Early life environmental exposures and children’s growth

A longitudinal study evaluating prenatal exposure for endocrine disrupting chemicals and nutrition in relation to children’s growth up to seven years of age

Katherine Svensson
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Early life is an important period for growth and development and therefore, sensitive to environmental exposures, such as chemicals and nutrition. Endocrine disrupting chemicals (EDCs) are man-made chemicals, common in everyday population exposure, and have been associated with unfavorable health effects and development. Additionally, optimal nutrition during pregnancy is important for both maternal and fetal health. But we need more knowledge on how these environmental exposures may influence children’s growth and if there are sex specific effects.

Twenty-six EDCs were measured in the urine and serum of pregnant women and their children’s growth was measured up to 7 years of age, including birthweight, height, weight, and body fat. Results show that higher levels of EDC mixtures were associated with lower birthweight, slower weight gain, and sex-specific effects on body fat. Also, better nutrition was associated with greater height and sex-specific effects on body fat.

The associations were small and not of concern for the individual, but from a population perspective it is an opportunity for improvement. Regulation of EDCs, both persistent and non-persistent, as well as adherence to nutritional guidelines, may be beneficial to promote healthy environments for children’s growth.
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ABSTRACT

Endocrine disrupting chemicals (EDCs) have the potential to disrupt the endocrine system, and of special concern is exposure during early life as it is a sensitive period for growth and development. Pregnant women are exposed daily to many EDCs concurrently, and there is need for research that focuses on mixtures. Additionally, optimal nutrition during pregnancy is critical for fetal growth and pregnancy outcomes. However, further knowledge is needed on the importance of EDC mixtures and nutrition during pregnancy, for birthweight and growth during childhood.

The overall aim of this thesis was to evaluate the associations between prenatal exposure to EDC mixtures and nutrition respectively, with birthweight, growth and body composition in early- and mid-childhood, and to determine if these associations differed by sex. Data from mother-child pairs in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study was analyzed, including 26 EDCs in prenatal urine and serum samples, children’s anthropometric and body composition measures up to seven years of age, and sociodemographic data from questionnaires and registers.

Results suggest that higher prenatal exposure to EDC mixtures were associated with lower birthweight, and slower weight gain in early childhood, including a later peak growth velocity among girls. At 5.5 and 7 years of age, EDC mixtures were associated with lower BMI, less odds of overweight and less body fat among girls, but more body fat among boys at 7 years of age. Chemicals of concern in the mixture were e.g., phthalates, bisphenols, perfluorinated alkyl substances (PFASs) and pesticides. Finally, maternal nutrition during pregnancy, i.e., better adherence to nutritional guidelines, was associated with more body fat for boys but less body fat for girls.

In conclusion, prenatal exposure to both EDC mixtures and nutrition suggests to have an influence on birthweight, and children’s growth. Several of the found associations also differed by sex.
Hormonstörande kemikalier (endocrine disrupting chemicals, EDCs) har visats kunna störa det endokrina systemet, och exponering tidigt i livet är speciellt känsligt eftersom det är en period av tillväxt och utveckling. Gravida är dagligen exponerade för många kemikalier samtidigt och därför behövs forskning med fokus på blandningar av EDCs. Därutöver är optimal näring under graviditeten viktig för fostrets tillväxt. Dock behövs mer forskning om blandningar av EDCs och nutrition under graviditeten för att få mer kunskap om dess betydelse för födelsevikt och barns tillväxt.

Det övergripande syftet med avhandlingen är att utvärdera betydelsen av prenatal exponering av EDCs och nutrition, i relation till födelsevikt, tillväxt och kroppssammansättning tidigt och i mitten av barndomen, och utvärdera om det finns köns skillnader. Data analyserades från mammor och barn i studien Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA), inklusive halter av 26 EDCs i urin- och serumprover hos gravida kvinnor, barnens längd, vikt, och kroppssammansättning, samt sociodemografiska data från enkäter och registerdata.

Resultatet tyder på att högre prenatal exponering för blandningar av EDCs under graviditeten var associerade till lägre födelsevikt och mer långsam viktuppgång tidigt i barndomen. Vid 5,5 och 7 års ålder var blandningar av EDCs associerade med lägre BMI, lägre odds för övervikt och mindre kroppsfett bland flickor, men för pojkar var associationerna de motsatta. Kemikalier av särskild vikt för de funna associationerna var t.ex. ftalater, bisfenoler, perfluorerade alkylsubstanser (PFASs) och bekämpningsmedel. Slutligen påvisades att mammans näringsintag under graviditeten, d.v.s. bättre nutrition i relation till näringsrekomendationerna, var associerat med mer kroppsfett för pojkar men mindre kroppsfett för flickor.

Sammanfattningsvis visar avhandlingen att exponering för blandningar av EDCs och mammans näringsintag under graviditeten är associerade till barns födelsevikt och tillväxt. Flera av de påvisade associationerna skilde sig åt för pojkar och flickor.
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LIST OF PAPERS

This thesis is based on the following four original manuscripts which are referred in the text with Roman numerals (I-IV).


III. Svensson, K., Gennings, C., Lindh, C., Kiviranta, H., Wikström, S., & Bornehag, C.G. EDC mixtures during pregnancy and body fat at 7 years of age in a Swedish cohort, SELMA study. (Manuscript)


The manuscripts have been published in Open Access journals which allow for reprints.
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AUTHOR CONTRIBUTIONS

Below is a summary of the contribution from each author in the four studies. All authors have reviewed and agreed to the publication of the final version of the four manuscripts.

Katherine Svensson: conceptualization, interpretation of results, writing of the manuscripts, and statistical analysis.
Chris Gennings: conceptualization, interpretation of results, review of the manuscript. Development of the Weighted Quantile Sum (WQS) regression, development of My Nutrition Index, and development of the repeated holdout validation method in the WQS regression.
Eva Tanner: statistical analysis, conceptualization, interpretation of results, review of the manuscript. Calculation of weight trajectories, and development of the repeated holdout validation method in the WQS regression.
Lars Hagenäs: conceptualization, interpretation of results, review of the manuscript.
Alicja Wolk: conceptualization, interpretation of results, review of the manuscript.
Niclas Håkansson: statistical analysis, review of the manuscript. Calculation of nutrient composition values based on the food frequency questionnaires.
Christian Lindh: analytical method, review of the manuscript.
Hannu Kiviranta: analytical method, review of the manuscript.
Panu Rantakokko: analytical method, review of the manuscript.
Sverre Wikström: conceptualization, planning of the study, data collection, interpretations of results, review of the manuscript.
Carl-Gustaf Bornehag: conceptualization, planning of the study, data collection, interpretation of results, review of the manuscript.
INTRODUCTION

Early life, especially the fetal period, represents a sensitive period of growth and development (Hanson et al., 2011). According to the Developmental Origins of Health and Disease (DOHaD) hypothesis, prenatal environmental exposures may lead to long-term health effects in childhood and adulthood (Gluckman et al., 2010). Endocrine disrupting chemicals (EDCs) and nutrition are two environmental exposures important to consider. Most EDCs are man-made chemicals, and because of their various utilities they are present in our everyday environment. Exposure to these chemicals have been associated with unfavorable health effects, both in observational and experimental studies (Kabir et al., 2015). In contrast, optimal nutrition is vital during pregnancy and has been associated with favorable pregnancy outcomes and fetal growth (Schwarzenberg & Georgieff, 2018).

Birthweight and weight gain in childhood are early markers of importance for children’s health and development. Both fetal growth and birthweight are associated with postnatal growth and health later in life (Cameron & Demerath, 2002). Infants born small may have higher risk for metabolic and cardiovascular disease later in life (Barker et al., 2002; Knop et al., 2018). Furthermore, fast weight gain in early infancy has been associated with a higher risk for obesity in childhood (Druet et al., 2012).

DOHaD hypothesis: a paradigm to study early life exposures

The first 1,000 days, from the time of conception until 2 years of age are critical for healthy growth and development of the child (Mameli et al., 2016; Schwarzenberg & Georgieff, 2018; Woo Baidal et al., 2016). From a life-time perspective, the fetal period is a critical period of growth, and the growing fetus has the ability to adapt, to a certain degree, to its environment (Hanson et al., 2011). Therefore, environmental stressors during this time of development may have long-term health effects (Gluckman et al., 2010).

David Barker, a physician and epidemiologist (1938–2013), first provided indication that stressors early in life could lead to higher risk of cardiometabolic diseases during adulthood (Barker, 2007). Ecological studies in England and Wales showed a correlation pattern between infant mortality during the years
1921–1925 and death rates of heart disease in the years 1968–1978 (Barker & Osmond, 1986). Infant mortality was generally associated with poor social conditions, whereas heart disease was associated with wealth and adult lifestyle (e.g., smoking). This unexpected correlation pattern suggested there must be other explanations besides lifestyle. As this correlation pattern, was particularly seen among infants born small (i.e., small-for-gestational age) it provided a link to fetal undernutrition (Barker et al., 1993). These results led to the hypothesis that adverse environmental exposures in utero and infancy may permanently change the body structure, e.g., regarding physiology and metabolism, and increase the risk of disease later in life (Barker, 2007). The DOHaD Society has the following definition on the DOHaD hypothesis:

The Developmental Origins of Health and Disease is a multidisciplinary field that examines how environmental factors acting during the phase of developmental plasticity interact with genotypic variation to change the capacity of the organism to cope with its environment in later life” (Heindel et al., 2015).

Studies on the Dutch famine also provided further ground for the DOHaD hypothesis. The famine lasted about 5 months during the last part of World War II (1944–1945) in the western part of the Netherlands and food rations varied between 400 and 800 calories. Research on the Dutch famine show that infants born during the famine, had smaller birth size, and higher risk of developing cardiovascular disease in adulthood, such as higher glucose intolerance (de Rooij et al., 2006; Ravelli et al., 1998), coronary heart disease (Roseboom et al., 2000) and inconsistently with blood pressure (Roseboom et al., 1999; Stein et al., 2006). Some of these associations were more evident for infants born with low birthweight (Ravelli et al., 1998; Roseboom et al., 2000; Stein et al., 2006).

During the fetal stage, developmental adaptations to the environment occur through gene-environment interaction (Bateson et al., 2004; Padmanabhan et al., 2021). This means that one genotype (i.e., an organism with a specific genetic makeup) can result in a range of different phenotypes in response to environmental conditions. This type of developmental plasticity is later lost in adolescence and adulthood. Current research indicates that the maternal milieu (e.g., health, genetics, nutrition, and toxins) may influence the intrauterine environment and placental function, and consequently fetal growth and metabolism (Blasetti et al., 2022; Tarrade et al., 2015). For example, the
development of fetal adipose tissue is sensitive to maternal nutritional and hormonal changes (Moreno-Mendez et al., 2020). Furthermore, adipose tissue at birth also influences adiposity later in life (Orsso et al., 2020). If fetal adaptation is mismatched to a later environment, it may induce susceptibility to metabolic diseases later in life, such as diabetes type 2, or metabolic syndrome (Hanson et al., 2011). One mechanism, through which fetal programming occurs, is through epigenetic mechanisms, for example, DNA methylation, histone modification, non-coding RNA’s, RNA modification, and chromatin structure (Hanson et al., 2011; Heindel et al., 2022).

The DOHaD hypothesis initially focused on lack of nutrition (i.e., famine) during pregnancy and the association with later health outcomes. However, the hypothesis has later expanded to include other stressors, such as environmental chemicals (Haugen et al., 2015). The DOHaD hypothesis provides an integral approach to understanding how both nutrition and environmental chemicals may influence health and disease from early life across the lifespan (Heindel et al., 2015).

**Endocrine disrupting chemicals**

Humans are exposed to chemicals every day, and there is special concern in regard to EDCs. These chemicals have the capacity to interact and interfere with the natural hormones in the human body (Kabir et al., 2015). EDCs may interfere by binding to hormone receptors and result in both agonist or antagonist actions, or they may bind to non-active sites (i.e., allosteric site) of an enzyme (Zoeller et al., 2012). But EDCs can also interfere with hormone synthesis or metabolism, transport or degradation. In this way, EDCs can interact with the hormone system and result in different effects at very low concentrations. There are several definitions for EDCs, but the most comprehensive definition is the one according to the Endocrine Society, which focuses on the ability of EDCs to interact with hormone action as a predictor for adverse outcomes.

An exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action (Gore et al., 2015; Zoeller et al., 2012).

EDCs have many utilities (e.g., solvents, plasticizers and pesticides) and can be found in an abundant variety of daily used products such as personal care
products, plastics, fabrics, electronics and building materials (Gore et al., 2014). EDCs are normally not bound to the chemical structure of the products, so they leak into the environment and are routinely found in air, dust and contaminated food and water (Ribeiro et al., 2017). The exposure routes by which humans and animals get in contact with EDCs are through skin contact, inhalation, ingestion or through biological transfer from the placenta and breast milk (Gore et al., 2014). The half-life of EDCs can vary. Some are short-lived and can be metabolized by the human body in 12–48 hours, such as phthalates and bisphenols (Benjamin et al., 2017). Although some EDCs have a short half-life, the concern is that sources of exposure are many and the daily exposure is constant, for which they become “pseudo-persistent” (Daughton, 2003). Other EDCs are persistent with a much longer half-life of several years (Gore et al., 2015), such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs) and perfluoroalkyl substances (PFASs). DDT and PCBs were banned more than 50 years ago but can still be found in humans and the environment (Ghisi et al., 2019; Kabir et al., 2015).

**Exposure to EDCs during pregnancy and childhood**

Pregnant women and children are especially vulnerable populations as they represent life stages where physical changes occur (Padmanabhan et al., 2021). Exposure to EDCs may influence the hormonal milieu; presenting a potential risk for both maternal and child health (Padmanabhan et al., 2021; Vrijheid et al., 2016). The exposure of the pregnant woman is also a proxy for fetal exposure, as many EDCs have the potential to pass the placenta (Mamsen et al., 2019; Padmanabhan et al., 2021). If hormone disruption occurs during fetal development, it can affect organ development and function (Gore et al., 2015; Zoeller et al., 2012). There may be a long latency period between the time of exposure until associated health effects may be observed (Diamanti-Kandarakis et al., 2009).

In 2012, the WHO published a report on possible health effects on the reproductive system and neurodevelopment associated with exposure to EDCs specifically in early life (WHO, 2012). The report included a summary from both human studies (e.g., observational, and clinical), and experimental studies in animals and cell systems. It highlighted that certain EDCs can influence fetal
development by disrupting the natural testosterone and thyroid hormones. Since this report was published, there has been growing research pointing to additional health effects associated with exposure to EDCs in early life, such as fetal growth, metabolism, and respiratory health (Kiess et al., 2021).

**Challenges with the regulation of EDCs**

Traditional risk assessment is focused on single chemicals and their potential health effects (Kortenkamp, 2008). However, the general population is concurrently exposed to many different EDCs from different sources (e.g., food, water, personal care products, indoor dust, and air) (Kabir et al., 2015). Human biomonitoring studies, as well as pregnancy cohorts, show that pregnant women and children from the general population are routinely exposed to a vast number of chemicals (Casas et al., 2011; Govarts et al., 2018; Morck et al., 2015; Norström et al., 2020; Woodruff et al., 2011; Ye et al., 2008). Health effects, which are not detected with single chemical exposure, may be detected when considered in a mixture (Caporale et al., 2022; Le Magueresse-Battistoni et al., 2018; Ribeiro et al., 2017; Silva et al., 2002). Even at low doses of exposure, the joint effect of EDCs could be synergism, antagonism or additivity (Kortenkamp, 2008).

The regulation of chemicals within the European Union (EU), established by the Regulation on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) since 2007 (European Commission, 2006), aims to protect human health, assess the safety of chemicals, promote innovation, and promote alternative methods to assess hazardous chemicals. Although there is a current effort to regulate chemicals on a group level (European Chemicals Agency, 2023), current regulations are based on a single chemical approach. One difficulty of regulating one single chemical at the time, is that replacement chemicals may have very similar chemical structures and hence, similar health effects. One example is the replacement chemicals of bisphenol A (BPA), (e.g., bisphenol F (BPF) and bisphenol S (BPS)), which may present similar health effects as BPA (Bornehag et al., 2021; Engdahl et al., 2021; Ji et al., 2021; Trasande, 2017). Therefore, further research with a mixture approach is needed to enhance knowledge for improved regulation of EDCs (Kortenkamp, 2008; Padmanabhan et al., 2021).
Diet and nutrition during pregnancy

Nutrition is important for human health, especially during pregnancy with importance for both maternal, fetal and child health (Christian et al., 2015). The recommended nutritional guidelines for pregnant women in Sweden, the Nordic Nutrition Recommendations (NNR), involve both macronutrients (i.e., protein, fat and carbohydrates) as well as micronutrients (e.g., iron, calcium, and vitamins) (Nordic Council of Ministers, 2014, 2023). The nutritional guidelines provide the recommended intake (RI) of nutrients to maintain a good nutritional status for the general population. The RI may change depending on an individual’s characteristics including age, sex, height, weight, pregnancy and lactation. The NNR also provides food-based dietary guidelines which is a translation of the nutrient recommendations that can be acquired through foods, and take into account the food composition of available foods in the Nordic countries.

Deficiencies of macro- and micronutrients have been associated with unfavorable pregnancy outcomes (e.g., preeclampsia) and fetal development (e.g., brain, thyroid) (Danielewicz et al., 2017). Micronutrients play different roles in fetal development and therefore, the need for certain micronutrients such as iron or folic acid changes during pregnancy (Jouanne et al., 2021; Koletzko et al., 2019). If the quality of the diet is suboptimal then supplementation may be needed (Jouanne et al., 2021). Results from intervention studies, in settings with food insecurity, show that both food and micronutrient supplementation is associated with reduced maternal mortality and improved birth outcomes (e.g., birth weight) (Christian et al., 2015).

The Early Nutrition project, funded by the European Commission, conducted a systematic review of current dietary guidelines for the time before and during pregnancy, as well as early childhood (Koletzko et al., 2019). Their recommendation for pregnant women is to eat a balanced and healthy diet, with an increase of dietary energy intake by no more than 10% of what is recommended for the general population. Especially during early pregnancy, it is more important to eat healthy with a focus on foods rich in nutrients, rather than to eat more (Koletzko et al., 2019). They also endorse the recommendation of eating fish weekly, dietary supplementation if there is a poor supply of the
micronutrients (i.e., iron, vitamin D, B₁₂, and A, and folic acid), and avoiding raw foods. Results from pregnancy cohorts show that adequate nutrition during pregnancy is associated with favorable health outcomes, such as higher birthweight and lower odds of delivering low birthweight or SGA infant (Ancira-Moreno et al., 2020; Chen et al., 2021a; Chen et al., 2021b; Gennings et al., 2020; Gonzalez-Nahm et al., 2019; Yisahak et al., 2021). The association between maternal nutrient intake and children’s growth after birth is still unclear and more research is needed (Gonzalez-Nahm et al., 2019; Tahir et al., 2019).

**Children’s growth**

Healthy fetal and infant growth are important to promote neurodevelopment, and health later in life (Adair et al., 2013; Piwoz et al., 2012; Ross et al., 2009). To weight and measure children is important in order to follow each individual child’s growth over time and is part of routine health care visits. Fetal growth is strongly related to birthweight, and also a predictor for growth in early infancy. Children born with low birthweight, or either small or large for gestation (i.e., small-for-gestational age (SGA) or large-for-gestational age (LGA)) have higher risk for metabolic disorders and cardiovascular disease in adulthood (Cauzzo et al., 2023; Knop et al., 2018; Ni et al., 2021).

Research studies suggest that periods of faster weight gain in early infancy may be related to the development of obesity in children and adults (Druet & Ong, 2008; Druet et al., 2012). In fact, a cohort in the Netherlands of approximately 6,000 children found that accelerated growth during the fetal period and early childhood may be a risk for obesity by 6 years of age (Gishti et al., 2014). A meta-analysis of 10 cohorts in Europe and the US, showed that children with greater weight gain in the first year of life had greater odds of obesity in both child- and adulthood (Druet et al., 2012). While early catch-up growth may improve neurodevelopment among infants born with low birthweight or SGA, it may come with a risk for future cardiovascular disease (Eriksson et al., 1999; Singhal, 2017).

Using population growth curves to follow a child’s growth is useful to identify changes in height and weight over time, and if there are deviations from the
population distribution (Cameron & Schell, 2021). Curves are usually drawn on a chart to represent the population distribution, by showing the mean, and standard deviations (SDs) from the mean (Holmgren & Nylander, 2022). In general, children that are within the 95% confidence interval (CI) of the population distribution are considered to fall within the norm for that specific population. The further away a child falls from that interval the more likely a child has a condition affecting their growth, for example stunting, overweight or obesity (Rogol et al., 2000). Children usually follow their curve, in terms of their position within the population distribution, but it can happen that children also change position, not only when SGA infants catch up. Growth curves are a tool that aids in identifying if any conditions influence the child’s growth, or if there is a need of special care.

The internationally used definition for child overweight and obesity is based on the age- and sex-adjusted body mass index (ISO-BMI) cut off points implemented by the International Obesity Task Force (IOTF) (Cole et al., 2000). ISO-BMI provides cut off points specific to age and sex for children 2–18 years of age and would correspond to a BMI of 25 for overweight and BMI of 30 for obesity in adults.

There are sex-differences in the metabolism, cardiovascular and anthropometry of males and females (Arfai et al., 2002; Ayyavoo et al., 2014; Kirchengast, 2010; Taylor et al., 1997; Taylor et al., 2010). Growth velocity is also different by sex, and tends to be slower for girls from birth up to approximately 7 months of age, and is then faster until the age of four, as compared to boys (Rogol et al., 2000). Sex differences in body fat distribution are more distinct in adults but are also observed in pre-pubertal children (Ayyavoo et al., 2014; Taylor et al., 1997; Taylor et al., 2010). Females tend to have more peripheral fat as compared to males who have more fat around the waist (Taylor et al., 2010).
Public Health concerns

**The Swedish national public health goals**

There are eight Swedish national goals of public health as guidance for prevention and health promotion. One of them focuses on early life and includes good and equal access to health care, a high-quality education and the promotion of children’s health (Swedish Agency of Public Health, n.d). The World Health Organization (WHO) also states that healthy environments are important for children to grow and develop without environmental factors that may increase the risk for disease (WHO, 2017).

A public health concern related to children’s growth, is that one in every five children between the ages of 6–9 years in Sweden have overweight (ISO-BMI ≥ 25) or obesity (ISO-BMI ≥ 30) (Public Health Agency of Sweden, 2021, May 17). Among 4-year-olds, the prevalence is 11% for overweight and 13% for obesity (Miregård et al., 2023). The percentage of children with overweight and obesity in the county of Värmland has plateaued over the last six years and is similar to the rest of Sweden (Region Värmland, 2019). Here, the prevalence of overweight and obesity among 6-year-olds was 23% in 2017/2018 and decreased to 20% in 2018/2019 according to the regional school health system (Region Värmland, 2019). However, it is still a concern as childhood overweight and obesity may be risk factors for metabolic- and cardiovascular diseases in adulthood (Umer et al., 2017; Weihrauch-Blüher & Wiegand, 2018). Children with greater weight-for-age in early infancy, or with overweight in mid-childhood, are more likely to also have overweight in adolescence and adulthood (Bjerregaard et al., 2014; Eriksson et al., 2001)

As healthy environments are important for children’s health, it would be important to investigate both beneficial and unfavorable effects of environmental exposures, such as EDCs and nutrition, on children’s growth.

**Sustainable development goals for 2030**

The Sustainable Development Goals (SDGs) for 2030 are international goals with several health targets (Figure 1) (WHO, 2017). Especially four of the goals are related to nutrition and chemical exposure in early life.
The goal of good health and wellbeing (Goal 3#) highlights the importance of promoting health and identifying risk factors to prevent disease. This goal is more or less related to all the other SDGs. The common and daily exposure to EDCs is of concern as certain EDCs have been linked to several health effects, including lower birthweight (Johnson et al., 2014; Wikström et al., 2019), and obesity in childhood (Heindel et al., 2022).

Clean water (Goal #6) and sustainable consumption (Goal #12) are important to maintain clean and healthy environments. EDCs are used in many products and unsustainable patterns of consumption increase the amount of chemicals used worldwide. Even when certain chemicals are banned, they are replaced with other chemicals which have similar properties and therefore, may have similar health effects (Norström et al., 2020). There is also evidence that persistent chemicals are present in drinking water in Sweden with varied concentrations across the country (Wiberg et al., 2017). Even though they are at low levels, the exposure to these chemicals is still of concern.

Finally, good nutrition is critical both for the health of the mother, fetus and child (Goal #2, and Goal #3). In high-income countries, the access to food may be good but there is evidence that pregnant women may suffer from vitamin D and iron deficiency (Lips et al., 2019; Milman et al., 2017).

Figure 1. Sustainable Development Goals (WHO. Regional Office for Europe, 2019)
**Social determinants of health**

One of the key questions in public health is to identify different risk factors associated with health and disease (Public Health Agency of Sweden, 2022, March 24). This allows the identification of risk groups and develops prevention strategies to reduce risk factors and consequently reduce health inequalities. The model by Dahlgren and Whitehead (Dahlgren & Whitehead, 2021), summarizes the main determinants of health (Figure 2). It includes factors related to the individual lifestyle factors but also more overriding factors such as social, economic and cultural conditions. The individual factors, such as sex, are largely fixed but the outer layers in the models can be influenced by public policies, to improve the conditions surrounding the individual. The determinants of health are all interconnected and influence the population’s health.

In regard to children’s birthweight, growth and body composition, there are several important determinants of health, such as genetics (e.g., parental characteristics), gestational age, birthweight, feeding practices (e.g., breastfeeding), sickness and nutritional status of the child (Balasundaram & Avulakunta, 2023). In the case of child overweight and obesity, risk factors include, for example, genetics, diet, sleep and physical activity, parental weight, and socio-economic status (Ang et al., 2013). The social and built environment has an influence on access to healthy foods (e.g., fresh fruits and vegetables) and access to green spaces that promote physical activity (Davison & Birch, 2001). Child overweight and obesity are also correlated with socioeconomic status, and urbanization level (Moraeus et al., 2012). This shows the complexity of various factors influencing children’s growth. With this in mind, environmental exposures, such as EDCs and nutrition, represent one part in the complexity of factors influencing children’s growth.
Figure 2. Social determinants of health (Dahlgren & Whitehead, 2021)

Source: adapted from Dahlgren and Whitehead, 1991
Questions at issue

The DOHaD hypothesis suggests, that exposures in early life may play a role for later health outcomes in childhood and adulthood (Gluckman et al., 2010; Hanson et al., 2011). Environmental exposures, such as EDCs and nutrition, may influence fetal growth and development, and may potentially lead to long-term health effects.

Previous research shows that higher exposure to certain EDCs is associated to unfavorable pregnancy outcomes, such as lower birthweight, and overweight (Ferguson et al., 2022; Johnson et al., 2014; Stratakis et al., 2022; Wikström et al., 2019). However, there are still very few longitudinal studies with a mixture approach on these specific outcomes. Therefore, research is needed to evaluate associations between EDC mixtures with both birthweight, and children's growth.

Nutrition is vital for healthy pregnancy outcomes (Christian et al., 2015; Gennings et al., 2020). However, there is lack of consensus regarding potential long-term effects on children's growth. As both exposure to EDCs and nutrition have effects on pregnancy outcomes, it is relevant to examine if these environmental exposures would have long-term effects on children's growth.

EDCs interfere with the naturally occurring hormones, which may result in anti-androgenic and estrogenic activity (Gore et al., 2015). Additionally, there is indication that prenatal nutrition may have sex-specific effects on fetal and placental growth (Alur, 2019; Dearden et al., 2018; Tarrade et al., 2015). Therefore, it is important to investigate if long-term associations with children's growth differs by sex.
OVERALL AND SPECIFIC AIMS OF THE THESIS

The overall aim of the thesis is to examine the importance of environmental exposures - specifically prenatal exposure to EDC mixtures and maternal nutrition during pregnancy - for children’s growth and body composition up to seven years of age.

The thesis has the following specific aims (Figure 3):

• To evaluate if prenatal exposure to EDC mixtures is associated with measures of growth and body composition in children
• To identify EDCs of concern
• To evaluate if prenatal nutrition is associated with measures of body composition in children
• To determine if the associations differ by sex
Figure 3. Representation of the specific aims of the thesis within the context of Developmental Origins of Health and Disease (DOHaD)

<table>
<thead>
<tr>
<th>Longitudinal design - Developmental Origins of Health and Disease (DOHaD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenatal period</strong></td>
</tr>
<tr>
<td>EDC mixture (10 weeks)</td>
</tr>
<tr>
<td>Environmental exposures</td>
</tr>
</tbody>
</table>

*Figure 3. Representation of the specific aims of the thesis within the context of Developmental Origins of Health and Disease*
MATERIALS AND METHODS

Study design and population

The SELMA study

The Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study is an ongoing pregnancy cohort study with the aims to investigate early life exposure to environmental stressors and its importance for children’s health and development. The study focuses on four health domains including metabolism and growth, sexual development, neurodevelopment and immunological responses. A more detailed description of the SELMA study has previously been published (Bornehag et al., 2012).

Briefly, from 2007 to 2010 a total of 6,658 pregnant women were invited and 2,582 women decided to participate in the study (39% participation rate). Participants, as compared to non-participants, tended to be older (32 vs. 31 years), have a college or university education (50% vs. 36%), lower prevalence of smoking (14% vs. 19%), live in a single-family house (69% vs. 50%) and more likely to report allergy symptoms (58% vs. 38%) (Bornehag et al., 2012). The pregnant women were all registered at one of the 25 antenatal care centers in the county of Värmland. Women were recruited during early pregnancy at approximately 10 weeks’ gestation during their first antenatal care visit, and followed-up during pregnancy. Their children were then followed-up from birth until school-age years. A comprehensive battery of health and exposure data was collected through biological samples, register data, questionnaires and routine health care visits.

In 2015-2017, participating mother-child pairs were invited to a follow-up visit when children were seven years of age. Examinations of health and development of the child were performed at a child health care center by qualified personnel, and data was collected on anthropometric and body fat measures, sexual development, psychometric tests, biological samples, and the child’s health status
and family lifestyle through interviews and questionnaires. A total of 1,006 children attended the study visit, corresponding to 51% out of the mother-child pairs followed-up at birth (n=1,954).

**Study population**

The study population for each study is based on participants with complete data for the exposure and outcome of interest, as well as information for covariates. A total of 2,582 women were participants in the SELMA study at the time of recruitment: early prenatal visit at median of 10 week’s gestation. Thereafter, a total of 1,954 mother-child pairs were participants at the time of birth. During pregnancy, data was collected through bio-sampling, and questionnaires. Information in regard to maternal characteristics and pregnancy outcomes was retrieved from the Swedish national birth medical register (Swedish National Board of Health and Welfare, n.d). We also used available information on EDCs assessed in urine and serum samples collected at recruitment, and information from food frequency questionnaires (FFQ) collected during mid-pregnancy (25 weeks of gestation). Sociodemographic characteristics was also collected through questionnaires at the time of recruitment and during pregnancy.

Children’s growth measures were collected through routine health care visits at regular appointments from birth up to 5.5 years of age and were retrieved through patient records. All participants followed postnatally were invited to participate in a follow-up study when children were seven years of age, and body fat measures were collected as part of this study visit.

The combination of all the data sources resulted in the study populations for each study (Figures A1-A4). First, participants with complete information in regard to exposure and outcomes were identified. Then, those participants with complete information on covariates were selected for statistical analysis.
Data collection

This section includes a description of the collection process of the biological samples, and data from questionnaires and routine health care visits. The data collection spans from first trimester of pregnancy until the 7-year-old follow-up examination of the child.

Collection of prenatal urine and serum samples

Women participating in the study provided urine and blood (serum) samples during their first prenatal care visit at a median of 10 week’s gestation. Blood (serum) samples were collected during the visit by trained staff in a polypropylene tube. Urine samples were collected at home by the woman before visiting the antenatal care center. They were asked to collect the first morning urine void in a clean glass container and fill two polypropylene tubes provided by mail. Both tubes were filled up to 8mL each and kept refrigerated until they were brought by the women to the antenatal care center. All samples were then stored frozen until analytical assessment at -80 °C for serum samples and -20 °C for urine samples at the biobank at Karlstad’s Central Hospital.

Analytical assessment of EDCs in prenatal samples

There were 54 different analytes analyzed which had proven or suspected endocrine disrupting properties. Of these, 41 analytes were detected in more than 75% of the samples (Table 1 and 2). Those 41 chemicals included 14 phthalates and non-phthalate plasticizers: monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), di-(2-ethylhexyl) phthalate (DEHP) metabolites (mono-2-ethylhexyl phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MEOCP)), diisononyl phthalate (DINP) metabolites (mono(hydroxyisononyl) phthalate (MHiNP), mono(oxoisononyl) phthalate (MOiNP), mono(carboxyisooctyl) phthalate (MCiOP)), monohydroxyisodecyl phthalate (MHiDP), monocarboxyisononyl phthalate (MCiNP), 2–4-methyl-7-oxyooctyl-oxycarbonyl-
cyclohexane carboxylic acid (MOINCH), and diphenylphosphate (DPP). Chemicals also included four phenols: 2,4,4′-trichloro-2′-hydroxydiphenyl ether (Triclosan), bisphenol A (BPA), 4,4-bisphenol F (BPF), bisphenol S (BPS); one PAH: 2-hydroxyphenanthrene (2-OHPH), and two pesticides: 3,5,6-trichloro-2-pyridinol (TCP), and 3-phenoxybenzoic acid (3-PBA). Also, six PFAS: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonic acid (PFHxS), three organochlorine pesticides: hexachlorobenzene (HCB), trans-nonachlor, DDT and its metabolite dichlorodiphenyldichloroethylene (DDE), and ten PCBs congeners 74, 99, 118, 138, 153, 156, 170, 180, 183, and 187.

The metabolites of DEHP and DINP were summed to total exposure variables (Tanner et al., 2020b). The sum of the metabolites from DEHP and DINP were calculated on a molar basis. Also, DDT was summed with its metabolite DDE, and all the PCB congeners were summed to represent total exposure. After summation of metabolites from the same parent compound a total of 26 chemicals were included in the analyses. These 26 chemicals were analyzed as a mixture and as single-compound in sensitivity analyses (Figure 4).

The values below level of quantitation (LOQ) were replaced by the value of LOQ/√2 for the chemicals measured in serum. For PFAS, also measured in serum, and chemicals measured in urine, the available machine read values were used (Tanner et al., 2020b). All urinary metabolites were creatinine adjusted for urinary dilution in units of nmol per mmol creatinine for regression analysis.

The analytical assessment of the metabolites in urine and serum samples has previously been described in more detail and only summarized here briefly (Gyllenhammar et al., 2017; Koponen et al., 2013; Norén et al., 2021). Urinary metabolites of nonpersistent chemicals with short half-life were analyzed using liquid
chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS; QTRAP 5500, AB ScieX, Framingham, MA, USA) (Gyllenhammar et al., 2017). PFAS and cotinine were analyzed in serum samples using LC-MS/MS (Norén et al., 2021). These analyses were performed at the Laboratory of Occupational and Environmental Medicine (OEM) at Lund University in Lund, Sweden. Finally, persistent organic pollutants were analyzed in serum samples using gas chromatography - high triple quadrupole mass spectrometry (Agilent 7010 GC–MS/MS system (Wilmington, DE, USA), GC column DB-5MS UI (J&W Scientific, 20 m, ID 0.18 mm, 0.18 μm)) (Koponen et al., 2013). These analyses were conducted at the Finnish Institute for Health and Welfare in Finland.

Figure 4. Representation of the collection of urine and serum samples and analytical assessment of EDCs
<table>
<thead>
<tr>
<th>Matrix Type</th>
<th>Chemical Type</th>
<th>Parent Compound (if applicable)</th>
<th>Analyte</th>
<th>Abbreviation</th>
</tr>
</thead>
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<td>Urine</td>
<td>Phthalates</td>
<td>DEP monoethyl phthalate</td>
<td>MEP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DBP monobutyl phthalate</td>
<td>MBP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BBzP monobenzyl phthalate</td>
<td>MBzP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phthalates</td>
<td>DEHP mono(2-ethylhexyl) phthalate</td>
<td>MEHP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mono(2-ethyl-5-hydroxyhexyl) phthalate</td>
<td>MEHHP</td>
<td></td>
</tr>
<tr>
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<td>Phthalates</td>
<td>mono(2-ethyl-5-oxohexyl) phthalate</td>
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<td></td>
</tr>
<tr>
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<td>Phthalates</td>
<td>di-(2-ethylhexyl) phthalate</td>
<td>ΣDEHP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phthalates</td>
<td>DINP monohydroxy-isonoal phthalate</td>
<td>MHiNP</td>
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</tr>
<tr>
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<td>monooxo-isonoal phthalate</td>
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<td>diisononyl phthalate</td>
<td>ΣDINP</td>
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<td>Non-phthalate plasticizers</td>
<td>DiNCH 2,4,4′-trichloro-2′-hydroxydiphenyl ether</td>
<td>Triclosan</td>
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<td></td>
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<td></td>
<td>Phthalates</td>
<td>TPP dihexylphosphate</td>
<td>DPP</td>
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<tr>
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<td>2,4,4′-trichloro-2′-hydroxydiphenyl ether</td>
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<tr>
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<td>Bisphenols</td>
<td>4,4-bisphenol F</td>
<td>BFF</td>
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<td></td>
<td>Bisphenols</td>
<td>bisphenol S</td>
<td>BFS</td>
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<tr>
<td></td>
<td>PAH a</td>
<td>2-hydroxyphenanthrene</td>
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<td>3,5,6-trichloro-2-pyridinol</td>
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<td></td>
<td>Pyrethroids</td>
<td>3-phenoxycarboxylic acid</td>
<td>3-FBA</td>
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</tr>
</tbody>
</table>

a Polycyclic aromatic hydrocarbon
b Molar sum of metabolites: mono-2-ethylhexyl, mono(2-ethyl-5-hydroxyhexyl), mono(2-ethyl-5-oxohexyl), and mono(2-ethyl-5-carboxypentyl) phthalates.
c Molar sum of metabolites: mono(hydroxyisononyl), mono(oxoisononyl), and mono(carboxyisooctyl) phthalates.
<table>
<thead>
<tr>
<th>Matrix Type</th>
<th>Chemical Type</th>
<th>Parent Compound (if applicable)</th>
<th>Analyte</th>
<th>Abbreviation</th>
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<td>perfluorooctane sulfonic acid</td>
<td>PFOS</td>
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<td>perfluorononanoic acid</td>
<td>PFNA</td>
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</tr>
<tr>
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<td>perfluorodecanoic acid</td>
<td>PFDA</td>
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<td>perfluoroundecanoic acid</td>
<td>PFUnDA</td>
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<td>perfluorohexane sulfonic acid</td>
<td>PFHxS</td>
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<td>Organochlorine pesticide</td>
<td>hexachlorobenzene</td>
<td>HCB</td>
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<td>Nonachlor</td>
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<td>total polychlorinated biphenyls</td>
<td>ΣPCB b</td>
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</table>

* Sum of DDT and its metabolite dichlorodiphenyldichloroethylene.

b Sum of PCB congeners 74, 99, 118, 138, 153, 156, 170, 180, 183, 187

**Prenatal diet and nutrition**

Information on maternal diet was collected during mid-pregnancy through self-reported FFQ (Gennings et al., 2020) (Figure 5). The questionnaire included questions of commonly eaten foods. Categories of food items included grain and cereals, meat, poultry, fish, eggs, vegetables, potatoes, legumes, fruit and berries, cakes and sweets, and other food items (e.g., salad dressing, coffee cream, extra salt). Women were asked to report if, and if so, how often, they consumed these food items during the current pregnancy. There were also open questions in regards to daily or weekly consumption of type of milk, yogurt, bread, cheese. The collected information was used to calculate the frequency of consumption of every food item per day, and the amount of each food item consumed per day.
item was calculated based on an estimated portion size. The portion size was estimated in a previous study based on food records 4x7 days among 213 randomly selected Swedish women (Khani et al., 2004). The food compositions values are provided in the Swedish national food composition database (Swedish Food Agency, n.d.), and was used to calculate the nutrient composition values and total energy. The FFQ used in the SELMA study is an extended version (98-items) of a validated FFQ for nutrients estimates (Messerer et al., 2004).

My Nutrition Index

The calculated nutrient intake was then summarized into an index, “My Nutrition Index” (MNI) to estimate adherence to recommended nutrient intake during pregnancy (Gennings et al., 2020). The following macro- and micronutrients considered were total fat, saturated fat, monounsaturated and polyunsaturated fat, protein, carbohydrates, sugar, dietary fiber, vitamin E as α-tocopherol, vitamin C, cholesterol, potassium, sodium, calcium, magnesium, iron, phosphorus, zinc, thiamin, riboflavin, niacin, vitamin B6, vitamin B12, vitamin A, vitamin D, folate and selenium. MNI assigns a higher score if the nutrient intake is within the recommended intake, and lower score if the nutrient intake is higher or lower than recommended. This results in a score ranging from 0 to 100, where a higher score reflects better adherence to the recommended guidelines. The recommended guidelines vary according to individual characteristics and therefore, MNI takes into account the age, height, weight, pregnancy status, physical activity and smoking status of an individual. MNI was calculated for all the women who had answered the FFQ in the SELMA study (n=1,692). MNI is based on the American Dietary Guidelines for 2015-2020 and is very similar to the NNR 2012, which were used in Sweden until 2023 when new updated guidelines were released (Nordic Council of Ministers, 2014; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). A previous publication from the SELMA study, provides a comparison on both guidelines and shows that the two guidelines are very similar, except that the NNR 2012 recommends less saturated fat and higher
potassium intake as compared to the American guidelines for 2015-2020 (Gennings et al., 2020). The advantage of using an overall nutrition index, like MNI, is that it reduces the complexity of manipulating many nutrient variables.

Figure 5. Representation of the collection of information on prenatal diet and calculation of nutrient intake summarized in My Nutrition Index (MNI).

Measures of growth and body composition

Children’s growth

Children’s anthropometry was measured at the following time points. Birth weight and length were measured at birth and retrieved from the Swedish national birth medical register (Swedish National Board of Health and Welfare, n.d). After birth, weight and height were measured during routine visits at a Child Health center during the following time points: 4–6 weeks of age, and at 2, 3, 4, 5, 6, 8, 10, 12, 18, 30, 36, 48, and 66 months of age (Bornehag et al., 2012). Children’s weight measures were used to calculate weight trajectories between birth and 5.5 years (66 months) of age (Tanner et al., 2020a). Weight measures were modelled using a double-logistic growth model, which assumes a sigmoidal shape with two consecutive growth periods.
(Figure 6). The model derives several growth parameters indicative for the shape of the curve. The parameters in the first growth period were selected for analysis as weight was measured more frequently in early infancy. The more observations, the more precise is the estimation of the curve.

Figure 6. A double-logistic growth model was applied in this analysis to predict children’s weight trajectories from birth to 5.5 years of age. The infant period is captured by the first of two logistic functions. The two logistic functions have slopes $\beta_1$ and $\beta_2$ and inflection points at $\delta_1$ and $\delta_2$, respectively. The intermediate asymptote $\alpha_1$ separates the two growth spurts. (Tanner et al., 2020a)

The estimated curve is a representation of each child’s weight trajectory. The parameters selected for analysis were the infant growth spurt rate and the age at peak growth velocity form the first growth period (Figure 7). These parameters are indicators for the shape of the curve of each child’s weight trajectory between birth and 5.5 years of age. A more detailed description of the statistical modelling has previously been published (Tanner et al., 2020a).
Figure 7. A representation of the first function in a double-logistic growth model used to predict children’s weight trajectories. The first function is exponential with weight (kg) increasing with age (months) and undertakes an inflection point where the rate constant is determined by the slope of the tangent line ($\beta$). This slope is in this analysis labeled as the infant growth spurt rate (kg/month). Growth velocity is the first derivative of the logistic growth model, and the peak growth velocity (PGV) is reached at age = $\delta$. Adapted from Tanner et al., (Tanner et al., 2020a).

Overweight and obesity

The Swedish national growth reference was used to calculate birthweight $z$-scores (Niklasson & Albertsson-Wikland, 2008), and indicates the child’s birthweight in relation to the reference values for the Swedish population, taking into account the gestational age and sex. Children’s height and weight was used to calculate BMI and BMI $z$-scores according to the WHO child growth standards both for the ages 0–59 months and 5–19 years of age (Onis et al., 2007; WHO Multicentre Growth Reference Study Group, 2006). Values of BMI $z$-
scores greater than ±5SD were considered as biologically implausible values and excluded from the analysis. The BMI z-scores show the distribution of the study population in relation to the age and sex adjusted reference values of the WHO child growth standards. Children with overweight and obesity were identified using the ISO-BMI cut-off levels for age and sex (Cole et al., 2000).

**Body composition**

Body composition, as well as weight and height, were collected during a follow-up visit at seven years of age. A stadiometer was used to collect height and an eight-electrode method bioelectrical impedance analysis (BIA) was performed for weight and body fat (TANITA BC-418 MA). As body fat is less conducive of electric current than water, the BIA can estimate the percent body fat using a constant high-frequency electrical current source (50 kHz, 500 µA). At the time of measurement “standard male” or “standard female” body type was selected and the child’s age was entered to the instrument according to the instruction manual. The BIA equations estimates several components of body composition (e.g., fat free mass, body water) of which we selected percent body fat for analysis.

Additionally, skinfold thickness was measured to assess subcutaneous fat. Measurements were performed using a caliper instrument (Harpenden) on the right side of the body. For better precision, measures were performed twice on each of the following body sites: biceps, triceps, subscapular and suprailiac, and then averaged for analysis. The sum of triceps and subscapular was also calculated. All measurements during the seven-year follow-up visit were performed in underwear, before breakfast and after emptying the bladder.

**Covariates**

The SELMA study retrieved prenatal- and delivery-care information from the Swedish national medical birth register which keeps information on all deliveries in Sweden (Swedish National Board of Health and Wellfare, n.d). This information included women’s age,
weight at first prenatal care visit, parity, child’s sex and gestational age at birth based on ultrasound examination. Cotinine was measured in serum collected during the first prenatal visit. Self-administered questionnaires were used to collect sociodemographic information including maternal height, education level, and smoking status. Maternal BMI was calculated using height and weight (kg/m²).

Statistical analysis
This section includes a description of the statistical analysis performed in the studies including descriptive, bivariate and multivariate analysis. A summary of the statistical analysis in each study is presented in Table 3.

Descriptive and bivariate statistics
Descriptive statistics such as central tendency and frequency were calculated for all variables considered for analysis. Descriptive statistics of the sociodemographic characteristics, exposure and outcome variables are reported in the published articles of each study (I-IV). Bivariate analysis was also conducted to evaluate the relationship between outcome, exposure and covariates.

Linear and logistic regression
Linear regression models were applied in study IV, where the outcome was MNI which is a continuous variable. Both simple and multivariate linear regression was conducted. Also, in sensitivity analysis in studies I, II and III, linear regression models were applied to test the association between single chemicals and the outcome.

WQS regression
A Weighted Quantile Sum (WQS) regression was used in the studies I, II and III, to evaluate the mixture effect of prenatal exposure to 26 EDCs in relation to the selected outcomes of birth weight, growth trajectories, BMI and body composition (Figure 8). The WQS regression also identifies chemicals of concern driving the associations by estimated weights (Carrico et al., 2015; Czarnota et al., 2015).
The WQS regression calculates a weighted index, \( \sum_{i=1}^{c} w_i q_i \) that represents the mixture term, such that \( w_i \) is the mean weight of each chemical over 100 bootstrap samples, and \( q_i \) is the quantile of each chemical per subject (here, deciles). The higher the weights the greater the contribution of that chemical to the weighted index. The effect of the weighted index associated with each outcome of interest (e.g., birth weight, BMI) was then tested in a generalized linear or logistic model as follows:

\[
g(\mu) = \beta_0 + \beta_1 \left( \sum_{i=1}^{c} w_i q_i \right) + z' \varphi
\]

where, \( g(\mu) \) is the link function (in this case generalized linear or logistic model), \( \mu \) is the mean of the outcome, \( z' \) is the vector of covariates and \( \varphi \) is the vector of parameters associated with the covariates. It is possible to derive a weighted index associated with the outcome both in the positive and negative directions, sequentially, through constraints in the nonlinear estimation algorithm. When there was enough scientific literature suggesting a direction of the association, then the direction was decided \textit{a priori}, and the constraint was used to evaluate the specified direction. If the direction of the association was not clear then both indices were derived.

When possible, the estimation of the index and the weights are done in a training dataset, which commonly is 40% of the data, and then validated in the remaining 60% of the data. In study I, splitting of the data into training and validation was not applied due to unstable weights. In study II and III, a recently developed validation technique was applied with 100 repeated holdout validation sets, which randomly splits the data 100 times (i.e., the data are split 100 times with 40% used to estimate the weights in each split, and the remaining 60% used as a holdout validation set). The mean estimates of the 100 beta estimates with 95% confidence interval and mean chemical weights across the 100 splits are then reported (Tanner et al., 2019).
The WQS regression identifies the chemicals of concern driving the associations based on estimated weights. If the chemicals had a weight above the $1/c$ threshold (i.e., equal weighting), where $c$ is the number of chemicals in the mixture, then they were considered chemicals of concern. In study II and III, an additional criteria was applied to identify chemicals of concern, the “Busgang criteria” (Busgang et al., 2022b). The chemicals with a mean weight above the threshold in more than 50% of the holdouts were classified as “possible” chemicals of concern and if more than 90% of the holdouts they were classified as “probable” chemicals of concern. Therefore, only chemicals with weights above the threshold in at least 50% of the holdouts were considered chemicals of concern in study II and III.

In order to evaluate potential sex-differences of the association, an interaction term, WQS*sex, was added and estimated weights were stratified by sex. This allowed to estimate different slopes, as well as different rankings of the weights for boys and girls (Busgang et al., 2022b). Relative weights were calculated by dividing each chemical weight by the total weights within the strata (here, by sex).
Figure 8. Weighted Quantile Sum regression is a model designed to evaluate the mixture effect of components with complex correlation patterns, such as EDCs. Here, the mixture was comprised by 26 EDCs. Based on the measured concentration levels from each participant, the concentrations are first ranked into quantiles (here, deciles). The model splits the data into training (40%) and validation (60%). In the training set, the mean weight of each chemical is estimated across 100 bootstrap samples. A weighted index is then derived by multiplying the mean weight by the quantile of each component per subject. The higher the weights the higher the contribution to the weighted index. The weighted index can be derived in a positive or negative direction associated with the outcome of interest. Finally, the weighted index is tested in the validation set of the data in a generalized linear model. (Figure designed by Stefanie Busgang)

Covariate assessment

Parity was dichotomized into the categories of primiparous and multiparous based on the order of the child in the study. Smoking status was first determined by cotinine levels and secondly on self-reported smoking status. In the first study, women were categorized as active smoker and non-smoker based on the cotinine levels above or equal to 15 µg/dl and self-report of current smoking. In the studies II-IV, smoking status was updated to reflect smoking exposure based on cotinine levels above or equal to 0.2 µg/dl, and if the woman reported that she or anyone else in the household currently smoked. Maternal level of education was categorized as less than college and college or
more. For studies II–IV, the category of “other” was reviewed and because many women in this category had reported education levels equivalent to less than college, this group was moved to the category “less than college”. These were 112 women out of 1,874 who had reported information on education level (6%). For study I, covariates were selected based on previous literature or statistical significance in the WQS regression models (p-value<0.05). However, for study II–IV, the covariates used in the analysis were selected based on DAG analyses. In study III, the outcome of percent body fat was already age- and sex-adjusted, as this information is entered at the time of measurement. However, the outcome of “overall body fat” included additional measures of BMI and skinfolds, which are not age- and sex-adjusted. Therefore, this model was additionally adjusted for child’s sex and age as covariates. Similarly, in study IV, sensitivity analysis were conducted with the outcomes that were not age- and sex-adjusted to compare if the results differed when adjusting for child’s age and sex. All statistical analyses were performed using the statistical software R in the versions 3.5.2 to 4.1.2. The R package used to run WQS analysis was “gWQS: generalized weighted quantile sum regression” in the versions 2.0.0 to version 3.0.4 (Renzetti et al., 2021).

**Sensitivity analyses**

For study I, II and III, a single-chemical analysis was performed as sensitivity analysis to compare with the EDC mixture approach. Linear regression models with each chemical as exposure variable and the selected outcome was modelled. For this analysis the chemicals were log_{10} transformed to approximate normal distribution. In study IV, sensitivity analyses with additional covariate adjustment were performed adjusting for paternal BMI, and children’s age. Also, to remove any influential points, additional analyses were performed excluding parts of the data, for example, children with the highest and lowest BMI (highest and lowest 5th percentile), or women with obesity (BMI≥30).
Ethical approval and considerations

The international ethics codes of Nuremberg first drafted in 1947 and the Declaration of Helsinki adopted by the World Medical Society in 1964 provides rules of ethics to be followed when conducting research (United States Holocaust Memorial Museum, n.d.; World Medical Association, 2013). These international ethical codes and principals also provide the foundation of laws and regulations in Sweden.

According to the Swedish Act concerning the Ethical Review of Research Involving Humans, and according to the international ethics codes, all research involving human subjects need to provide written informed consent to all subjects before enrolling in a research study (Swedish Research Council, 2017). Information including the objectives, methods, risks and benefits, and the principal investigator of the study is to be provided to the subjects. It also needs to be clear that participation is voluntary and the subject can interrupt their participation at any time without any consequences (Swedish Research Council, 2017; World Medical Association, 2013). For children under 15 years of age, both parents must consent to the child’s participation. Also, as far as possible, the child should receive information in such a way that the child can understand it (Swedish Research Council, 2017).

In the SELMA study, all women provided their written consent to participate, and parental consent was provided in regards to the children’s participation (Bornehag et al., 2012). Also, at the 7-year-old follow-up visit, the information to the children was age adapted in written format and video format (SELMA study, n.d).

The SELMA study has collected and stores sensitive personal data, for example, data from health examinations, register data, interviews, biological samples and questionnaires. According to the Declaration of Helsinki, it is important to protect the integrity of study participants (World Medical Association, 2013). Therefore, data has to be stored and utilized in a way that ensures confidentiality in agreement with both Swedish and European law (Swedish Research Council, 2017). For
this, a principle in the SELMA study is to remove the individual personal identifiers (e.g., name, address, birth date) and store separately from the data, and replace it with a study identifier that cannot be connected back to the participant. In this way, de-identified data is stored and analyzed.

The four studies included in this thesis has used data from the SELMA study, which has been approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr: 2007/062 and Dnr: 2015/177).
Table 3. Statistical analysis for each study

<table>
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<tr>
<th>Study and sample size</th>
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<th>Outcome</th>
<th>Covariates</th>
<th>Statistical analysis</th>
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<tr>
<td>I.</td>
<td>Prenatal exposure to 26 EDCs (mixture)</td>
<td>Birthweight z-scores</td>
<td>Maternal characteristics:</td>
<td>WQS linear regression model:</td>
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<tr>
<td></td>
<td></td>
<td>Growth trajectory from birth until 5.5 years of age</td>
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<td>Children’s characteristics:</td>
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<td>II.</td>
<td>Prenatal exposure to 26 EDCs (mixture)</td>
<td>BMI and overweight (ISO-BMI ≥ 25) at 5.5 years of age</td>
<td>Maternal characteristics:</td>
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<td>- Parity</td>
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<td>Validation with 100 repeated holdouts</td>
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43
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<tr>
<th>Study and sample size</th>
<th>Exposure</th>
<th>Outcome</th>
<th>Covariates</th>
<th>Statistical analysis</th>
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</thead>
</table>
| III. n=737            | Prenatal exposure to 26 EDCs (mixture) | Body composition at 7 years of age | Maternal characteristics:  
  - BMI  
  - Education  
  - Smoking  
  - Parity  
  Children’s characteristics:  
  - Age  
  - Sex | WQS linear regression model:  
  - By sex  
  Validation with 100 repeated holdouts |
| IV. n=808             | Prenatal diet (My Nutrition Index) | Body composition at 7 years of age | Maternal characteristics:  
  - BMI  
  - Education  
  - Smoking  
  - Parity  
  - Total energy (kcal)  
  Children’s characteristics:  
  - Age  
  - Sex | Linear regression models:  
  - Overall  
  - By sex |

*Adjustment by child’s sex and age were applied in models with measures not adjusted by age and sex, including BMI and skinfolds (Study III, IV). In study III, models were stratified by sex, and in addition the model with “overall body fat” was adjusted by child’s age as it included BMI and skinfolds. In study IV, models were stratified by sex and a sensitivity analysis was conducted adjusting for child’s age.
RESULTS AND DISCUSSION

Prenatal exposure to EDC mixtures and children’s growth and body composition

Three studies addressed the aims related to exposure of EDC mixtures in early pregnancy with children’s growth and body composition. These studies found that prenatal exposure to EDC mixtures was associated with lower birthweight, a slower weight gain, and also a growth spurt at a later age, specifically for girls (Figure 9). It was further found that EDC mixtures was associated with BMI and body composition in a sex-specific manner. Such that, higher prenatal exposure to EDC mixtures was associated with lower BMI, lower odds of overweight, and less body fat for girls. Whereas for boys, the opposite was found with higher prenatal exposure to EDC mixtures associated with more body fat. The results of the associations found and the sex-specific chemical weights are summarized in Table 4 and 5.

Birthweight and weight trajectory (Study I)

EDC mixtures and birthweight

Results from study I, showed that higher prenatal exposure to EDC mixtures, as indicated by the WQS index, was associated with lower birthweight (Table 4). This association was mostly driven by PFOA, followed by Triclosan, HCB, 2-OHPH and MCNP, BPS, PFDA and MBP. These chemicals contributed differently to the association among boys and girls, and can be observed in the pattern of the sex-specific chemicals weights (Table 5).

Chemicals of concern

There are only a few pregnancy cohorts which have evaluated prenatal exposure to EDC mixtures and birthweight (Chiu et al., 2018; Govarts et al., 2016; Hu et al., 2021; Pearce et al., 2021; Woods et al., 2017). Although, these studies found associations with lower birthweight for certain chemicals, the results have been both statistically significant
(Govarts et al., 2016; Hu et al., 2021; Pearce et al., 2021) and non-significant (Chiu et al., 2018; Woods et al., 2017). These studies have identified different chemicals of concern contributing to lower birthweight depending on the selected chemicals in their mixture. Specifically, PFAS mixture (e.g., only PFASs and in combination with heavy metals) (Govarts et al., 2016; Woods et al., 2017), phthalates mixture (e.g., DEHP and low molecular weight) (Chiu et al., 2018; Govarts et al., 2016), organochlorine chemical mixture (e.g., DDE, PCBs, HCB, Trans-Nonachlor) (Hu et al., 2021), and organochlorine chemicals together with PBDEs in a mixture (Pearce et al., 2021). This suggest there are several chemicals contributing to the association between EDC mixtures and lower birthweight. Our results are in line with the Barker and DOHaD hypothesis that prenatal environmental stressors may play a role on lower birthweight (Barker et al., 1993).

Sensitivity analysis with single chemicals resulted in very few significant associations. We found that higher concentrations of BPS, PFOA, PFDA and HCB was associated with lower birthweight. Further, previous studies evaluating single chemicals, also show that prenatal exposure to PFASs has consistently been associated with lower birthweight (Johnson et al., 2014; Wikström et al., 2019) and risk for SGA (Govarts et al., 2018). There is also indication of lower birth size in association with higher prenatal concentrations of HCB (Govarts et al., 2018), and BPS (Goodrich et al., 2019).

Sex differences

We did not find any significant sex differences in the association between EDC mixtures and birthweight. However, the estimated chemical weights (the contribution to the WQS index) differed by sex. For example, PFOA had a higher weight among girls as compared to boys. In contrast, Triclosan had a higher weight among boys as compared to girls. This pattern was also observed for the other chemicals of concern (Table 5). In a previous mixture study, there is also indication that prenatal exposure to EDCs may result in sex-
specific differences, with organochlorine chemicals and PFOS having a stronger association with lower birthweight among girls (Hu, 2021).

**EDC mixtures and weight trajectory**

We further evaluated if prenatal exposure to EDC mixtures was associated with children’s growth, specifically with weight trajectory, between birth and 5.5 years of age. Our results showed that prenatal exposure to EDC mixtures was associated with the two selected growth parameters (\( \beta_1 \), \( \partial_1 \)), indicative of children’s weight trajectory (Figure 7). Higher prenatal exposure to EDC mixtures was associated with a slower growth spurt rate (\( \beta_1 \)) for all children (Table 4). We also found an association with a later growth spurt (\( \partial_1 \)), specifically for girls. These associations were driven by PFOA, PFDA, DPP, Triclosan, MOiNCH, \( \Sigma \)PCBs, BPA, BPF, MEP, MBzP, and 3-PBA. The chemical weights also differed by sex (Table 5).

The time of the peak growth velocity for the children in the SELMA study occurred on average at 3 months of age. Therefore, we can conclude that prenatal exposure to EDC mixtures may influence children’s weight gain in early infancy (3 months), hence also influence the shape of the growth curve. This is similar to a mother-daughter cohort in the UK, which evaluated prenatal exposure to EDC mixtures of persistent chemicals (e.g., PFAS, OCPs and PCBs) (Marks et al., 2021a). They found that exposure to a mixture of persistent EDCs was associated with lower weight-for-age z-score between birth and 19 months of age. Also, their time specific analysis showed that the associations were significant at birth and at 2 months of age. Consequently, suggesting that the strongest association with lower weight was in early infancy.

Our findings are in agreement with the DOHaD hypothesis, suggesting that early life exposure to environmental stressors (e.g., EDCs) may have long-term health effects in childhood (Heindel et al., 2015). In terms of the direction of the association, previous research shows that a period of faster weight gain during infancy is a risk factor for
childhood obesity (Druet et al., 2012; Gishti et al., 2014). In contrast, our results suggest that prenatal exposure to EDC mixtures is associated with slower weight gain. However, we did not analyze the second function of the double-logistic growth model (Figure 6), as it was more imprecise due to less frequent weight measures. Therefore, it is not possible to draw any conclusions on the long-term health risk for overweight and obesity in childhood. Future studies may want to explore further the association between prenatal EDC mixtures and later growth spurts.

Chemicals of concern

As mixtures studies are few, studies using single-chemical analysis provide initial knowledge on the association between EDCs and children’s weight gain. A pooled study of seven European cohorts found that higher prenatal exposure to PCBs was associated with lower weight-for-age z-score between birth and two years of age (Iszatt et al., 2015). However, the results are imprecise across studies as the opposite association has been found with higher BMI during the first three years of life (Verhulst et al., 2009). In addition, prenatal exposure to Triclosan has been associated with higher weight-for-age z-score at two years of age (Wu et al., 2018), which is opposite to our findings. Finally, prenatal exposure to BPA was associated with lower BMI among girls, although BMI was measured later in childhood, at nine years of age (Harley et al., 2013).

Sensitivity analysis identified significant associations between higher prenatal PFOA concentrations and slower weight gain and a later growth spurt. This is consistent with a previous single-chemical analysis in the SELMA study, showing that PFOA was associated with slower weight gain and a later growth spurt, specifically for girls (Tanner et al., 2020a). In our study, using a mixture approach, we further found that PFOA was one of the dominant chemicals even in a mixture. A review on studies using single-chemical analysis (Johnson et al., 2014), and a pregnancy cohort study evaluating mixtures of persistent chemicals (Marks et al., 2021a), suggest prenatal exposure
to PFASs is associated with lower birthweight, and also lower weight in early infancy.

**Sex differences**

We also found sex-specific associations with a later growth spurt, specifically for girls. There is an indication from previous epidemiological studies that prenatal exposures to certain EDCs may influence weight gain during the first year of life with sex differences. For example, higher prenatal exposure to PFASs has been associated with lower weight during the first year of life, although, in contrast to our results, with more pronounced association among boys (Andersen et al., 2010; Zhang et al., 2022). On the other hand, higher prenatal exposure to phthalates (i.e., MBzP) have been associated with lower percent fat mass among boys in early infancy (Stevens et al., 2023).

Possible mechanisms for sex specific association may be through disruption of the endocrine homeostasis or epigenetic changes. For example, PFASs have shown potential to interfere with both estrogen and androgen receptors in vitro and in vivo studies, (Benninghoff et al., 2011; Kjeldsen & Bonefeld-Jørgensen, 2013). Also, a review of human studies, summarized that PFASs have been associated with DNA methylation on regions involved in growth and development, with incipient evidence for sex differences (Perng et al., 2023). Also, BPA has resulted in DNA methylation marks in animal studies (i.e., rats, mice), which were more pronounced in males (McCabe et al., 2017). Another possible mechanism is by inducing the expression of peroxisome proliferator-activated receptor gamma (PPARγ), which is considered the “master” regulator of adipogenesis. A review summarized that BPA may interact with PPARγ, and perhaps with sex-specific differences in the expression of PPARγ (Hoepner, 2019). Finally, phthalates such as MEP, MBzP and DEHP metabolites, have been associated with lower thyroid hormones among children, 4–9 years of age, more significantly among girls, and may indirectly influence children’s growth (Boas et al., 2010). However, further
research is needed to identify mechanisms that may explain the observed results in our studies.

Figure 9. The figure represents the first function of a double-logistic growth model used to predict children’s weight trajectory. See figures 6 and 7 for more detailed explanation of the model. The arrows in red represent the direction of the associations found between prenatal exposure to EDC mixtures and children's birthweight and weight trajectory. Higher levels of EDCs in the mixture were associated with i) lower birthweight, ii) slower weight gain, and, iii) a growth spurt at a later age.
Overweight and body composition (Study II and III)

EDC mixtures and overweight and body composition

The results in study II and III showed that prenatal exposure to EDC mixtures was associated with children’s BMI, odds of overweight and body fat. We found a significant interaction by sex, suggesting the associations differed for boys and girls. Higher prenatal exposure to EDC mixtures was associated with lower BMI, less odds of overweight, and less body fat among girls. However, among boys, the opposite association was found with more body fat. We also found sex differences for the chemical weights.

Among girls, the associations with lower BMI and less odds of overweight at 5.5 years of age, were driven by a phthalate metabolite (MEP), PAH (2-OHPH), bisphenols (BPF, BPS), a non-phthalate plasticizer (DPP), and a PFAS (PFNA). The chemicals of concern for less body fat at 7 years of age was driven by a bisphenol (BPF), phthalate metabolites (MBP, ΣDEHP), PAH (2-OHPH), PFAS (PFOA), and phenol (Triclosan).

Among boys, the associations with more body fat, was driven by bisphenols (BPA, BPF, BPS), phthalates (MBP, MBzP, MHiDP, MCiNP, MOiNCH), PAH (2-OHPH), PFAS (PFNA), and pesticides (TCP and ΣDDT/DDE).

Results from previous mixture studies with children’s weight and body fat have been inconclusive. A pregnancy cohort with a mixture approach on phthalates, phenols, and parabens found significant associations with higher BMI z-score and overweight among children between 4–12 years of age (Berger et al., 2021). Also, mixtures of persistent chemicals (e.g., DDE, HCB, PCBs) have been associated with higher BMI z-score at 7 and 12 years of age (Agay-Shay et al., 2015; Rouxel et al., 2023). In contrast, one pregnancy cohort found a higher mixture of persistent chemicals (e.g., PFASs, organochlorine pesticides, and PCBs) with lower weight earlier in infancy, from birth.
to 19 months, and only among girls (Marks et al., 2021a). Whereas, null associations have also been found (Kupsco et al., 2022; Vrijheid et al., 2020) These studies did not find or report significant sex differences, and one study was not able to evaluate sex differences due to the study design only including girls (Marks et al., 2021a).

**Sex differences**

Our findings showed that the sex-specific associations were more distinct at 7 years of age, as compared to 5.5 years of age. At the age of 5.5 years of age the association for boys only showed a trend for higher BMI and odds of overweight, whereas at 7 years of age the associations for boys and girls were both borderline significant. It is possible that the sex-specific associations are more pronounced as children approach puberty when sex differences are more distinct. An example, are three follow-up studies within a pregnancy cohort in the US, evaluating prenatal exposure to DDT and children’s odds of obesity at the ages of 7, 9 and 12 years (Warner et al., 2013; Warner et al., 2014; Warner et al., 2017). They found significant associations only among boys and not among girls. The associations were non-significant when children were 7 years of age, but significant when children were older at 9 and 12 years. Another possible explanation is that associations are expressed only at certain time points of development. One example is a pregnancy cohort in Mexico, which evaluated the association between prenatal exposure to bisphenols and phthalates, and BMI trajectories from birth to 14 years of age. They found that prenatal exposure to phthalates was associated with high BMI trajectory at 14 years of age, but low BMI trajectory before the age of 9 (Yang et al., 2018).

In addition, the data sources for study II and III are different. Study II used register data from routine child health care visits, whereas study III uses data from a follow-up visit for research participants. During the follow-up visit, additional efforts were made to standardize the collection of body fat measurements to reduce the variability between personnel. Also, study III uses additional body fat measures (e.g., BIA, skinfolds) with more reliability than BMI (Jensen et al., 2016).
Although BMI is a good screening tool, it is only a proxy for body fat and does not distinguish between fat mass and lean mass (Must & Anderson, 2006; Prillaman, 2023). With improved quality of the data and body fat measures, the direction of the found associations at 5.5 and 7 years of age remained the same; higher exposure to prenatal EDC mixtures was associated with more body fat for boys and less for girls.

From a public health perspective, child overweight and obesity is a concern as it increases the risk for cardiovascular disease and metabolic disorders in adulthood (Park et al., 2012). Therefore, the identified trend and borderline associations of more body fat among boys is of concern. On the other hand, it is also a concern that prenatal exposure to EDCs may influence growth and body fat among girls, and the implications of potential long-term effects among girls are still important to explore. Future studies are needed to explore other metabolic markers to find further insight on the effect of EDCs exposure with growth and metabolism among children of both sexes.

**Chemicals of concern**

The chemicals of concern that contributed most in the association with lower BMI and less odds of overweight among girls, were MEP and 2-OHPH (study II). This is in agreement with a study in the US, that found higher prenatal MEP and ΣDEHP concentrations associated with lower BMI among girls at 4–7 years of age (Buckley et al., 2016). Among boys, MBzP had the highest chemical weight in the association with higher BMI and odds of overweight. Although the association with EDC mixtures was not significant among boys, MBzP was significantly associated with higher BMI and overweight in the single-chemical analysis (study II). It is in agreement with associations found in a previous pregnancy cohort between phthalates and high BMI trajectory (Yang et al., 2018). However, although these two studies are in agreement with our results, a recent review on prenatal exposure to phthalates and phenols concluded that the direction of the association still remains inconclusive (Nidens et al., 2021).
Finally, in the associations with “overall body fat” and percent body fat, BPF and PFNA had the highest chemical weights among boys (study III). In contrast, among girls the chemicals of concern (BPF, MBP, ΣDEHP, 2-OHPH, PFOA, Triclosan), contributed almost equally (chemical weights ≈ 7.0) to the association with lower “overall body fat” and percent body fat.

Sensitivity analysis with single-chemical analysis resulted in a significant association only for DDT and more body fat among boys. This is consistent with a pregnancy cohort, evaluating single-chemicals exposure, where DDT was associated with higher odds for overweight in children (Warner et al., 2017). When we conducted the analysis with a mixture approach, a significant association was found with the EDC mixture, where DDT and additional chemicals were identified as of concern for children’s body fat among boys.

**Prenatal nutrition and children’s body composition**

One study addressed the aim related to prenatal nutrition and children’s body composition. Nutrition was assessed during mid-pregnancy and body composition at seven years of age. The main interest was to evaluate the association with body fat, but also secondary analyses were done to evaluate additional body fat measures. In the following section the results from study IV are discussed.

**Height (Study IV)**

Results from study IV showed that a more nutritious diet during pregnancy, in line with recommended nutritional guidelines, was associated with greater height among all children (Table 4). Previously, studies have shown beneficial effects of better nutrition during pregnancy on offspring, such as higher birthweight (Chen et al., 2021b; Gennings et al., 2020). Our results suggest that better nutrition during pregnancy may also be beneficial long-term for children’s growth.
**Body composition (Study IV)**

We did not find any significant associations between prenatal nutrition and measures of children’s body composition in overall. However, when we examined differences by sex there was significant interaction between MNI and sex.

**Sex differences**

We found sex-specific associations, where a more nutritious diet during pregnancy was associated with more body fat for boys and less body fat for girls at 7 years of age. The results among girls, are in agreement with previous studies reporting that better diet quality during pregnancy is associated with lower body fat (e.g., BMI, percent body fat, skinfolds) in offspring (Chatzi et al., 2017; Fernández-Barrés et al., 2016; Tahir et al., 2019). In contrast, the results among boys were in the opposite direction, suggesting that more nutritious diet is associated with more body fat. The secondary analyses, which included additional measures of body fat, affirmed the associations for boys and girls were in the opposite direction.

A large study on seven European birth cohorts found that diet with inflammatory potential, measured by an index (e.g., dietary inflammatory index), was associated with higher fat mass index among girls and less fat free mass among boys (Chen et al., 2021a). in contrast, they also found that a better diet quality was associated with lower fat mass among girls and higher fat free mass among boys. The association found in this study among girls, is consistent with our findings suggesting that better diet quality was associated with less body fat at 6 years of age. A difference from our results is that their findings among boys were at a later age; at ten years of age.

Two other studies observed diverging results at different ages. The Dutch cohort, Generation R, did not find any associations between maternal dietary pattern and children’s body composition (e.g., BMI, fat free mass, fat mass index, percent body fat) at six years of age (vanden Broek et al., 2015). However, a Mexican cohort, ELEMENT, found
sex-specific and trimester specific associations between maternal dietary patterns and children’s body composition at 10-17 years of age (Fossee et al., 2023). This suggests that associations may be observed at different ages of development for boys and girls. Moreover, there is also indication that prenatal nutrition may influence metabolic markers, such as adipokine levels (e.g., adiponectin and leptin) (Fossee et al., 2023). Future studies are needed to further evaluate the influence of prenatal diet and children’s growth and metabolism by also evaluating metabolic markers in addition to body composition.

Based on our results, maternal nutrition during pregnancy may have an influence on children’s body composition in school age. As adjustments could not be made for children’s diet, it is not clear if this is due to early life exposure, which could indicate a mechanism of fetal programming, or if it is due to a nutritional home environment that is inherited by the child. Previous studies show that maternal diet during pregnancy is associated with children’s diet in childhood (Shrivastava et al., 2013) and adolescence (Bjerregaard et al., 2019). Mothers who during pregnancy were in the highest quartile of a diet score, indicating better diet quality, had twice the odds that their child also was in the highest quartile diet score (Bjerregaard et al., 2019). Maternal pre- and postnatal nutrient intake (Shrivastava et al., 2013) as well as family meals may influence children’s dietary habits (Mahmood et al., 2021). On the other hand, one study found that the association between maternal diet and children’s body composition did not change even after adjusting for the child’s diet (Chen et al., 2021a). Further studies are needed to disentangle the effect of prenatal and child nutrition in relation to children’s body composition. It is also important to evaluate if there is an interaction between the nutritional fetal environment and child’s nutrition home environment in association to their body composition.
Table 4. Summary of results

<table>
<thead>
<tr>
<th>Study</th>
<th>Exposure</th>
<th>Outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Prenatal exposure to 26 EDCs (mixture)</td>
<td>Birthweight z-scores</td>
<td>Higher exposure to EDC mixtures was associated with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weight trajectory from birth until 5.5 years of age</td>
<td>- Lower birthweight z-scores</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Growth spurt rate</td>
<td>- Slower growth spurt rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Age at peak growth velocity</td>
<td>Sex-specific associations:</td>
</tr>
<tr>
<td>II.</td>
<td>Prenatal exposure to 26 EDCs (mixture)</td>
<td>BMI and overweight (ISO-BMI ≥ 25) at 5.5 years of age</td>
<td>Higher exposure to EDC mixtures was associated with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sex-specific associations:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Boys: Not significant</td>
<td>- Girls: Lower BMI, lower odds of overweight</td>
</tr>
<tr>
<td>III.</td>
<td>Prenatal exposure to 26 EDCs (mixture)</td>
<td>Body composition at 7 years of age:</td>
<td>Higher exposure to EDC mixtures was associated with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- “Overall body fat”</td>
<td>Sex-specific associations:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Percent body fat</td>
<td>- Boys: More body fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Girls: Less body fat</td>
</tr>
<tr>
<td>Study</td>
<td>Exposure</td>
<td>Outcome</td>
<td>Results</td>
</tr>
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</tr>
</tbody>
</table>
| IV.   | Prenatal nutrition (My Nutrition Index) | Body composition at 7 years of age:  
- Height  
- Body fat | Better adherence to nutritional guidelines was associated with:  
- Greater height  
Sex-specific associations:  
- Boys: More body fat  
- Girls: Less body fat  
Secondary analysis:  
- Boys: More weight, BMI, greater skinfolds  
- Girls: Less weight, BMI, less skinfold |
Table 5. Summary of chemicals of concern for the associations found between EDC mixtures and children’s growth and body composition, stratified by sex

<table>
<thead>
<tr>
<th>Chemicals in the EDC mixture</th>
<th>Birthweight z-scores</th>
<th>Infant growth spurt rate</th>
<th>Age at infant peak growth velocity</th>
<th>BMI</th>
<th>Overweight</th>
<th>&quot;Overall body fat&quot;</th>
<th>Percent Body fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-persistent Chemicals</td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
</tr>
<tr>
<td>MEP</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>MBP</td>
<td>9.0</td>
<td></td>
<td>10.9</td>
<td>5.2</td>
<td>11.2</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>MBzP</td>
<td>7.0</td>
<td>3.8</td>
<td>4.0</td>
<td>14.4</td>
<td>18.9</td>
<td>7.7</td>
<td>7.6</td>
</tr>
<tr>
<td>∑DEHP</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∑DINP</td>
<td></td>
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<td></td>
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<tr>
<td>MHxDP</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MCiNP</td>
<td>4.6</td>
<td>9.9</td>
<td>7.4</td>
<td>4.3</td>
<td>8.0</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>MOINCH</td>
<td>10.7</td>
<td>4.1</td>
<td>9.5</td>
<td>5.3</td>
<td>7.8</td>
<td>5.5</td>
<td></td>
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<tr>
<td>DPP</td>
<td></td>
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</tr>
<tr>
<td>Triclosan</td>
<td>16.7</td>
<td>4.0</td>
<td>8.0</td>
<td>5.8</td>
<td>7.2</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>BPA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPF</td>
<td>3.9</td>
<td>9.1</td>
<td></td>
<td></td>
<td>5.9</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>BPS</td>
<td>9.2</td>
<td>3.8</td>
<td></td>
<td></td>
<td>5.2</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>2-OHPH</td>
<td>7.8</td>
<td>7.4</td>
<td>4.1</td>
<td>4.8</td>
<td>8.1</td>
<td>6.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Chemicals in the EDC mixture</td>
<td>Birthweight z-scores</td>
<td>Infant growth spurt rate</td>
<td>Age at infant peak growth velocity</td>
<td>BMI</td>
<td>Overweight</td>
<td>&quot;Overall body fat&quot;</td>
<td>Percent Body fat</td>
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<td>-----------------------------</td>
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<tr>
<td>TCP</td>
<td>5.9</td>
<td>5.4</td>
<td>5.1</td>
<td>6.0</td>
<td>5.0</td>
<td>6.8</td>
<td>5.8</td>
</tr>
<tr>
<td>3-PBA</td>
<td>7.4</td>
<td>4.1</td>
<td>10.2</td>
<td>5.9</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFOA</td>
<td>5.8</td>
<td>11.2</td>
<td>16.1</td>
<td>6.0</td>
<td>22.7</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.4</td>
</tr>
<tr>
<td>PFOS</td>
<td>5.5</td>
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<td></td>
<td></td>
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<tr>
<td>PFNA</td>
<td>4.8</td>
<td></td>
<td>4.2</td>
<td></td>
<td></td>
<td>5.7</td>
<td>10.0</td>
</tr>
<tr>
<td>PFDA</td>
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<tr>
<td>PFUnDA</td>
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<tr>
<td>PFHxS</td>
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<tr>
<td>HCB</td>
<td>8.2</td>
<td>6.9</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans-Nonachlor</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>DDT/DDE</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>∑PCB</td>
<td>5.4</td>
<td>5.4</td>
<td>7.0</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Cell value represents the estimated chemical weights. Chemicals with larger weight contributes more to the association between the EDC mixture and children's growth and body composition. The three chemicals with highest weight within each column are marked in bold.
Discussion of methods

Study design

The SELMA study is a pregnancy cohort that have followed mothers and children from pregnancy up to school age. The longitudinal study design and early recruitment in pregnancy in the SELMA study fits well to evaluate associations within the framework of the DOHaD hypothesis, which suggest that early risk factors have an effect on health later in life; childhood, puberty or adulthood (Hoffman et al., 2021). In this case, risk and protective factors (EDC exposure and nutrition) were collected during pregnancy and health outcomes were measured at birth and during childhood.

The strength of a longitudinal study design is the possibility to measure exposure before the health outcomes of interest (Andersson, 2006). In this case exposures were measured before birth, and before the growth measurements were collected during childhood. A longitudinal cohort starts with a healthy population (e.g., without the outcome of interest) and follow participants over time without any intervention. Participants are categorized according to risk or protective factors (e.g., smoking) in order to compare unexposed vs. exposed participants. If the exposure occurs on a gradient, participants with high vs. low exposure are compared, as is the case for EDCs. Another strength of a longitudinal cohort study is a greater ability to identify associations between risk factors and health outcomes as compared to other epidemiological study designs (e.g., cross-sectional, case-control) (Andersson, 2006).

On the other hand, a cohort study with a longitudinal study design, has the limitation of participant drop-out over time (Andersson, 2006). It is a known effect that participants who stay longer also tend to be healthier and of higher socio-economic status (Howe et al., 2013).
**Missing data**

In the SELMA study, data was collected from several sources of information, for example, biological samples, health examinations, questionnaires and medical registers. To address the issue of drop-out, we conducted sensitivity analysis in study I (Table S3), comparing the sociodemographic characteristics of the study population with the number of mother-child pairs followed up until birth. Similar to results presented in the cohort profile analysis (Bornehag et al., 2012), the participating women had higher education, and smoked less. In our analysis we additionally found that participants were more likely to be primiparous and their children had slightly lower birthweight as compared to non-participants. This suggest that there is self-selection bias in the SELMA study (Bornehag et al., 2012), and also in the follow-up visits in regards to sociodemographic and lifestyle characteristics. As the characteristics of education and smoking are correlated with health outcomes (Johansson & Sundquist, 1999), it may be possible that the SELMA cohort is a healthier cohort as compared to the general population. On the other hand, it may also be that participants are more worried about their health, as participants reported more allergy in the family as compared to non-participants (Bornehag et al., 2012). Consequently, there is limited representativity of the general population, and limited generalizability of our results on the general population. However, our aim was to examine associations between the prenatal exposures and health outcomes, and not to infer estimations in the general population.

We also see an adequate distribution of the exposure variables, EDCs and MNI. EDCs were detectable in 90–100% of the biological samples (except for Trans-Nonachlor detected in 77%), which are indicative of a common exposure. The MNI score had a mean of 61 ± 13.1, which is somewhat higher when compared to the MNI score in a US population-based study, which had a mean of 50.8 (NHANES 2007-2014). But the interquartile range was within a score of 50 and 70, which is the same as in the NHANES population. In terms of the proportion of children with overweight, there is a lower percentage of children with
overweight and obesity in the SELMA study (16.1% at 5.5 years) as compared to the general population in Värmland (20% for 6-year old’s) (Region Värmland, 2019). This may be a result from the study participants being a healthier cohort. However, BMI among the children had an approximately normal distribution. These descriptive statistics indicates that there was not a narrow distribution in the study population, which could have affected the estimation of associations.

Other sources of missing data may be due to non-response to questions in the questionnaires, and missing data from medical registers. Specifically, we found missing data in the covariates of maternal age, BMI, education, and smoking. The reason of this type of missing data is unknown. However, the population study still has a good distribution of the covariates, and we do not have any indication for suspecting systematic missing data of the covariates.

There are different approaches to handle missing data (e.g., complete case analysis, imputation). In the SELMA study, only complete cases were analyzed. The efficiency of using complete cases may be limited by less precision and power but this is less of a problem for large studies where the study population is still large after constriciting to complete cases (Hughes et al., 2019). Despite missing data, we were able to find significant associations.

For study II and III, we applied the validation technique of WQS regression with repeated holdout. This technique indirectly considers the variation from covariates. Because in each holdout a different set of participants is selected in the split of 40%/60% training and validation set. This validation method assessing the distribution of the associations estimated from different sets of the data, also have different data in terms of the covariates. Compared to the approach of imputation analysis for missing data, imputed results would be derived from the distribution of the observations in the data. This is the same variance of the covariates we are addressing in the repeated holdout validation of the WQS.
In summary, we do not have indications that missing data would be systematic, nor that the missing data would be associated with the exposure (e.g., EDCs, nutrition) nor the outcomes of interest (children’s growth).

**Assessment of EDCs in prenatal urine and serum**

Maternal exposure to EDCs is a proxy for fetal exposure. EDCs have been found in both maternal urine and blood, as well as in placenta, cord blood and fetal tissue, which are indicative that many EDCs have the potential to cross the placental barrier with different efficiency (Mamsen et al., 2019; Padmanabhan et al., 2021). Therefore, a way to assess fetal exposure is to measure maternal EDC concentrations in biological samples.

The collection of samples was conducted early in pregnancy, at a median of 10 week’s gestation; a sensitive period of development, and just before the development of fetal adipose tissue (Desoye & Herrera, 2021). However, a limitation is that samples were only collected once during pregnancy. Therefore, it was not possible to assess variation in exposure levels of EDCs throughout pregnancy. The exposure levels are more stable for persistent chemicals (e.g., PFAS, DDT, PCBs), which accumulate in the human body and have longer half-life of years (Gore et al., 2015). In regards to non-persistent chemicals, previous studies show that one sample may be representative of a short period of time (Fisher et al., 2015; Townsend et al., 2013), although there may be greater variation and less reproducibility of the chemicals from diet sources (e.g., canned food) (Fisher et al., 2015; Preau et al., 2010). To reduce misclassification, urine samples were collected in a standardized way (i.e., first morning void urine samples).

Based on this knowledge, we cannot rule out the possibility of misclassification, as it could be that mothers may have had a different exposure the day before urine collection (e.g., diet, use of personal care products). As the study is longitudinal, the misclassification of exposure would most likely not be related to the health outcomes.
measured in the child. Therefore, any misclassification would not be systematic but would draw the associations towards the null. In despite of this limitation, we were still able to find associations between the EDC mixture and children’s growth.

**Assessment of prenatal nutrition**

Prenatal nutrition was collected through a food-frequency questionnaire during mid-pregnancy at approximately 25 weeks of gestation. Women were asked to report their average diet during the current pregnancy. As collection of prenatal nutrition was only collected once during pregnancy, it was not possible to evaluate differences throughout pregnancy or identify sensitive windows of exposure. The data collected from this FFQ represents an average of the woman’s diet during early- and mid-pregnancy. Previous research suggest that early pregnancy may be a more sensitive period than late pregnancy in regard to nutrition and metabolic diseases (Roseboom et al., 2000). Consequently, one way to improve data collection in future studies could be to apply FFQ with shorter time of recall (e.g., 7-day or 30-day recall) in early-, mid- and late pregnancy. This would allow to identify diet variation in each trimester, and also identify differences in association with health outcomes.

The FFQ was an extended version (98-item) of a validated FFQ, which has shown good validity of specific nutrients (Messerer et al., 2004). The two versions are very similar, except for certain items which were divided into individual food items for more specific information (i.e., types of grains, or meat).

Nutritional intake was assessed using the MNI. This index assesses adherence to the recommended nutrient intake. A strength is that it takes into account several individual characteristics (e.g., pregnancy status, age, height, weight, physical activity, and smoking) which changes the recommended intake for an individual. Additionally, MNI is based on the recommended guidelines as compared to data driven approaches, such as PCA, which characterize diet patterns which can
vary across populations. Therefore, MNI is a more personalized way of evaluating adherence to the guidelines. Another strength is the benefit of reducing the dimensionality of the nutrient data to one index, which makes it easier to use in statistical modelling.

**Outcome measures**

In regards to the children’s weight and body fat indicators, these were measured at several timepoints from birth until seven years of age. This allowed for three studies to evaluate associations with children’s weight and body composition at different windows of development, and to evaluate if associations change or persist over time.

The measures of height and weight collected after birth until 5.5 years are part of routine health care visits and retrieved through medical register data. In general, all children in Sweden attend these routine health care visits, and therefore, the data is representative of the measures done regularly in the general population.

Before the study visit at seven years of age, personnel went through training sessions to collect measures in a standardized way. Also, additional body fat measures were collected, such as BIA and skinfolds, which are more reliable than BMI. The most widely used indicator for body fat is BMI and is used in clinical settings to identify child overweight or obesity (Cole et al., 2000). Although BMI is a measure of weight in relation to height, it can still be considered a good measure of body fat (Jensen et al., 2016). However, there are more accurate instruments available to estimate body fat, such as BIA and skinfolds. The performance of these methods is commonly tested against reference methods of dual X-ray absorptiometry (DEXA), and isotope dilution and air-displacement plethysmography (ADP), which provide valid estimates of body composition. Both BIA and skinfold estimation of body fat have shown good correlations with DEXA among children and make them feasible in large epidemiological studies in a child population (Boeke et al., 2013; Jensen et al., 2016).
The novel statistical methods of using a double-logistic growth model to estimate weight trajectories allowed to evaluate children’s weight gain longitudinally. In addition, using principal component analysis reduces the dimensionality of multiple body fat measures and removes the challenge of multiple testing. When compared to the model using only percent body fat as outcome the results were very similar and provide motivation for using PCA as a modelling strategy.

**Mixtures approach**

We used WQS regression to evaluate the mixture of 26 EDCs, which included both persistent and non-persistent chemicals. This statistical model has several advantages. It provides an index which has easy interpretability and application, as the WQS index is applied as a predictor variable in a regression model. The modelling is hypothesis-driven in the sense that the direction of the association is decided a priori. This allows to constrain the direction of the association based on previous literature and focuses the inference to a more powerful single degree of freedom test. For the outcomes where there is not a consensus of the direction, the index can be derived in both positive and negative directions (Carrico et al., 2015). Another strength, shown in previous studies, is WQS can handle chemicals with high and complex correlation patterns through the use of an ensemble step based on a large number of bootstrap samples or random subsets of components (Curtin et al., 2019; Czarnota et al., 2015).

Also, in recent years, extensions of WQS regression have been developed, which allows for additional research questions to be evaluated (Busgang et al., 2022b; Curtin et al., 2019; Renzetti et al., 2021; Tanner et al., 2019). In our studies, we applied a WQS model with interaction term (WQS*sex) to allow the associations to vary by sex, and applied stratified chemical weights in order to find differences in chemical exposure patterns by sex most associated with the outcome (Busgang et al., 2022b). Another extension is the validation technique of repeated holdouts (Tanner et al., 2019), which was applied in the studies II and III. This method produces more generalizable data as it
calculates the mean betas and mean chemical weights across 100 WQS models with repeated holdouts (40%/60% training and validation).

A limitation of using a WQS regression model is that we were not able to evaluate interactions between chemicals. Other methods such as Bayesian kernel machine regression (BKMR) may be a better fit for this purpose. However, our main question was to evaluate if there is a mixture effect and which chemicals are driving the associations, which is the focus of WQS regression. Future research questions may include evaluating interactions between chemicals. Another limitation, is that the model requires a large dataset to apply the partition of training and validation to get stable estimates and weights (Tanner et al., 2019). However, one way to stabilize results is by using the validation step of repeated holdouts (Tanner et al., 2019).

Previous research has shown that models evaluating EDC mixtures are able to identify the joint effect of chemicals at low concentrations, which otherwise would not have been identified with single-chemical analysis (Bornehag et al., 2019; Le Magueresse-Battistoni et al., 2018). That is, characterizing the mixture effect addresses the “something from nothing” phenomenon, where signals from individual components may not be significant, but by measuring the joint action, an effect is detected (Silva et al., 2002). In our analyses, we also observed significant associations with the EDC mixture and in contrast, none or less significant findings when running single chemicals analysis (e.g., linear or logistic regression). Hence, it would be a reason to continue evaluating mixture effects in future studies as well.
IMPLICATIONS

For the population health

From a public health perspective, the results suggest that prenatal exposure to EDCs and nutrition, have an influence on birthweight and children’s growth. Even though the associations are small in magnitude, it provides awareness that environmental exposures are important to consider in order to promote a healthy environment both during pregnancy and during childhood, which are part of the national and international goals of public health (Swedish Agency of Public Health, n.d; WHO, 2017).

We do not know about the potential long-term health effects; if the associations found in childhood may persist or change in adolescence or adulthood. It may be that lifestyle factors become more important as children grow older. A study following children up to 16 years of age, found that prenatal diet was associated with children’s fat mass but the associations were attenuated when adjusting for confounders (Yin et al., 2012). On the other hand, children’s body fat at seven years of age has been correlated with body fat in adolescence and adulthood (Eriksson et al., 2001).

In regards to communication concerning EDC exposure to pregnant women and the general population, it has to be done within the context of each person. It is important to provide information but also discuss which possibilities there are to apply the information. Certain modifications in lifestyle and daily habits cannot be done without the economical means, health literacy, access to health, and social support from family and friends (Hildreth et al., 2023). As we know from the model of determinants of health (Dahlgren & Whitehead, 2021), the greater impact is done through legislation and not on the individual level. Therefore, changes to reduce exposure to EDCs need to be done through public policy in order to promote a healthy environment and support the health of pregnant women and children.
For regulation of EDCs

We found significant associations between prenatal EDC mixtures and children’s birthweight, weight gain, and body fat. Few or no associations were found when evaluating single-chemicals. Analyzing mixtures instead of one single chemical at the time, may provide important knowledge for risk assessment and regulation. Previous research shows that associations not detected in single chemical analysis could be found in mixtures relevant to human exposure and validated in experimental settings (Caporale et al., 2022). Research studies with a mixture approach is growing and are important to evaluate the joint effect of EDCs, especially for low levels of exposures, and for associations with small effect size.

Our models identified chemicals of concern of both persistent and non-persistent nature, and suggest that there is a mixture effect of both types of chemicals. In terms of interventions to reduce exposure, chemicals with short half-life do not accumulate in the human body and therefore, there is potential to reduce the level of exposure and, hence their associated health effects. On the other hand, persistent chemicals accumulate in the human body and the environment, and makes reduction of exposure more complicated. One way would be to restrict the various uses of persistent chemicals on a broad scale to reduce the influx of chemicals into the environment. A strategy of this kind is the recent law proposal to regulate PFAS use (e.g., manufacture, consumer products, textile) in the European Union (EU) countries (European Chemicals Agency, 2023). The law is projected to come into force in 2026/2027 and will regulate approximately 10,000 PFASs.

One consequence of the results from mixture studies would be the recommendation to remove groups of chemicals instead of single chemicals. Multiple chemicals, sometimes jointly acting, are present in the environment and can have health effects not found with single chemicals (Le Magueresse-Battistoni et al., 2018). In addition, replacement chemicals have shown to have similar or sometimes worse health effects than the original chemical (Bornehag et al., 2021; Ji et
al., 2021). Often less is known about the toxicity profile of substitution chemicals. Therefore, removing groups of chemicals with similar structure, just like the recent EU proposal for PFAS, is a better approach then removing one chemical at the time.

**For prenatal nutrition**

My nutrition index is a measure of adherence to the recommended guidelines for nutrient intake (Busgang et al., 2022a; Gennings et al., 2020). One advantage in research is that it evaluates the nutrient intake of each individual’s diet without being dependent on a specific diet, which can vary across populations. On the other hand, the challenge is to translate the acquired information into practical steps for the general population to improve their nutrient intake (e.g., a higher MNI score). A suggestion for future work, is to translate our results into practical recommendations, and provide examples on how changes in the diet can enhance nutrient intake (e.g., higher MNI scoring). This could be accomplished through a dietary tracking app that evaluates the MNI. Another idea for future work would be to identify diet patterns (e.g., prudent diet, Western diet) associated with a higher MNI score. These types of analyses may also provide results that enhance translation of results into practical recommendations for the general population.

Previous research suggest that certain foods may also be sources of exposure to EDCs (Giuliani et al., 2020; Marks et al., 2021b). In SELMA it was, for example, showed that fish consumption in during pregnancy was associated with PFAS serum levels in the pregnant women (Shu et al., 2018). So, even though foods may have nutritional value it may also come with the downside of chemical exposure. A suggestion for future work would be to conduct studies that evaluate the favorable level of intake for certain foods without ingesting unfavorable levels of chemicals in association with different health outcomes. A previous analysis in the SELMA study, provides an example in regards to fish consumption and EDCs in relation to health outcomes (e.g., birthweight), where low to moderate levels of fish
consumption improved nutritional quality, which outweighed the adverse effect of PFOS exposure (Gennings et al., 2020). Recently updated NNR guidelines were published in June of 2023 (Nordic Council of Ministers, 2023), and provide recommendations that promote good health and furthermore, a sustainable food consumption with less impact on the environment, for example, to consume less meat and a more plant-based diet. The current recommendations, and previous recommendations of 2012, take into account dietary exposure to certain EDCs, including PCBs, PFASs and PAHs. For foods that may contain PCBs and PFASs (e.g., fish, meat) there are specific recommendations for children and pregnant women. However, more research is needed on the exposure of other EDCs through diet, for example phthalates (Giuliani et al., 2020).

**Future studies**

Below are suggestions for future studies related to prenatal exposure to EDC mixtures and nutrition, with children’s growth and metabolism. Certain suggestions are of a descriptive or of technical nature but has the potential to improve the evaluation of associations between exposure and outcome.

- A descriptive study, describing adherence to guidelines in terms of food frequency consumption during pregnancy and identify diet patterns (e.g., Western or prudent diet)
- Identify diet patterns associated with EDC concentrations of specific chemicals and/or mixtures during pregnancy
- Analyze associations between EDC mixtures and additional markers of growth trajectories, such as height- or BMI-trajectories in early infancy
- Evaluate the associations between prenatal nutrition and weight trajectories
• Evaluate the associations between weight trajectories and children's body composition

• Evaluate associations between children's BMI trajectory and cardiometabolic markers (e.g., blood pressure)

Our results are based on observational data. Therefore, experimental studies are needed to find possible biological mechanisms, which may explain the associations found, especially for the association of lower BMI and body fat among girls. In addition, to explore the possible mechanisms for which EDC mixtures, at human-relevant exposures, may influence metabolic markers. This type of collaboration between epidemiology and experimental studies is already at work in the SELMA study (Lizunkova, 2023), and continued research is important.
CONCLUSIONS

In summary, we found that prenatal exposure to environmental exposures, EDCs and nutrition, may play a role in children’s birthweight and growth. The magnitude of effects is small and may not be of clinical importance for the individual. However, from a population perspective it is of interest as EDC is a common exposure and nutrition is of importance especially during the time of pregnancy.

Results show that a mixture approach is key when examining associations with health outcomes. The significant associations that were found with a group of 26 EDCs, were non-significant for the majority of chemicals in single-chemical analyses. In a mixture based on 26 EDCs, we identified several chemicals of concern for children’s birthweight, growth and body fat. They included both short-lived and persistent chemicals, and suggest that both current and past exposure may be of importance during pregnancy for health effects in children.

Results also suggest there are sex-differences in the association between EDC mixtures and children’s growth, which is in agreement with results from single-compound analysis and animal studies. We also found differences by sex in the association between maternal nutrition and children’s body composition. Given that both the environmental exposures, EDCs and nutrition, may influence children’s growth and body fat differently for boys and girls, it would be of interest to also evaluate sex differences in future studies.

The outcomes of interest were measured at birth and in early- and mid-childhood, up to seven years of age. The long-term health effects are still to be explored and future studies are needed to evaluate if the associations persist over time.
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Figure A1. Study population in study I

- Participating women in the SELMA study, n = 2,582
- Children in the SELMA study, n = 1,954
- Child routine health care visits: Children with available data on child’s weight at ages 0–5.5 years, n = 1,549
- Mother-child pairs with available data on EDC mixture and child’s weight at age 0–5.5 years, n = 1,323
- Mother-child pairs with available data on EDC mixture, child’s weight at 0–5.5 years and covariates, n = 1,118
- Missing data on covariates (n=205): Maternal education (n=185), Maternal BMI (n=52)
Figure A2. Study population in study II

1. Participating women in the SELMA study, n = 2,582
2. Children in the SELMA study, n = 1,954
3. Child routine health care visits: Children with available data on height and weight at age 5.5 years, n = 1,513
4. Mother-child pairs with available data on EDC mixture and child BMI at 5.5 years, n = 1,279
5. Missing data on covariates (n=170): Maternal age (n=39), Maternal education (n=42), Maternal BMI (n=92)
6. Mother-child pairs with available data on EDC mixture, child BMI at 5.5 years and covariates, n = 1,105

Children in the SELMA study, n = 1,954

Child routine health care visits:
Children with available data on height and weight at age 5.5 years, n = 1,513

Mother-child pairs with available data on EDC mixture and child BMI at 5.5 years, n = 1,279

Missing data on covariates (n=170): Maternal age (n=39), Maternal education (n=42), Maternal BMI (n=92)

Mother-child pairs with available data on EDC mixture, child BMI at 5.5 years and covariates, n = 1,105
Figure A3. Study population in study III

- Participating women in the SELMA study
  n = 2,582

- Children in the SELMA study
  n = 1,954

- Child follow-up visit at 7 years of age:
  n = 1,006

- Mother-child pairs with available data on
  EDC mixture and child’s body composition at 7 years
  n = 769

- Missing data on covariates (n=32):
  Maternal education (n=16),
  Maternal BMI (n=16)

- Mother-child pairs with available data on
  EDC mixture, child’s body composition at 7 years and covariates
  n = 737
Participating women in the SELMA study
n = 2,582

Children in the SELMA study
n = 1,954

Child follow-up visit at 7 years of age:
n = 1,006

Mother-child pairs with available data on maternal nutrition and child body composition at 7 years
n = 830

Mother-child pairs with available data on maternal nutrition, child’s body composition at 7 years and covariates
n = 808

Missing data on covariates (n=22):
Maternal smoking (n=2),
Maternal education (n=10),
Maternal BMI (n=10)

Figure A4. Study population in study IV
Early life environmental exposures and children’s growth

Early life is an important period for growth and development and therefore, sensitive to environmental exposures, such as chemicals and nutrition. Endocrine disrupting chemicals (EDCs) are man-made chemicals, common in everyday population exposure, and have been associated with unfavorable health effects and development. Additionally, optimal nutrition during pregnancy is important for both maternal and fetal health. But we need more knowledge on how these environmental exposures may influence children’s growth and if there are sex specific effects.

Twenty-six EDCs were measured in the urine and serum of pregnant women and their children’s growth was measured up to 7 years of age, including birthweight, height, weight, and body fat. Results show that higher levels of EDC mixtures were associated with lower birthweight, slower weight gain, and sex-specific effects on body fat. Also, better nutrition was associated with greater height and sex-specific effects on body fat.

The associations were small and not of concern for the individual, but from a population perspective it is an opportunity for improvement. Regulation of EDCs, both persistent and non-persistent, as well as adherence to nutritional guidelines, may be beneficial to promote healthy environments for children’s growth.

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