identify whether there are differences in growth from hormonal exposures that begin during these developmentally sensitive periods. Given the literature suggesting that being born small or early may be associated with future metabolic syndrome, additional studies exploring growth trajectories into school-aged and adolescent children may prove informative.

Despite the large sample size of the present study, future studies exploring these associations could benefit from an even larger study cohort sample size to estimate associations with greater precision and to model associations for formulations that were less commonly used in this cohort. Prescription, birth registry, and birth cohort data collected in

Denmark could offer the opportunity to explore these associations in a larger cohort. For evaluation of the association between hormonal contraceptives and birth outcomes, linkage to the Danish National Birth Cohort (DNBC) study would offer a sample of approximately 97,000 births. Linkage to the DNBC study would offer about 57,000 children at age 7, roughly twice that of the sample in the present study, for evaluation of the association between hormonal contraceptive use and offspring overweight or obesity. The DNBC study is also conducting follow-up with children once they reach age 11, thus providing the opportunity for assessing potential long-term effects of hormonal contraceptive use on offspring growth and development.

To assess the possibility that the associations observed in this study represent unaddressed confounding, particularly confounding by indication, studies will need to be conducted on prescribing practices as they relate to maternal characteristics. The present study described differences in maternal characteristics by type of hormonal contraceptive prescribed, but these differences should be described by specific formulation, including progestin component. Furthermore, there may be other maternal characteristics that merit consideration, such as maternal menstrual cycle differences that may be predictive of the formulation of hormonal contraceptive prescribed. These differences in menstrual cycles may suggest underlying differences in maternal metabolic milieu, a possible confounder in any relationships observed between hormonal contraceptive use and offspring developmental outcomes.

5.5 Public health impact

The primary implication of this research is the potential that hormonal contraceptive use, for some types and formulations, may be associated with adverse pregnancy outcomes and altered growth and development of offspring. For offspring overweight and obesity, the association observed was strongest for exposure in early pregnancy. Exposure in early pregnancy is relatively uncommon; however the potential that hormonally active agents

could be associated with offspring development is informative to other research, such as the potential for environmental sources of exposure to hormonally active agents to confer developmental effects on offspring. Much of the literature on potential developmental effects of exposure to exogenous, hormonally active agents is confined to animal model data. This research translates what has been demonstrated in experimental data into observational, human research and supports the assertion that developmental effects from these exposures are possible.

This research may also be informative to research on other pharmacologic sex hormone agents. There are other, commonly used, pregnancy-related pharmacologic sex hormones, such as hormonal agents in infertility treatment or for prevention of preterm birth. The results of this research suggest we should assess the potential for long-term developmental effects of these agents.

For preterm birth, associations were observed for users of certain hormonal contraceptives during early pregnancy, but also before conception. Assuming that other studies can offer additional assessment of the potential for confounding by indication and replicate the current findings, this research may have implications for prescribing practices. For women who may be planning a pregnancy, some contraceptive formulations may offer less of a potential for developmental effects to offspring.

This area of research is in its infancy, but provides the rationale for additional investigation exploring these associations. Hormonal contraceptive use is prevalent, yet little is known about long-term, formulation-specific effects on offspring.

APPENDIX

Table S3.1. Study population characteristics by type of hormonal contraceptive used within 12* months before conception

Characteristic		No hormonal contraceptive n=23,727 **%**	Combination OC n=14,012 %	Progestin-only OC n=4,572 %	Vaginal ring n=1,215 %	Transdermal n=832 %	Other** n=376 %
Maternal age							
	14-19	0.5	1.8	0.3	0.6	1.2	0.0
	20-29	33.1	56.5	37.7	54.3	59.0	47.9
	30-39	63.1	41.3	60.6	44.0	39.4	49.7
	40-49	3.3	0.4	1.4	1.1	0.5	2.4
Maternal BMI (kg/m^2)							
	<18.5	3.3	3.1	3.3	2.8	4.1	2.9
	18.5-24.9	65.6	66.8	65.7	68.8	65.0	60.1
	25.0-29.9	21.3	21.9	22.8	22.6	21.3	28.2
	≥30.0	9.8	8.2	8.3	5.8	9.6	8.8
Parity							
	0	37.8	70.6	17.2	67.4	57.2	29.5
	1	39.6	20.9	63.9	21.0	27.5	41.2
	2	17.6	7.0	15.0	9.3	12.7	21.8
	3	3.8	1.1	3.1	1.7	1.9	5.3
	4 or more	1.3	0.4	0.8	0.7	0.6	2.1
Maternal education							
	More than 4 years of university or technical	29.5	23.4	30.0	25.8	16.4	17.8
	4 year university degree, regional technical	40.0	40.5	44.7	46.0	42.0	33.8
	3 years high school, junior college	12.7	15.3	10.6	13.2	17.7	17.3
	Technical high school	10.3	12.8	9.5	10.5	14.7	16.8
	1-2 years high school	3.9	4.3	2.8	2.1	4.9	7.5
	9-year secondary	2.3	2.3	1.3	1.1	2.6	5.3
	Other	1.4	1.4	1.2	1.4	1.8	1.6
Maternal smoking (at 17 weeks)							
	None	80.0	76.8	85.0	77.8	75.0	70.5
	Quit	13.9	16.5	10.9	16.5	16.6	18.6
	Daily	1.3	1.6	1.2	1.7	2.2	2.4
	Sometimes	4.8	5.1	3.0	4.1	6.3	8.5

*Defined by any hormonal contraceptive use within 12 months prior to conception

**Includes the intrauterine, injectable, emergency, and implant hormonal contraceptives

Table S3.2. Covariate balance by combination oral contraceptive use within decile rank for exposure within 12 months before conception

	Propensity score decile rank																			
	1		2		3		4		5		6		7		8		9		10	
	Exposed (n=2791)		Exposed (n=2792)		Exposed (n=2791)		Exposed (n=2792)		Exposed (n=2791)		Exposed (n=2792)		Exposed (n=2792)		Exposed (n=2792)		Exposed (n=2791)		Exposed (n=2791)	
Covariate	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Maternal age (yrs)																				
14-19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.3	0.1	3.8	5.4
20-29	0.0	0.0	0.2	0.6	1.7	4.0	11.6	9.1	49.1	50.5	71.2	68.8	2.6	4.6	2.6	4.6	43.0	42.0	96.2	96.4
30-39	81.5	87.2	99.0	99.4	97.0	94.0	86.6	89.0	49.4	48.3	28.5	30.9	97.4	95.4	97.4	95.4	56.8	57.9	0.1	0.2
40-49	18.5	12.8	1.2	0.0	1.3	2.0	1.8	1.8	1.5	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maternal BMI (kg/m^2)																				
<18.5	3.8	4.3	3.3	2.9	3.6	5.5	2.6	3.2	2.3	2.8	1.5	1.7	5.7	5.5	5.7	5.5	5.6	5.1	0.5	0.8
18.5-24.9	62.9	65.0	66.1	55.4	65.1	55.3	66.4	64.4	63.0	57.7	59.9	61.5	70.3	71.9	70.3	71.9	72.5	73.0	65.0	61.1
25.0-29.9	21.1	20.5	20.3	24.6	21.5	24.6	20.2	23.3	25.1	29.7	27.8	24.1	16.0	15.8	16.0	15.8	14.3	16.5	26.8	30.1
≥30.0	12.2	10.3	10.2	17.1	9.8	14.6	10.8	9.1	9.4	9.8	10.9	12.8	8.0	6.8	8.0	6.8	7.6	5.4	7.7	8.0
Parity																				
0	0.6	0.0	1.2	0.0	1.4	2.0	1.9	1.8	2.8	4.7	28.1	30.3	100	100	100	100	100	100	100	100
1	36.5	34.2	45.9	56.6	60.9	56.3	71.5	69.9	81.2	79.2	69.0	68.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	44.8	47.9	41.2	30.9	31.1	35.2	22.8	24.7	13.9	14.5	2.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	13.5	11.1	9.7	10.9	4.9	5.5	2.9	2.7	1.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 or more	4.7	6.8	2.2	1.7	1.7	1.0	1.0	0.9	1.0	1.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maternal education																				
More than 4 years of university	41.3	32.5	39.0	28.0	37.5	27.1	29.9	24.7	16.3	14.2	15.7	18.4	50.9	51.9	50.9	51.9	41.4	45.4	16.0	20.6
4 year university degree	29.9	24.8	36.8	40.0	39.1	35.7	45.0	44.8	52.7	44.8	42.1	34.8	29.6	30.6	29.6	30.6	36.6	36.2	48.4	50.8
3 years high school	11.7	20.5	10.1	13.7	10.0	13.1	11.3	12.8	13.9	16.7	14.4	15.3	9.9	9.8	9.9	9.8	10.4	9.9	15.2	14.5
Technical high school	8.0	8.6	7.3	6.9	6.1	10.1	8.4	10.5	11.5	15.8	21.0	23.0	4.3	3.8	4.3	3.8	4.5	4.0	10.5	7.5
1-2 years high school	4.9	9.4	3.5	6.9	3.7	8.0	3.0	3.7	3.0	5.4	4.1	5.1	2.1	0.8	2.1	0.8	2.8	1.3	4.5	2.0
9-year secondary	2.7	4.3	2.3	4.0	2.1	4.0	1.4	1.4	1.5	2.2	1.7	1.7	1.5	1.6	1.5	1.6	2.0	1.5	3.9	2.8
Other	1.6	0.0	1.1	0.6	1.4	2.0	1.1	2.3	1.1	1.0	0.9	1.7	1.7	1.6	1.7	1.6	2.3	1.8	1.6	2.0
Maternal smoking (at 17 weeks)																				
None	84.0	78.6	85.5	79.4	84.8	75.4	84.1	79.0	81.7	78.2	76.9	72.2	80.0	84.2	80.0	84.2	79.2	81.7	66.6	66.2
Quit	9.6	16.2	9.6	12.6	10.5	14.6	10.9	12.8	12.6	15.8	15.3	17.3	15.5	12.5	15.5	12.5	16.7	14.1	22.6	23.5
Daily	0.9	1.7	0.8	1.7	1.2	1.0	0.9	0.5	1.7	1.3	2.1	2.8	0.7	1.3	0.7	1.3	0.9	1.5	3.3	2.5
Sometimes	5.6	3.4	4.1	6.3	3.6	9.1	4.1	7.8	4.0	4.7	5.7	7.6	3.8	2.1	3.8	2.1	3.2	2.8	7.9	7.4

Table S3.3. Hormonal contraceptive use and weight for gestational age at birth

Exposure	Small for gestational age (SGA)				Birthweight z-score	
	Exposed (n)	SGA (n)	OR (95% CI)	aOR* (95% CI)	β (95% CI) unadjusted	β (95% CI) adjusted*
None	23,421	605	referent	referent	referent	referent
Within 12 weeks after conception						
Combination OC	1,062	42	1.55 (1.14, 2.13)	1.10 (0.78, 1.55)	0.20 (0.14, 0.27)	0.02 (-0.05, 0.09)
Other **	576	15	1.01 (0.60, 1.69)	1.02 (0.60, 1.73)	0.07 (-0.02, 0.17)	0.08 (0.01, 0.18)
Within 1 month before pregnancy						
Combination OC	4,660	142	1.19 (0.98, 1.43)	0.96 (0.80, 1.17)	0.15 (0.12, 0.19)	0.01 (-0.03, 0.04)
Progestin-only OC	1,204	14	0.44 (0.26, 0.76)	0.57 (0.33, 0.97)	-0.09 (-0.16, -0.03)	-0.03 (-0.09, 0.03)
Vaginal ring	356	11	1.20 (0.66, 2.20)	0.99 (0.54, 1.81)	0.07 (-0.05, 0.18)	-0.09 (-0.20, 0.03)
Other **	245	3	0.47 (0.15, 1.46)	0.40 (0.13, 1.27)	0.09 (-0.05, 0.22)	-0.001 (-0.13, 0.13)
Within 4 months before pregnancy						
Combination OC	3,833	103	1.04 (0.84, 1.29)	0.82 (0.66, 1.02)	0.11 (0.07, 0.15)	-0.05 (-0.08, 0.01)
Progestin-only OC	1,284	11	0.33 (0.18, 0.59)	0.41 (0.22, 0.75)	-0.10 (-0.16, -0.04)	-0.03 (-0.09, 0.03)
Vaginal ring	352	16	1.80 (1.08, 2.98)	1.48 (0.88, 2.49)	0.15 (0.03, 0.28)	0.01 (-0.11, 0.14)
Other **	271	6	0.85 (0.38, 1.93)	0.73 (0.32, 1.68)	0.08 (-0.06, 0.21)	0.004 (-0.14, 0.13)
Within 12 months before pregnancy						
Combination OC	4,633	147	1.24 (1.03, 1.48)	0.95 (0.79, 1.15)	0.12 (0.08, 0.15)	-0.05 (-0.09, -0.01)
Progestin-only OC	1,795	33	0.71 (0.50, 1.01)	0.95 (0.66, 1.36)	-0.08 (-0.13, -0.03)	0.01 (-0.04, 0.06)
Vaginal ring	424	10	0.91 (0.48, 1.71)	0.76 (0.40, 1.42)	0.07 (-0.03, 0.17)	-0.06 (-0.16, 0.03)
Other **	618	9	0.56 (0.29, 1.08)	0.52 (0.26, 1.01)	0.01 (-0.08, 0.10)	-0.02 (-0.11, 0.06)

*Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth
**Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

Table S3.4. Hormonal contraceptive use by progestin type and route of administration and small for gestational age

Exposure	Exposed (n)	SGA (n)	OR (95% CI)	aOR* (95% CI)
None	23,421	605	referent	referent
Within 12 weeks after conception				
Combination OC				
drospirenone and EE	368	16	1.71 (1.03, 2.05)	1.24 (0.74, 2.08)
levonorgestrel and EE	545	19	1.36 (0.86, 2.17)	0.96 (0.59, 1.56)
Other**	725	22	1.18 (0.77, 1.82)	1.08 (0.69, 1.69)
Within 1 month before conception				
Combination OC				
desogestrel and EE	295	10	1.32 (0.70, 2.50)	1.12 (0.59, 2.12)
drospirenone and EE	1,472	36	0.95 (0.67, 1.33)	0.75 (0.53, 1.05)
levonorgestrel and EE	2,521	82	1.27 (1.00, 1.60)	1.04 (0.82, 1.32)
norethisterone and EE	372	14	1.47 (0.86, 2.53)	1.24 (0.72, 2.13)
Vaginal ring				
etonogestrel and EE	356	11	1.20 (0.66, 2.20)	0.99 (0.54, 1.81)
Other**	1,449	17	0.45 (0.28, 0.73)	0.53 (0.32, 0.86)
Within 4 months before conception				
Combination OC				
drospirenone and EE	1,227	35	1.11 (0.78, 1.56)	0.88 (0.62, 1.25)
levonorgestrel and EE	2,107	61	1.12 (0.86, 1.47)	0.89 (0.68, 1.17)
Vaginal ring				
etonogestrel and EE	352	16	1.80 (1.08, 2.98)	1.48 (0.88, 2.49)
Other**	2,054	24	0.45 (0.30, 0.67)	0.46 (0.31, 0.70)
Within 12 months before conception				
Combination OC				
desogestrel and EE	215	12	2.23 (1.24, 4.01)	1.73 (0.96, 3.12)
drospirenone and EE	1,475	43	1.13 (0.83, 1.55)	0.87 (0.63, 1.19)
levonorgestrel and EE	2,605	82	1.23 (0.97, 1.55)	0.95 (0.74, 1.21)
norethisterone and EE	338	10	1.15 (0.61, 2.17)	0.89 (0.47, 1.68)
Progestin-only OC				
desogestrel	1,031	18	0.67 0.42, 1.08)	0.88 (0.55, 1.42)
norethisterone	640	12	0.72 (0.40, 1.28)	1.01 (0.56, 1.81)
Vaginal ring				
etonogestrel and EE	424	10	0.91 (0.48, 1.71)	0.76 (0.40, 1.42)
Other**	742	12	0.62 (0.35, 1.10)	0.61 (0.34, 1.08)

*Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth

**Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

Table S3.5. Sensitivity analysis for combination oral contraceptive use and preterm birth with restriction to nulliparous pregnancies

Exposure	Within 12 weeks after conception (n=9,761) aOR* (95% CI)	Within 1 month before conception (n=12,565) aOR* (95% CI)	Within 4 months before conception (n=12,127) aOR* (95% CI)	Within 12 months before conception (n=12,990) aOR* (95% CI)
None	referent	referent	referent	referent
Combination OC	1.39 (1.02, 1.90)	1.22 (1.02, 1.47)	1.39 (1.15, 1.68)	1.16 (0.97, 1.39)
Progestin-only OC	2.21 (1.03, 4.73)	0.80 (0.41, 1.56)	1.26 (0.71, 2.23)	1.18 (0.68, 2.04)
Vaginal ring	**	0.53 (0.24, 1.21)	0.82 (0.42, 1.62)	0.63 (0.31, 1.27)
Transdermal	**	**	**	0.73 (0.32, 1.67)
Injectable	**	**	**	1.97 (0.89, 4.35)
Other**	0.49 (0.15, 1.59)	0.49 (0.15, 1.55)	0.71 (0.29, 1.74)	†

*Adjusted for maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth
**Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category
†Too few to estimate association

Table S3.6. Sensitivity analysis for combination oral contraceptive and preterm birth with vaginal ring users as the comparator group

	Within 1 month before conception (n=5,099)		Within 4 months before conception (n=4,185)		Within 12 months before conception (n=5,057)	
Exposure	OR (95% CI)	aOR* (95% CI)	OR (95% CI)	aOR* (95% CI)	OR (95% CI)	aOR* (95% CI)
Vaginal ring	referent	referent	referent	referent	referent	referent
Combination OC	1.54 (0.89, 2.67)	1.55 (0.89, 2.71)	1.67 (0.92, 3.01)	1.72 (0.94, 3.15)	1.88 (1.05, 3.39)	1.78 (0.99, 3.21)

*Adjusted for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth

Table S3.7. Sensitivity analysis for association between combination oral contraceptive use and preterm birth with propensity score analysis approach*

Exposure	Within 12 weeks after conception (n=24,318)** aOR** (95% CI)	Within 1 month before conception (n=27,978)** aOR** (95% CI)	Within 4 months before Conception (n=27,242)** aOR** (95% CI)	Within 12 months before conception (n=27,915)** aOR** (95% CI)
None	referent	referent	referent	referent
Combination OC	1.35 (1.02, 1.77)	1.17 (1.00, 1.37)	1.34 (1.14, 1.57)	1.24 (1.06, 1.45)

*Doubly robust approach with generalized estimation equation models including both the predicted probability of combination oral contraceptive use (modeled as an indicator variable for decile rank) and with adjustment for parity, maternal education, maternal pre-pregnancy BMI, maternal smoking, and maternal age at birth
**Study population after trimming for non-positivity

Table S4.1. Distribution of hormonal contraceptive use by exposure period

	Within 12 weeks after*		Within 1 month prior*		Within 4 months prior*		Within 12 months prior*	
Exposure	underweight/ normal weight	Overweight /obese	underweight/ normal weight	Overweight/ obese	underweight/ normal weight	Overweight /obese	underweight/ normal weight	Overweight/ obese
None**	8,645	1,342	8,645	1,342	8,645	1,342	8,645	1,342
Combination OC								
desogestrel and EE	25	5	116	17	87	8	88	13
drospirenone and EE	112	15	579	88	486	58	608	77
levonorgestrel and EE	177	18	1,024	164	864	137	1,113	154
norethisterone and EE	28	0	153	17	116	20	126	33
Progestin-only OC								
desogestrel	53	15	266	49	331	43	395	67
norethisterone	52	6	204	28	169	32	235	44
lynestronol	1	0	7	2	8	2	33	5
levonorgestrel	0	0	6	0	11	2	18	5
Vaginal ring (etonogestrel and EE)	30	4	159	14	138	15	183	21
Transdermal (norelgestromin and EE)	28	0	87	13	94	9	103	15
Injectable								
(medroxyprogestin)	3	0	1	0	9	2	55	10
Implant (etonogestrel)	0	0	0	0	0	0	7	0
IUD (levonorgestrel)	3	0	3	0	3	0	44	5

*Represents discrete categories of exposure based on the last possible date of use as derived from date of prescription and the defined daily doses prescribed
**No use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

Table S4.2. Covariate distribution by contraceptive formulation and route of administration for use at any time within 12 months of conception

	No hormonal contraceptive n=10,099	Combination OC n=6,459	Progestin-only OC n=2,061	Vaginal Ring n=558	Transdermal n=335	Injectable n=78
Covariate	%	%	%	%	%	%
Maternal age						
14-19	0.2	0.7	0.2	0.0	0.6	0.0
20-29	30.8	54.9	35.0	53.4	53.1	50.0
30-39	65.3	43.9	63.6	45.3	46.3	48.7
40-49	3.7	0.4	1.3	1.3	0.0	1.3
Maternal BMI ($kg/m^{2)}$						
<18.5	3.1	2.7	3.0	2.5	3.6	0.0
18.5-24.9	66.6	68.2	67.2	67.6	66.3	61.5
25.0-29.9	21.5	21.6	22.7	24.0	23.3	28.2
≥30.0	8.9	7.5	7.1	5.9	6.9	10.3
Parity						
0	39.9	73.7	18.5	73.3	60.3	52.6
1	39.2	18.8	64.5	17.6	28.1	25.6
2	16.4	6.1	13.7	7.7	10.8	19.2
3	3.2	1.1	2.7	1.3	0.9	2.6
4 or more	1.3	0.3	0.6	0.2	0.0	0.0
Maternal education						
More than 4 years of university or technical	32.2	26.6	31.8	27.6	19.7	16.7
4 year university degree, regional technical	42.4	43.3	47.6	49.8	50.2	38.5
3 years high school, junior college	11.5	13.4	9.3	13.1	13.4	23.1
Technical high school	8.4	11.3	7.8	7.2	10.5	15.4
1-2 years high school	2.7	2.7	1.7	0.5	2.7	5.1
9-year secondary	1.0	1.3	0.8	0.5	1.8	0.0
Other	1.3	1.5	1.1	1.3	1.8	1.3
Maternal smoking (at 17 weeks gestation)						
None	83.6	80.5	87.6	79.8	82.4	68.0
Quit	12.1	14.2	9.2	14.9	10.8	20.5
Daily	1.1	1.4	0.9	2.0	1.2	5.1
Sometimes	3.2	3.9	2.3	3.4	3.6	6.4

Table S4.3. Distribution of hormonal contraceptive use for baseline population, study population, and proportion retained at follow-up

	Baseline population (n=44,649)				Study population (n=19,652)				Proportion retained at follow-up (overall=44.0%)*			
	Within 12 months before	Within 4 months before	Within 1 month before	Within 12 weeks after	Within 12 months before	Within 4 months before	Within 1 month before	Within 12 weeks after	Within 12 months before	Within 4 months before	Within 1 month before	Within 12 weeks after
Contraceptive	%	%	%	%	%	%	%	%	%	%	%	%
None[†]	75.8	80.3	78.4	93.5	74.3	79.1	76.9	94.6	42.7	42.7	42.7	42.7
Combination OC	15.0	13.2	15.6	4.2	16.5	14.1	16.6	3.6	47.9	46.4	46.4	35.8
Progestin OC	5.8	4.4	4.0	1.4	6.0	4.7	4.3	1.2	44.8	46.6	46.7	35.4
Vaginal Ring	1.4	1.2	1.2	0.4	1.5	1.2	1.3	0.3	48.3	43.5	48.7	33.0
Transdermal	1.0	0.8	0.8	0.4	0.9	0.8	0.8	0.2	40.3	44.0	42.2	22.7
Injectable	0.6	0.1	0.0	0.0	0.5	0.1	0.0	0.0	36.1	42.3	33.3	75.0
Other[‡]	0.5	0.0	0.0	0.0	0.4	0.0	0.0	0.0	39.2	30.0	60.0	30.0

*Represents the proportion of pregnancies retained at follow-up by contraceptive type – illustrates higher proportion lost to follow-up in early pregnancy among hormonal contraceptive users

**Represents discrete categories of exposure based on the last possible date of use as derived from date of prescription and the defined daily doses prescribed

[†]No use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

[‡]Includes intrauterine device, implant, and emergency contraceptive (for baseline population only)

Table S4.4. Association between hormonal contraceptive use within 1 month* of conception and offspring overweight or obese

Exposure	Exposed (n)	Overweight or obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC	2,158	286	0.99 (0.86, 1.13)	1.04 (0.90, 1.19)
Progestin-only OC	562	79	1.05 (0.83, 1.35)	1.03 (0.80, 1.31)
Vaginal ring	173	14	0.57 (0.33, 0.98)	0.60 (0.35, 1.04)
Transdermal	114	15	1.00 (0.58, 1.72)	1.03 (0.60, 1.77)
Other[†]	4	0	[‡]	[‡]

*Use within 1 month before, but not within 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

[‡]Data too sparse to estimate association

Table S4.5. Association between hormonal contraceptive use within 4 months* of conception and offspring overweight or obese

Exposure	Exposed (n)	Overweight or obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC	1,776	223	0.93 (0.79, 1.08)	0.96 (0.82, 1.13)
Progestin-only OC	598	79	0.98 (0.77, 1.25)	0.97 (0.76, 1.24)
Vaginal ring	153	15	0.70 (0.41, 1.20)	0.73 (0.43, 1.25)
Other[†]	117	11	0.67 (0.36, 1.25)	0.67 (0.36, 1.26)

*Use within 4 months before, but not within 1 month before and 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Table S4.6. Association between hormonal contraceptive use within 12 months* of conception and offspring overweight or obese

Exposure	Exposed (n)	Overweight or obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC	2,212	277	0.92 (0.80, 1.06)	0.98 (0.85, 1.13)
Progestin-only OC	802	121	1.14 (0.94, 1.40)	1.12 (0.91, 1.37)
Vaginal ring	204	21	0.74 (0.47, 1.16)	0.77 (0.49, 1.22)
Transdermal	118	15	0.94 (0.51, 1.62)	1.01 (0.58, 1.74)
Injectable	65	10	1.17 (0.60, 2.30)	1.14 (0.57, 2.26)
Other[†]	56	5	0.63 (0.25, 1.59)	0.59 (0.24, 1.48)

*Use within 12 months before, but not within 4 and 1 month before and 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Table S4.7. Association between hormonal contraceptive use in early pregnancy* and offspring overweight or obese by progestin type

Exposure	Exposed (n)	Overweight/obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC				
drospirenone and EE	127	15	0.86 (0.50, 1.48)	0.89 (0.52, 1.54)
levonorgestrel and EE	195	18	0.66 (0.40, 1.07)	0.69 (0.42, 1.15)
Progestin-only OC				
desogestrel	68	15	1.82 (1.03, 3.25)	1.87 (1.06, 3.32)
Other[†]	143	14	0.70 (0.40, 1.22)	0.70 (0.40, 1.22)

*Use within 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within 12, 4, and 1 month before conception and within 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

[†]Hormonal contraceptives with < 10 exposed cases were combined into an "other" category

Table S4.8. Association between hormonal contraceptive use within 1 month prior to conception* and offspring overweight or obese by progestin type

Exposure	Exposed (n)	Overweight/obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC				
desogestrel and EE	133	17	0.94 (0.57, 1.58)	0.97 (0.58, 1.62)
drospirenone and EE	667	88	0.98 (0.78, 1.23)	1.04 (0.82, 1.31)
levonorgestrel and EE	1,188	164	1.03 (0.87, 1.23)	1.10 (0.92, 1.31)
norethisterone and EE	170	17	0.72 (0.43, 1.19)	0.74 (0.45, 1.23)
Progestin-only OC				
desogestrel	315	49	1.19 (0.87, 1.62)	1.16 (0.85, 1.59)
norethisterone	232	28	0.88 (0.59, 1.32)	0.85 (0.57, 1.27)
Vaginal ring				
etonogestrel and EE	173	14	0.57 (0.33, 0.98)	0.60 (0.35, 1.04)
Transdermal				
norelgestromin and EE	100	13	0.96 (0.54, 1.72)	0.99 (0.55, 1.78)
Other[†]	19	2	0.76 (0.17, 2.28)	0.72 (0.16, 3.13)

*Use within 1 month before, but not within 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Table S4.9. Association between hormonal contraceptive use within 4 months prior to conception* and offspring overweight or obese by progestin type

Exposure	Exposed (n)	Overweight/obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC				
drospirenone and EE	544	58	0.77 (0.58, 1.01)	0.80 (0.60, 1.06)
levonorgestrel and EE	1,001	137	1.02 (0.84, 1.24)	1.06 (0.88, 1.29)
norethisterone and EE	136	20	1.11 (0.69, 1.79)	1.19 (0.73, 1.92)
Progestin-only OC				
desogestrel	374	43	0.84 (0.61, 1.16)	0.83 (0.60, 1.16)
norethisterone	201	32	1.22 (0.83, 1.79)	1.19 (0.81, 1.75)
Vaginal ring				
etonogestrel and EE	153	15	0.70 (0.41, 1.20)	0.73 (0.43, 1.25)
Other[†]	235	23	0.70 (0.45, 1.08)	0.71 (0.46, 1.10)

*Use within 4 months before, but not within 1 month before and 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception
**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity
[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Table S4.10. Association between hormonal contraceptive use within 12 months prior to conception* and offspring overweight or obese by progestin type

Exposure	Exposed (n)	Overweight/obese (n)	Crude OR (95% CI)	Adjusted** OR (95% CI)
None	9,987	1,342	referent	referent
Combination OC				
desogestrel and EE	101	13	0.95 (0.53, 1.71)	1.01 (0.56, 1.83)
drospirenone and EE	685	77	0.82 (0.64, 1.04)	0.86 (0.67, 1.11)
levonorgestrel and EE	1,267	154	0.89 (0.75, 1.07)	0.95 (0.79, 1.04)
norethisterone and EE	159	33	1.69 (1.15, 2.48)	1.78 (1.22, 2.62)
Progestin-only OC				
desogestrel	462	67	1.09 (0.84, 1.42)	1.07 (0.82, 1.39)
norethisterone	279	44	1.21 (0.87, 1.67)	1.18 (0.85, 1.65)
Vaginal ring				
etonogestrel and EE	204	21	0.74 (0.47, 1.16)	0.77 (0.49, 1.22)
Transdermal				
norelgestromin and EE	118	15	0.94 (0.54, 1.62)	1.01 (0.58, 1.74)
Injectable				
medroxyprogesterone	65	10	1.17 (0.60, 2.30)	1.14 (0.57, 2.25)
Other[†]	117	15	0.95 (0.55, 1.63)	0.89 (0.52, 1.54)

*Use within 12 months before, but not within 4 and 1 month before and 12 weeks after conception as compared to no use of a hormonal contraceptive within the discrete categories of within12, 4, and 1 month before conception and within 12 weeks after conception
**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity
[†]Hormonal contraceptives with < 10 exposed cases were combined into an “other” category

Table S4.11. Sensitivity analyses employing multiple imputation-GEE models

Exposure	Early pregnancy* adjusted OR** (95% CI)	Within 1 month* adjusted OR** (95% CI)	Within 4 months* adjusted OR** (95% CI)	Within 12 months* adjusted OR** (95% CI)
None	referent	referent	referent	referent
Combination OC	0.85 (0.66, 1.11)	1.06 (0.96, 1.17)	0.99 (0.85, 1.15)	0.99 (0.87, 1.14)
Progestin-only OC	1.27 (0.69, 2.33)	1.04 (0.84, 1.29)	1.06 (0.84, 1.35)	1.16 (0.92, 1.46)
Vaginal ring	‡	0.72 (0.45, 1.13)	0.82 (0.56, 1.21)	0.87 (0.60, 1.17)
Transdermal	‡	0.99 (0.57, 1.72)	‡	1.00 (0.61, 1.64)
Injectable	‡	‡	‡	1.04 (0.66, 1.65)
Other†	0.87 (0.52, 1.45)	‡	0.86 (0.17, 4.22)	0.75 (0.34, 1.64)

*Represents discrete categories of exposure based on the last possible date of use as derived from date of prescription and the defined daily doses prescribed as compared to no use of a hormonal contraceptive within 12, 4, and 1 month of conception and with 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

†Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

‡Data too sparse to estimate association

Table S4.12. Sensitivity analyses employing inverse probability weighting-GEE models

Exposure	Early pregnancy* adjusted OR** (95% CI)	Within 1 month* adjusted OR** (95% CI)	Within 4 months* adjusted OR** (95% CI)	Within 12 months* adjusted OR** (95% CI)
None	referent	referent	referent	referent
Combination OC	0.68 (0.47, 0.99)	1.04 (0.98, 1.21)	0.97 (0.82, 1.04)	0.99 (0.89, 1.15)
Progestin-only OC	1.16 (0.72, 1.86)	1.00 (0.78, 1.29)	0.99 (0.77, 1.27)	1.10 (0.90, 1.36)
Vaginal ring	‡	0.60 (0.34, 1.04)	0.67 (0.46, 0.97)	0.77 (0.49, 1.23)
Transdermal	‡	1.06 (0.57, 1.94)	‡	1.02 (0.59, 1.77)
Injectable	‡	‡	‡	1.06 (0.53, 2.12)
Other†	0.86 (0.38, 1.96)	‡	0.64 (0.34, 1.21)	0.58 (0.23, 1.46)

*Represents discrete categories of exposure based on the last possible date of use as derived from date of prescription and the defined daily doses prescribed as compared to no use of a hormonal contraceptive within 12, 4, and 1 month of conception and with 12 weeks after conception

**Adjusted for maternal age, maternal smoking at 17 weeks gestation, maternal pre-pregnancy BMI, and parity

†Hormonal contraceptive types with <10 exposed cases collapsed into a single "other" category

‡Data too sparse to estimate association

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