Dietary exposure to contaminants during pregnancy and fetal growth

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Aos meus pais

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ABSTRACT

Introduction
Maternal exposure to polycyclic aromatic hydrocarbons (PAH) and to acrylamide during pregnancy has been suggested to reduce fetal growth. However, the role of diet, the main source of exposure to these compounds among non-smokers and non-occupationally exposed, remains uncertain. This thesis aimed to characterize women's exposure during pregnancy to dietary PAH, specifically the genotoxic benzo(a)pyrene [B(a)P] and acrylamide, and to assess the effects of prenatal exposure to these compounds on fetal growth indicators.

Methods
This thesis was done within two large European population-based cohort studies conducted in Spain and Norway: the INfancia y Medio Ambiente (INMA – Environment and Childhood; n=657) and The Norwegian Mother and Child Cohort (MoBa; n=50651). Dietary B(a)P and acrylamide intakes were estimated based on information from food frequency questionnaires and constructed databases containing the concentrations of these compounds in foods. The associations between maternal intakes of B(a)P and acrylamide during pregnancy and fetal growth indicators were analyzed by multivariate linear or logistic models.

Results
(i) Smokers had higher dietary intakes of B(a)P and acrylamide compared to non-smokers; (ii) the main predictors of B(a)P intake were shellfish and processed/cured meats while the main predictors of acrylamide intake were snacks, fried potatoes, and crisp bread; (iii) higher prenatal exposure to dietary B(a)P and acrylamide may reduce birth weight and increase the risk of small for gestational age, independently of smoking-related exposure and (iv) significant interactions were found between vitamin C intake and dietary B(a)P during pregnancy: stronger effects of dietary B(a)P on fetal growth were observed among women with low vitamin C intake.

Conclusions

Higher maternal dietary B(a)P and acrylamide intakes during pregnancy may impair fetal growth, which might have implications for public health at earlier and later stages of life. While further study is needed to confirm these results, reducing the intake of these compounds from the diet should be recommended in dietary guidelines for pregnant women. Likewise, increasing intakes of fruits and vegetables rich in vitamin C should be recommended given its potential to prevent stronger adverse effects from exposure to such contaminants.

RESUMEN

Introducción

La exposición materna a los hidrocarburos aromáticos policíclicos (HAP) y a la acrilamida durante el embarazo ha sido asociada con la reducción del crecimiento fetal. Sin embargo, el papel de la dieta, la principal fuente de exposición a estos compuestos entre no fumadores y aquellos sin exposiciones ocupacionales, sigue siendo incierto. Los objetivos de esta tesis son caracterizar la exposición a través de la dieta a los HAP, específicamente el compuesto genotóxico benzo(a)pireno [B(a)P] y la acrilamida, durante el embarazo, y evaluar los efectos de la exposición prenatal a estos compuestos sobre indicadores del crecimiento fetal.

Métodos

Esta tesis se realizó dentro del marco de dos grandes estudios europeos de cohortes de base poblacional realizados en España y Noruega: INfancia y Medio Ambiente (INMA, n=657) y The Norwegian Mother and Child Cohort (MoBa, n=50651). La ingesta de B(a)P y acrilamida a través de la dieta fue estimada a partir de información de cuestionarios de frecuencia alimentaria y las concentraciones de estos compuestos en diferentes alimentos. Las asociaciones entre la ingesta materna de B(a)P y acrilamida durante el embarazo y los indicadores de crecimiento fetal se analizaron mediante modelos de regresión lineal o logística múltiple.

Resultados

(i) Las fumadoras tuvieron mayor ingesta de B(a)P y acrilamida a través de la dieta en comparación con las no fumadoras; (ii) los principales predictores de la ingesta de B(a)P fueron los mariscos y los embutidos, mientras que los principales predictores de la ingesta de acrilamida fueron los aperitivos, las patatas fritas y el pan crujiente; (iii) la exposición prenatal al B(a)P y la acrilamida pueden reducir el peso al nacer y aumentar el riesgo de pequeño para la edad gestacional, independientemente de la exposición relacionada con el tabaco y (iv) se encontraron interacciones

significativas entre la ingesta elevada de vitamina C y la exposición al B(a)P durante el embarazo: los efectos de la ingesta de B(a)P a través de la dieta sobre el crecimiento fetal fueron más fuertes entre las mujeres con baja ingesta de vitamina C.

Conclusiones

La elevada ingesta de B(a)P y acrilamida a través de la dieta durante el embarazo, puede perjudicar el crecimiento fetal, lo cual podría tener implicaciones para la salud pública a corto y largo plazo. Aunque futuros estudios son necesarios para confirmar estos resultados, recomendaciones para la reducción de la ingesta de estos compuestos a través de la dieta deberían ser incluidas en las guías dietéticas para mujeres embarazadas. Asimismo, debería recomendarse un aumento en la ingesta de frutas y verduras con alto contenido en vitamina C por su potencial para prevenir efectos mayores relacionados con la exposición a estos contaminantes.

PREFACE

Diet is the main source of exposure to polycyclic aromatic hydrocarbons (PAH) and acrylamide in the general population. These compounds are genotoxic contaminants that have been associated with adverse reproductive and child outcomes. Maternal dietary intake of these compounds during pregnancy results in prenatal exposure. The consequences of such exposure on fetal development are largely unknown. This thesis aims to assess the association between prenatal exposure to contaminants through maternal diet and fetal growth indicators in two population-based cohorts from Spain and Norway.

This thesis consists of a compilation of scientific publications according to the normatives of the Doctoral Program in Biomedicine of the Department of Experimental and Health Sciences at the University Pompeu Fabra. The first two publications derived from this thesis are based on data from the Spanish birth cohort study INMA – INfancia y Medio Ambiente (Environment and Childhood):

I. Smoking during pregnancy is associated with higher dietary intake of Polycyclic Aromatic Hydrocarbons (PAHs) and poor diet quality.
II. Dietary benzo(a)pyrene and fetal growth: effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism.

The last two publications are based on data from the MoBa – the Norwegian Mother and Child Cohort Study:

III. Dietary benzo(a)pyrene and birth weight: associations modified by vitamin C intakes in The Norwegian Mother and Child Cohort Study (MoBa).
IV. Impact of acrylamide intake on fetal growth – results from the Norwegian Mother and Child Cohort Study (MoBa).

This thesis includes an abstract, a general introduction, rationale, objectives, methods, results (the four above mentioned original articles), a global discussion, and final conclusions.

CONTENTS

1 INTRODUCTION

1 INTRODUCTION

Maternal diet during pregnancy is the main source of essential nutrients that are needed for optimal fetal and child development [1], and at the same time, it is a source of prenatal exposure to contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and acrylamide, which have been associated with adverse reproductive and child outcomes [2–4]. Diet has been identified as the main source of exposure to these contaminants among non-smokers and non-occupationally exposed individuals [5–8]. However, the role of prenatal exposure to PAHs and acrylamide through diet on fetal and child development is still unknown. This thesis aims to assess the effects of prenatal exposure to these contaminants through the maternal diet on fetal growth indicators in two European large population-based cohort studies conducted in Spain and Norway.

The evaluation of growth parameters at birth is undertaken to identify newborns who have suffered growth restriction *in utero* and therefore may have been exposed to adverse conditions [9]. Fetal growth is of interest not only as a predictor of pregnancy outcome and of infant and child health, but also of subsequent adult disease [10]. Variability in fetal growth parameters at birth is known to be associated with several factors, including the sex of the child and gestational age at birth [11], as well as maternal weight and length, age, parity, nutrition, genes, stress, smoking and other environmental exposures, placental structure and function.

1.1 Fetal growth indicators and long-term health implications

1.1.1 The developmental origins of health and disease (DOHaD) theory

The developmental origins of health and disease (DOHaD) theory, also known as “The Barker hypothesis”, proposes that adverse

events during *in utero* life can determine fetal development, which could result in long-term physiological and metabolic changes. This theory emerged during the late 1980s when David Barker and his colleagues observed an association between low birth weight and death rates from ischemic heart disease among men in a retrospective cohort study during 1911 and 1930 [12]. Later epidemiological studies in different populations worldwide have shown that birth weight is inversely associated with adult morbidity and mortality from cardiovascular disease [13–20], type 2 diabetes [21–23] and metabolic syndrome [24,25]. Recent studies have also reported a link between early growth and adult risk of development of other diseases, such as schizophrenia [26,27], depression [28,29], osteoporosis [30], autoimmune diseases [31], respiratory function [32], and cancers [33].

Although initial studies concentrated their attention on fetal life, subsequent works have demonstrated that the sensitive periods during which the environment could have long-term health effects also include fetal and early postnatal life. Both epidemiological and experimental evidence suggest that factors capable of originating developmentally-induced risk of subsequent health disorders before birth include the diet, body composition and endocrine status of the mother [34,35].

The DOHaD theory suggests that these observations, whereby early life influences affect later health, are originated due to developmental plasticity and fetal programming, in response to malnutrition or contaminant exposure during fetal life and infancy, that permanently shapes the body's structure, function, and metabolism (e.g.: promoting elevated fat storage, altered liver function, or faster development and earlier maturation) [36–38].

The biological mechanisms behind early life programming and the DOHaD are not fully understood. One of the most studied and known factors shown to have major implications for the long-term health of the babies has been maternal nutritional status during pregnancy. It has been reported that both maternal under- and over-nutrition can reduce placental-fetal blood flow and inhibit

fetal growth [39]. Mechanisms underlying programming of chronic diseases include diminished cell volume, abnormal gene structure, changes in gene expression, hormonal function and growth factors.

Since “The Barker hypothesis” was first suggested, and because we now know that the placenta does not protect the fetus from maternal exposure to environmental pollutants, researchers’ attention has increasingly shifted to the possible role of intrauterine and perinatal exposure to toxic compounds in the explanation of a wide range of child development outcomes and adult diseases [40].

1.1.2 Measurements of birth size as fetal growth indicators

Birth weight is the most commonly used indicator of fetal growth in epidemiological studies [41,42], mainly due to its relative convenience and availability. As mentioned above, it has recently been inversely associated with adult morbidity and mortality from chronic diseases [34,38]. Infants weighing less than 2500g at birth are defined as low birth weight (LBW) regardless of gestational age. Low birth weight has been strongly associated with perinatal morbidity and increased risk of long-term disability in both developed and developing countries [43]. Birth weight is a marker of growth and development *in utero*, which may be affected by genetic, placental and several maternal factors, including nutritional status and environmental exposures during pregnancy (WHO).

Small for gestational age (SGA) defines an infant who has failed to achieve a weight threshold for the baby's sex and gestational age. The most common definitions are: infants with a birth weight more ≥ 2 standard deviations (SD) below the mean, and infants with a birth weight below the 10^{th} percentile of a population specific weight versus gestational age plot [44]. However, some studies define SGA as birth weight below the 5^{th} or 3^{rd} percentile for gestational age [45]. Being SGA can be constitutional, without an underlying pathological cause, or secondary to intrauterine growth

restriction, which can be caused by many possible factors. Other commonly used measures of fetal growth include head circumference, birth length and ponderal index [birth weight (kg)/birth length $(cm)^3$].

1.2 Dietary contaminants and impaired fetal growth

1.2.1 PAHs – benzo(a)pyrene

a) Definition

Polycyclic aromatic hydrocarbons (PAHs) are a group of lipophilic chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat [2,5]. To date, hundreds of different PAHs have been identified, each of them containing two or more aromatic rings. Generally, they have high melting and boiling points, low vapour pressure, and very low water solubility. PAHs are one of the most widespread organic pollutants. A few PAHs are used in medicines, dyes, plastics, and pesticides. Others are emitted from the processing of coal, crude oil, petroleum, and natural gas, from production of aluminium, iron and steel, from heating in power plants and homes (oil, gas, charcoal-fired stoves, wood stoves), burning of refuse, wood fires, and from motor vehicle exhausts. Among these compounds, benzo(a)pyrene [B(a)P] (Figure 1) is the best known; it has been identified as human mutagen, carcinogen, and endocrine disruptor, and has been widely used as a marker of occurrence and carcinogenic effects of total PAHs. B(a)P has been recently classified as carcinogenic to humans by the International Agency for Research on Cancer [46].

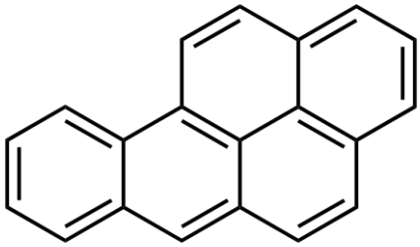

Figure 1. Chemical structure of benzo(a)pyrene [B(a)P]

Humans can be exposed to PAHs through different routes. Table 1 shows the levels of B(a)P intake estimated by different routes of exposure [2]. The major routes of exposure in the general population are ingestion of food and inhalation of contaminated air, as well as from tobacco smoke. Among individuals not exposed to high levels of tobacco smoke or with occupational exposures, it has been estimated that diet is the source of 70-90% of total PAH exposure [5–7,47–50].

Table 1. Estimated non-occupational mean daily intake of different B(a)P by different routes for an adult non-smoker (ng/person).

Sources of exposure	B(a)P
Diet: general population	50-290
Drinking water [a]	0.02-2
Air [b]	20
Cigarette smoking	2-20 (per cigarette)
	220 (per 20 cigarettes/day) [c]

[a] Assuming an ingestion of 2 l/day.

[b] Assuming a ventilation rate of 20 m^3/day.

[c] Assuming 11 ng as a mean delivery and that 80% of inhaled particle-bound B(a)P from mainstream smoke is deposited in the respiratory tract [51].

b) Occurrence in food and dietary intakes

Food can be contaminated by environmental PAHs that are present in air (by deposition), soil (by transfer) or water (deposition and transfer), by industrial food processing methods and by home food

preparation [5]. Industrial processing procedures, such as smoking and drying, and cooking methods at high temperatures, such as grilling, roasting and frying, are commonly thought to be the major sources of food contamination by PAHs. For cooking, the level of contamination depends on time, type of oil used, methods (grilling, frying, roasting), distance from the heat source and drainage of fat [6].

The levels of PAHs in raw food depend on the levels of PAHs in the air of the area where the food is grown. In rural areas, the levels of PAHs measured in unprocessed food reflect the background contamination, coming from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. In industrial areas or along highways, the contamination of vegetation can be ten-fold higher than in rural areas. This may be due to the deposition of small airborne particles containing PAHs from exhauts of motor vehicles and aircrafts, or industrial plants (e.g. aluminium, incinerators, etc) [2]. For this reason, leafy vegetables for example can be a significant source of PAHs in the human diet.

High PAH levels, including B(a)P, have also been found in shellfish [52–54]. Shellfish are contaminated as a result of short or long-term contamination of the sediment on which they grow, particularly if the shellfish beds are in proximity to oil refineries or areas heavily used by shipping. Moreover, it is known that mollusks are not able to metabolize PAHs, which is why they may accumulate them in their organisms, and are commonly used for biomonitoring aquatic pollution [55,56].

Several studies have previously been carried out to estimate dietary intake of total PAHs or B(a)P in order to identify the major sources of dietary exposure in the general population [3,57–62]. According to surveys conducted in six European countries published in 2002 by the Scientific Committee on Food of the European Commission, the mean dietary intake of B(a)P in adults has been estimated to be in the range of 0.05 to 0.29 µg/day [2]. However, these data were in some cases based on a limited

number of foods, and different methods were used in each survey to assess food intakes. Due to the ubiquitous presence of PAHs in food, the food categories that contribute significantly to the exposure are determined by a combination of the level of contamination and the amount of that food consumed. Previous studies have identified cereals, fats and oils (resulting from currently modified processing methods), vegetables, and seafood as the main contributors of total PAH and B(a)P intake [3,60,61]. The contribution to human exposure of each of the mentioned food categories can be either because of the frequency of intake or because of the levels of PAHs. For example, the contribution of fats and oils to PAH intakes is attributable to its high levels of contamination, while the contribution of cereals is due to the high intakes of this food group. Although barbecued and smoked food have shown very high levels of PAH, European studies show that the contribution of this type of food to the total intake of PAH is modest, since they are minor components of the usual diet. However, one study in the US found that grilled/barbecued meat intake was the second contributing food group (21%) to the mean daily intake of B(a)P [63]. Therefore, the contribution of specific food groups, such as barbecued meat or shellfish, to PAH intake varies with the extent to which these foods are consumed in different populations.

c) Prenatal exposure and fetal growth

Experimental studies have shown developmental toxicity of B(a)P after oral administration to pregnant mice. These studies indicate that prenatal dietary exposure to B(a)P can reduce fetal weight at birth, reduce weight gain during postnatal development, and can cause fetal death and malformations [2]. In humans, a few epidemiological studies have reported that prenatal exposure to PAHs may be associated with adverse reproductive or child outcomes, including low birth weight and length, preterm birth, reduced head circumference at birth, and lower scores on childhood tests of neurodevelopment [64–69].

Although diet is recognized as the main source of PAH exposure for non-occupationally exposed individuals and non-smokers [5–7,49], most epidemiological studies exploring the role of PAHs in fetal growth have estimated PAH exposure based on levels of bulky DNA adducts or personal measures of atmospheric PAH exposure. Bulky DNA adducts are a biomarker of overall PAH exposure, including PAHs from the diet, as well as from tobacco smoke and contaminated air [70]. Only two recent epidemiologic studies have examined the role of prenatal exposure to B(a)P specifically from diet on fetal growth [71,72]. One study estimated dietary PAHs based on a limited number of food items (smoked, grilled or barbecued meat intakes) and reported very weak inverse associations with indicators of size at birth . The other study found a negative effect of maternal intake of barbecued meat during the third trimester of pregnancy on birth weight but not birth length or head circumference [72]. Consequently, little is known specifically about dietary intake of these compounds among pregnant women, and how this route of exposure to PAHs may relate to birth outcomes.

Biological mechanisms

Exposure to contaminants during fetal life and early postnatal stages is of particular concern due to the unique fetal and infant susceptibility [64,73–75]. PAHs, including B(a)P specifically, have been shown to cross the human placental barrier [76–78]. Detectable levels of placental and fetal bulky DNA adducts have been found in both non-smokers and smokers. Higher levels have been measured in smokers compared with non-smokers, and also among women living in areas with high levels of air pollution compared with women in a less contaminated area [79,80]. However, mechanisms through which these compounds may influence fetal growth are not clear.

A number of mechanisms linking prenatal exposure to PAHs to fetal growth have been postulated. PAHs bind to receptors regulating the induction of P450 enzymes, and this binding may decrease the uptake of oxygen and nutrients. Moreover, similar

consequences may also be related to the binding of these chemicals to receptors related to insulin and growth factor metabolism [81]. Regarding B(a)P, its exposure affects early trophoblast proliferation due to the interaction with growth factor receptors in *in vitro* studies [69,82].

1.2.2 Acrylamide

a) Definition

Acrylamide is a chemical compound derived from acrylic acid that easily forms polymers (Figure 2). Glycidamide is the major metabolite of acrylamide and it is formed via the cytochrome P450 pathway, is glycidamide [83]. This metabolite is assumed to be the genotoxic agent of acrylamide [84].

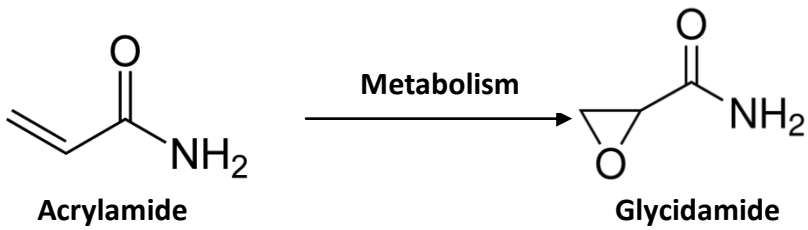

Figure 2. Chemical structure of acrylamide and its metabolite glycidamide

Acrylamide is recognized as a neurotoxic compound in humans; it has been shown to have mutagenic and carcinogenic properties in experimental studies in animals, and it has been classified as a probable human carcinogen (group 2A) by the International Agency for Research on Cancer (IARC). The production of acrylamide started in 1954 for commercial use. The wide-spread polymers of acrylamide have had applications in water and waste-water treatment, crude oil production processes, paper and paper pulp processing, mineral processing, concrete processing, as cosmetic additives, in soil and sand treatment, coating application, textile processing and other miscellaneous uses (photographic emulsion, adhesives and coatings) [85]. During manufacture of

polyacrylamide, a small amount of acrylamide monomer may be present in the final product because of incomplete polymerization [86]. Besides its industrial applications, acrylamide is also present in tobacco smoke [87–90], and has recently found to be naturally formed in certain foods during processing or cooking at high temperatures [8,91,92]. The discovery of acrylamide in foods generated a great debate about the possible health effects of the long-term exposure to acrylamide through diet.

Humans may be potentially exposed to acrylamide through food or drinks ingestion, dermal contact, and by inhalation of acrylamide vapors or particles. High doses of acrylamide have been measured in occupational exposures. The most common routes of exposure in the workplace are inhalation or dermal absorption. In the general population, non-smokers and non-occupationally exposed individuals, diet has been identified as the largest source of exposure [4]. Table 2 summarizes the estimated levels of exposure reported from different sources.

Table 2. Summary of exposure estimates (µg/kg bw/day) by sources and population groups

Sources of exposure	Mean or median	90th percentile or upper boundary
Diet: general population	0.43	0.92
2–5-year-olds	1.06	2.31
Drinking water	no data	<0.01
Personal care products	approx. 0.5	1.1 (female)
Cigarette smoking	0.67 (from cigarette data)	1.3
	2.6 (from adduct data)[a]	approx. 6
Occupational exposures	1.4–18	43(based on PEL)

Modified from Manson et al. 2005 [4]; PEL: permissible exposure limit.

[a] Acrylamide exposure in smokers based on adduct formation was estimated by taking the value for total exposure in smokers (3.4µg/kg bw/day) and subtracting the value for total exposure in non-smokers (0.85µg/kg bw/day).

b) Occurence in food and dietary intakes

In April 2002, the Swedish National Food Administration and researchers from Stockholm University reported for the first time that some foods could contain relatively high levels of acrylamide as a result of being heated at high temperatures during processing or preparation [92]. Since the Swedish report, several health and food safety agencies confirmed the detection of acrylamide in foods in numerous countries, including Norway (www.snt.no), the United Kingdom (British Food Standard Agency), Germany (www.bfr.bund.de), Switzerland (Swiss Federal Office of Public Health 2002), and the United States (www.cfsan.fda.gov). Although these agencies recognized dietary acrylamide exposure to be a matter of cancer for food safety, for the moment no specific recommendations have been made due to the lack of evidence of any adverse effects of this route of exposure in humans [93].

Acrylamide is formed during cooking or processing at high temperatures (e.g. frying, grilling, or roasting) of carbohydrate-rich foods containing the amino acid asparagine and reducing sugars [8]. Thus, acrylamide occurs predominantly as a natural process of cooking or processing methods rather than as a environmental contaminant [89], and there is variability in levels detected between and within food items [94]. Elevated levels of acrylamide have been detected in potato chips, French fries, crisp and soft breads, cereals, chocolate, and coffee [95,96]. It has also been shown that low amounts of acrylamide might migrate from food packaging material into the packed foodstuff [97]. Table 3 shows the levels detected in various foods from different countries reported recently in a review by Friedman and co-workers [89].

Table 3. Acrylamide concentrations in foods.*

Food	Acrylamide concentration (μg/kg)
Almonds, roasted	260
Asparagus, roasted	143
Baked products: bagels, breads, cakes, cookies, pretzels	70–430
Beer, malt, and whey drinks	30–70
Biscuits, crackers	30–3200
Cereals, breakfast	30–1346
Chocolate powder	15–90
Coffee powder	170–351
Corn chips, crisps	34–416
Crispbread	800–1200
Fish products	30–39
Gingerbread	90–1660
Meat and poultry products	30–64
Onion soup and dip mix	1184
Nuts and nut butter	64–457
Peanuts, coated	140
Potato boiled	48
Potato chips, crisps	170–3700
Potato, French fried	200–12000
Potato puffs, deep-fried	1270
Snacks, other than potato	30–1915
Soybeans, roasted	25
Sunflower seeds, roasted	66
Taco shells, cooked	559

*From Friedman 2003 [89].

Total dietary intake of acrylamide has been estimated in several epidemiological studies and national dietary surveys. In 2010, the mean acrylamide exposure for adults (>18 years) in Europe was estimated by the European Food Safety Authority to range between 0.31 and 1.1 μg/kg bw/day. This estimation was based on national dietary survey data from 17 countries [98]. The results were similar to those reported in the latest acrylamide risk

assessment report by the FAO/WHO also in 2010, in this report, acrylamide intake levels ranged between 0.2 and 1 µg/kg bw/day for the general adult population [99]. In both studies, French fries, coffee, bread, potato crisps, and biscuits were identified as the major contributors to overall acrylamide intake among European adults [98–100].

In Norway, authorities have reported a median acrylamide intake of 0.42 µg/kg bw/day for non-pregnant women aged 16-79 years using national food survey data [100]. Among pregnant women, dietary acrylamide intake has recently been reported for the first time also among Norwegian women [101]. In that study, the estimated total dietary acrylamide intake was 0.48 µg/kg bw/day, and the main food groups contributing to the intake were found to be crisp bread, potato crisps, snacks, and bread. Other important food groups were chocolate, waffles/pancakes, breakfast cereals and coffee (see Figure 3).

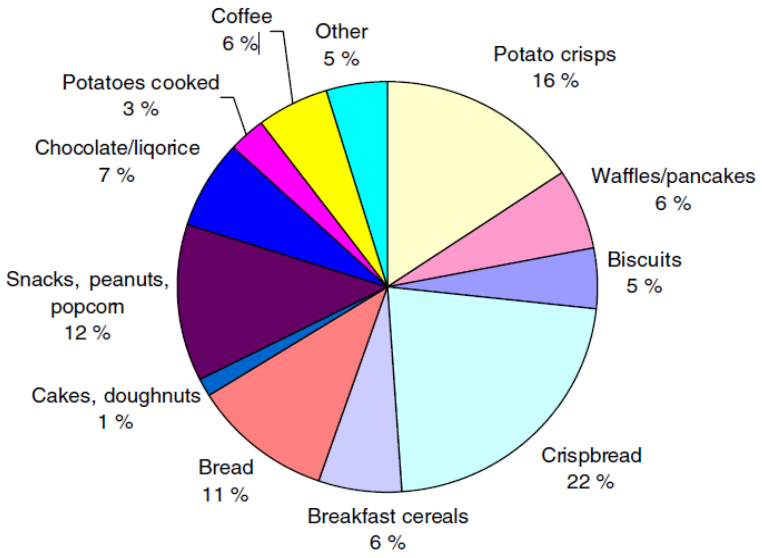

Figure 3. Contribution (%) of the most important food groups to dietary exposure to acrylamide in pregnant Norwegian women according to the MoBa FFQ [101]**.**

c) Prenatal exposure and fetal growth

In animal experiments, acrylamide has been shown to cause reproductive and developmental toxicity [4,102]. Several experimental studies in rats and mice, found a decrease in pup weight after maternal oral exposures to acrylamide during gestation [103–106]. El-Sayyad et al. also showed that pregnant mice fed with a diet containing 30% of fried potato chips, which has been found to contain high levels of acrylamide, gave birth to offspring with reduced birth weight [103].

In humans, to date, only one epidemiological study has assessed the association between prenatal exposure to acrylamide and fetal growth indicators. This study showed that higher acrylamide exposure among non-smoking pregnant women was associated with a significant reduction in birth weight and increased the risk of SGA [107]. The exposure assessment was based on acrylamide Hb-adduct levels in cord blood samples as well as applying a food score approach based on the intake of acrylamide-rich foods obtained from food frequency questionnaire (FFQ). To our knowledge, no previous study has analyzed the effects of prenatal exposure to acrylamide intake estimated from the total diet on fetal growth.

Biological mechanisms

Animal experiments show a negative effect of prenatal acrylamide exposure on fetal growth. However, the mechanisms by which acrylamide might exert this effect are not fully understood. Studies in humans have shown that exposure to acrylamide through diet begins *in utero* since it has been found to cross the placenta barrier [108–111]. Measurements of Hb adducts from acrylamide in mother and cord blood samples have demonstrated that acrylamide is circulated in the body of the fetus [110,111].

Both acrylamide and glycidamide are reactive electrophiles and thus have the potential to react with nucleophilic sites in biomacromolecules, which could affect cellular processes of

importance for growth. It is known that during heat-processing of foods, in parallel with acrylamide formation, other Maillard products with potential toxic effects are formed [112].

1.3 Maternal nutrition and fetal growth

Maternal nutrition during pregnancy plays a critical role in fetal growth and development [113], and may therefore influence children's health later in life [114]. Rapid growth, cell differentiation, and development of vital organ systems occur during the first weeks of gestation, thus, during this period, the fetus is particularly vulnerable to any external insult [115,116]. The energy used to create these systems comes from the energy and nutrients in the mother's circulation, and around the lining of the womb. Nutrient and oxygen supply have been identified as dominant determinants of fetal growth [13]. Thus, an adequate amount of nutrients and energy need to be consumed during pregnancy. Additionally, since the placenta is not yet formed during the early stages of pregnancy, there is no mechanism to protect the embryo from nutrient deficiencies which may be inherent in the mother's circulation. For this reason, adequate nutrient intake during pregnancy – including during the first few weeks after conception - is essential for optimal fetal development.

The association between maternal nutrition and birth outcomes is complex, it may be influenced by many biologic, socioeconomic, and demographic factors, which vary widely in different populations [117]. Several epidemiological and experimental studies have provided useful information about the effects of poor maternal nutritional status during pregnancy on fetal outcomes. Some of the first epidemiological studies to document child health effects linked to maternal deficiences specific to pregnancy came after a severe famine that occurred in the western Netherlands during the Second World War, in which calorie restriction was showed to cause dramatic effects on pregnancy weight gain and infant birth size in a well-developed society in which the population had generally been adequately nourished [118,119].

More recent studies suggest that deficiencies of antioxidant intakes during pregnancy associated with micronutrients such as selenium, zinc, manganese, or vitamins, can result in poor pregnancy outcomes including fetal growth restriction [120,121]. Fruits and vegetable intake during pregnancy is significantly associated with birth weight [120]. Vitamin C (ascorbic acid and dehydroascorbic acid) is an essential water-soluble vitamin found widely in fruit and vegetables, and known as an important antioxidant. The recommended dietary allowance of vitamin C for women in the general population is of 75 mg/day, and 85 mg/day for pregnant women [122]. It has been shown that the requirement of vitamin C for smokers is increased by 35 mg/day since smoking increase oxidative stress and metabolic turnover of vitamin C [122].

Additionally, studies have shown that the intake of antioxidant nutrients, including vitamin C, can attenuate the adverse effects of exposure to PAHs. Vitamins A, C or E, alpha-carotene and beta-carotene have been reported to reduce DNA damage related to PAH exposure, perhaps by inducing the activity of detoxifying enzymes such as glutathione S-transferase (GST), and could therefore help to protect against adverse health outcomes related to exposure to such contaminants [123–128].

1.4 Synthesis

Together, findings from experimental studies and a handful of epidemiological studies suggest that exposure to several contaminants commonly found in the diet may influence fetal growth, with implications for long-term health and well-being of these children. However, evidence on the effects of prenatal exposure to dietary contaminants such as acrylamide and PAH—to which exposure is widespread in many food cultures—is currently limited, and it remains uncertain whether beneficial nutrients may help to mitigate any such effects.

2 RATIONALE

2 RATIONALE

It is widely accepted that diet is the main source of nutrients with properties that help to reduce the risk of illnesses such as cancers, as well as nutrients essential for optimal reproductive health [1]. What is less well appreciated is that the diet is also a source of exposure to some contaminants with potentially harmful health effects, such as polycyclic aromatic hydrocarbons (PAHs) and acrylamide [2–4,6,7]. Although there are other sources of exposure to these compounds, such as contaminated air or tobacco smoke, previous studies have shown that diet is the main source of exposure among non-smokers and non-occupationally exposed individuals [5–8].

Previous evidence shows that human fetuses are exposed to PAHs and acrylamide, both genotoxic and carcinogenic compounds, from maternal circulation through the placenta [76–78,108–110]. Implications of exposure to PAH and acrylamide for fetal growth and development, as a consequence of maternal dietary intake during pregnancy, are largely unknown: only two small epidemiological studies, using very limited and crude proxy measures of exposure, have examined this issue [71,72]. Only one study to date has examined effects of prenatal exposure to acrylamide on fetal development [107]. It has been shown that fetuses and neonates may be particularly vulnerable to the effects of exposure to contaminants during *in utero* and during early neonatal life due to rapid growth, cell differentiation, and development of vital organ systems [115,116]. Because of fetal susceptibility, exposure to low doses of toxic compounds during intrauterine life may compromise the optimal development of the fetus [67,76,129,130], with implications for the future health of the child. Although maternal diet is likely to be the main source of prenatal exposure to PAHs and acrylamide, the role of this route of exposure to these compounds during pregnancy on child development remains uncertain.

Indicators of intrauterine development, such as birth weight and small for gestational age (SGA), have been shown to predict neonatal morbidity and mortality [131,132], increase the risk of delayed neurodevelopment [133], and to be related with the risk of chronic diseases during adulthood including cardiovascular diseases, type 2 diabetes, and insulin resistance [38,114]. Since PAHs and acrylamide are found in commonly consumed foods, and reduced fetal growth may further influence children's health later in life, the possible negative effect of prenatal exposure to these compounds through diet on fetal growth might have implications for public health at both earlier and later stages of life.

This study aims to contribute to a better scientific understanding of dietary PAHs, and acrylamide exposure during pregnancy, and its impact on fetal growth indicators, using data from two prospective European population-based pregnancy cohort studies conducted in Spain and Norway. In contrast to earlier studies, we characterize exposure using the whole diet, rather than a limited number of indicator foods, to better estimate exposure, and take into account possible interactions with beneficial nutrients which may help to modify any negative effects of these compounds.

3 OBJECTIVES

3 OBJECTIVES

3.1 General objectives

The overall aim of this project is to assess the effects of prenatal exposure to two classes of contaminants through the maternal diet on fetal growth indicators in two large European population-based birth cohort studies, the Environment and Childhood (INMA – INfancia y Medio Ambiente) and The Norwegian Mother and Child Cohort (MoBa) studies.

3.2 Specific objectives

- To estimate dietary intake during pregnancy of total polycyclic aromatic hydrocarbons (PAHs) and benzo(a)pyrene [B(a)P], frequently used as an indicator of exposure to genotoxic and carcinogenic PAHs, and to characterize factors associated with higher intake within these two cohorts.

- To assess associations between intake of B(a)P during pregnancy and indicators of fetal growth impairment, focusing on whether maternal vitamin intakes and the glutathione S-transferase P1 (*GSTP1*) (Ile105Val) polymorphism, both potentially associated with the ability to reduce adverse effects of this contaminant, may modulate this association in the INMA study.

- To assess associations between maternal dietary intakes of B(a)P during pregnancy and birth weight in the MoBa study, focusing on whether associations vary depending on levels of maternal vitamin intakes.

- To assess associations between prenatal exposure to dietary acrylamide and fetal growth indicators in the MoBa study, and to describe population characteristics associated with higher acrylamide intakes during pregnancy.

4 METHODS

4 METHODS

This thesis is based on data from two large European population-based birth cohort studies (Figure 4). This section provides a brief summary of the two different study populations included in this thesis. Further methodological details can be found in the methods section of each paper, included in the results section of this thesis.

Figure 4. Geographic location of the two cohort studies included in this thesis.

The INMA study included a sample of 657 women from the city of Sabadell, located 20 km north-west of Barcelona in Spain. The MoBa study included 50651 women from many geographically different areas in Norway. Spain has a total population of roughly

46 million inhabitants, a population density of 93/km^2, and is characterized by the Mediterranean diet. Norway's population is around 5 million people, and is the second least densely populated (15.5/km^2) country in Europe.

4.1 The INfancia y Medio Ambiente (INMA) Project

The INMA - INfancia y Medio Ambiente - (Environment and Childhood) Project (www.proyectoinma.org) is a network of prospective population-based Spanish cohorts that aim to study the role of environmental exposures during pregnancy and early childhood in relation to child growth, health and development [134]. The study population includes pregnant women and their children recruited from the general population in seven study areas: Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias and Gipuzkoa (Figure 5). Extensive assessments were carried out in pregnant women and children. The study was approved by the Clinical Research Ethical Committee of the Municipal Institute of Health Care (CEIC-IMAS), and informed consent was signed by all participants.

Figure 5. Geographic location of the seven INMA study areas in Spain.

The analyses presented in this thesis are based on data from the cohort of Sabadell. Criteria for inclusion of the mothers were: (i) to be resident in one of the study areas, (ii) to be at least 16 years old, (iii) to have a singleton pregnancy, (iv) to not have followed any programme of assisted reproduction, (v) to wish to deliver in the reference hospital and (vi) to have no communication problems [134]. In the city of Sabadell, between July 2004 and July 2006, 657 women were recruited during their first trimester of pregnancy and were followed every trimester of the pregnancy until the moment of birth. The participation rate was 60%. Data were collected from: study questionnaires administered in person by trained staff, which included dietary intake assessments; clinical records including ultrasound scans and physical examination data; and environmental measurements to assess air and water pollutants. Biological samples (blood, placenta, urine, saliva, hair, nails and mother's milk) were collected to measure nutrients and to estimate exposure to a number of pollutants. Detailed

information on the sample selection and dietary assessment methods can be found in papers I and II.

4.2 The Norwegian Mother and Child Cohort study (MoBa)

The Norwegian Mother and Child Cohort Study (MoBa) was initiated by and maintained at the Norwegian Institute of Public Health [135]. The main purpose of MoBa is to test specific aetiological hypotheses by estimating the association between exposures and diseases in mothers and children, aiming at identifying strategies for prevention. MoBa is a nation-wide pregnancy cohort that in the years from 1999 to 2008 included 108000 children, 90700 mothers and 71500 fathers. Women were recruited to the study through a postal invitation in connection with a routine ultrasound examination offered to all pregnant women in Norway during weeks 17–18 of gestation. There were no exclusion criteria. The participation rate was 38.5%. Pregnancy and birth records from the Medical Birth Registry of Norway (MBRN) are linked to the MoBa database [136]. Informed consent was obtained from each participant before the study. The Regional Committee for Medical Research Ethics in South-Eastern Norway approved the study.

The study is based on questionnaires administered to the mother and father, with biological samples being collected from mother, father and child. Questionnaires covered a wide range of information on exposures and health of parents and child. Exposure variables include genes, psychosocial factors, infections, use of medication, nutrition, life styles, occupational exposure, use of health services, substance abuse and socioeconomic factors as well as chemical and physical factors in the environment. Health variables include maternal and paternal history and health outcomes for the mother and child detected during and after pregnancy. Health outcomes are also collected from hospital discharge registries as well as other health registries such as the MBRN.

The work presented in this thesis uses the quality-assured MoBa data files released for research in 2010 (version 5). After excluding women with missing information needed for this thesis, the final study sample was 50651 women and child. Detailed information on the sample selection and dietary assessment can be found in papers III and IV.

4.3 Estimation of dietary PAH and acrylamide intakes

Dietary PAH and acrylamide intakes were estimated based on the combination of the data on food intake, which was available from FFQs, and approximate values of contaminant levels in each food item, which came from food composition tables developed using published values of these contaminants in foods.

a) Total PAHs and B(a)P

In order to construct a food composition table with estimated levels of PAH in food, a compilation of all available published data in scientific journals and reports on food concentrations of the sum of up to sixteen PAHs (benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, cyclopenta[c,d]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, 5-methylchrysene, 7H-benzo[c]fluorene) and B(a)P individually were undertaken, having taken into account processing and cooking methods associated with the formation of these compounds.

Exclusion criteria for the selection of eligible values to include in our food composition table included: (i) values published before 1990, shortly after regulatory frameworks influencing PAH emissions and food contamination were changed, (ii) values from possibly highly polluted settings (e.g.: foods locally produced in oil-producing countries such as Kuwait [137]), and (iii) values published for food groups instead of individual food items. In addition, several extreme outliers (e.g. three fold higher than all

other published data in foods with elevated concentrations) were also excluded, to avoid unduly influencing mean values (see Table 4). Additionally, for olive oil, values published before 2001 were excluded, since in July of that year the Spanish authorities approved an order laying down limits for certain PAHs in olive oil [138]. Also in 2001, the International Olive Oil Council recommended a maximum concentration of 2µg/kg of B(a)P and other PAHs in olive-pomace oil [139]. For this reason, levels of PAHs detected in olive oil after this period were substantially lower than before (mean of published values for B(a)P before and after 2001 were 0.254µg/100g and 0.036µg/100g, respectively). Similarly, the exclusion of values for PAH in foods from studies published before 1990 was applied because in the end of the 1980s' regulations were introduced that set maximum levels for PAHs emissions along with regulations requiring changes in food production methods in many developed countries, which contributed to a substantial reduction in the levels of PAHs in the environment and the food chain [3]. For example, in 1988 the Council of the European Communities set a maximum level of 0.03 µg/kg for B(a)P in foodstuffs as a result of the use of smoke flavourings. Similarly, in western Germany, the B(a)P emissions due mainly to residential heating were about 10 tons in 1981, 7 tons in 1985, and 2.5 tons in 1988, as a consequence of these policy shifts. Please see the appendix 1 of Paper I in the results section for the complete list of studies included in the PAH food composition table developed for this thesis.

When published values for specific foods were below detection limits, or not detectable, half the detection limit was assigned in the food composition table [140]. Average concentrations for the sum of PAHs (hereafter "total PAH") and B(a)P in each food were calculated using all available eligible data. When no data was available for a food item in the FFQ, concentrations of PAH were imputed from similar items (e.g.: values for skimmed milk were imputed from semi-skimmed milk). Daily PAH intake was estimated by multiplying food item concentrations of B(a)P and total PAH by intake in grams for each woman. For example, for women who report consuming a standard serving of egg (55 grams, one unit)

once per day, given the concentration of 0.004µg/100g (see Table 5), the mean daily intake of B(a)P from this food would be 0.002µg. Total dietary intake of these compounds was assessed by summing the intake from all food items.

Table 4. List of references for the excluded values.

Values published before 1990
1. Dennis MJ, Massey RC, McWeeny DJ, Knowles ME, Watson D. Analysis of polycyclic aromatic hydrocarbons in UK total diets. Food Chem Toxicol 1983; 21: 569-74.
2. Hischenhuber C, Stijve T. Determination of Benzo(a)pyrene in roasted coffe brews by HPLC with fluorescence detection. Deutsch Lebensmittel-Rundschau 1987; 83 (1): 1-4.
3. de Kruijf N, Schouten T, van der Stegen GHD. Rapid determination of benzo(a)pyrene in roasted coffe and coffe brew by high-performance liquid chromatography with fluorescente. J Agric Food Chem 1987; 35: 545-9.
4. Tuominen JP, Pisalo HS, Sauri M. Cereal products as a source of polycyclic aromatic hydrocarbons. J Agric Food Chem 1988;36(1):118-20.
5. Kolarovic L, Traitler H. Determination of polycyclic aromatic hydrocarbons in vegetable oils by caffeine and glass capillary gas chromatography. J Chromatogr 1982; 237:263-72.
6. Lawrence JF, Weber DF. Determination of polycyclic aromatic hydrocarbons in Canadian samples of processed vegetable and dairy products by liquid chromatography with fluorescence detection. J Agric Food Chem 1984;32(4):794-7.
7. Joe FL, Salemme JJr, Fazio T. Liquid chromatographic determination of trace residues of polynuclear aromatic hydrocarbons in smoked foods. J Assoc Off Anal Chem 1984;67(6): 1076-82.
Values from possibly highly polluted settings or extreme outliers
1. Kuwait: Husain A, Naeemi E, Dashti B, al Omirah H, al Zenki S. Polycyclic aromatic hydrocarbons in food products originating from locally reared animals in Kuwait. Food Addit Contam 1997;14:295-9.
2. Jánská M, Hajslová J, Tomaniová M, Kocourek V, Vávrová M. Polycyclic aromatic hydrocarbons in fruits and vegetables grown in the Czech republic. Bull. Environ. Contam. Toxicol 2006; 77:492-9.
3. Aygün SF, Kabadayi F. Determination of benzo[a]pyrene in charcoal grilled meat samples by HPLC with fluorescence detection. Int J Food Sci Nutr 2005; 56(8): 581-5.
4. Lin D, Tu Y, Zhu L. Concentrations and health risk of polycyclic aromatic hydrocarbons in tea. Food Chem Toxicol 2004; 43(1): 41-8

Table 5. Examples of the total PAH and B(a)P Food Composition Table.

CATEGORY	FOOD (items included)	Cooking Method	Preservation Method	PAH/B(a)P (µg/100g): Mean concentrations		Published data: concentrations		SOURCES OF B(a)P AND PAH DATA		
				B(a)P	PAH	B(a)P (µg/100g)	Total PAH (µg/100g)	YEAR	AUTHOR	COUNTRY
EGGS	**Eggs**			0.004	0.311					
	Egg hen	NA	NA			0.009	0.855	2008	Martí-Cid et al	ES
	Egg hen	NA	NA			0.002	0.242	2003	Falco et al	ES
	Egg (chicken)	NA	NA			0.002	0.059	1995	Ludovici et al	IT
	Eggs	NA	NA			0.002	0.086	2002	COT	UK
LEGUMES	**Legumes**			0.005	0.665					
	Haricot bean	NA	NA			0.003	1.130	2008	Martí-Cid et al	ES
	Lentil	NA	NA			0.003	0.590	2008	Martí-Cid et al	ES
	Pulses	NA	NA			0.006	0.274	2002	COT	UK
	Peas	NA	CA			0.009		2001	Kazeuroni et al	US
SEAFOOD: FATTY FISH	**Fatty fish**			0.010	0.577					
	Red mullet	NA	NA			0.008	0.310	2006	Llobet et al	ES
	Tuna	NA	NA			0.007	0.400	2006	Llobet et al	ES
	Anchovy	NA	NA			0.008	0.680	2006	Llobet et al	ES
	Salmon	NA	NA			0.011	0.600	2006	Llobet et al	ES
	Mackerel	NA	NA			0.012	0.940	2006	Llobet et al	ES
	Sardine	NA	NA			0.007	0.530	2006	Llobet et al	ES
	Herring	NA	FR			0.015		2005	Yurchenko et al	ET

NA: not available; CA:canned; FR:fresh; RA: raw; SM: smoked; NE: cooked; BA: barbecued; ra: rare; me: medium; wd: well done; vwd: very well done.

Table 5 (continued). Examples of the total PAH and B(a)P Food Composition Table.

CATEGORY	FOOD (items included)	Cooking Method	Preservation Method	PAH/B(a)P (µg/100g): Mean concentrations		Published data: concentrations		SOURCES OF B(a)P AND PAH DATA		
				B(a)P	PAH	B(a)P (µg/100g)	Total PAH (µg/100g)	YEAR	AUTHOR	COUNTRY
SEAFOOD:	**White fish**			0.008	0.209					
WHITE FISH	Sole	NA	NA			0.008	0.250	2006	Llobet et al	ES
	Hake	NA	NA			0.007	0.320	2006	Llobet et al	ES
	Codfish	NA	NA			0.0014	0.058	1995	Lodovici et al	IT
	Silver hake	NA	FR			0.015		2005	Yurchenko et al	ET
SEAFOOD:	**Crustaceans**			0.170	1.260					
CRUSTACEANS	Shrimp	NA	NA			0.044	1.590	2006	Llobet et al	ES
	Shrimp	RA	SM				0.930	1993	Gomaa et al	US
	Cockles	NA	FR			0.317		2002	COT	UK
	Scallops	NA	FR			0.148		2002	COT	UK
MEAT: STEAK	**Steak**			0.049	0.590					
(no cooking methods)	Veal steak	NA	NA			0.037	0.614	2008	Martí-Cid et al	ES
	Beef	NE	NA			0.061	0.566	1995	Lodovici et al	IT
MEAT: STEAK -	**Steak**	BA	NA	0.304	4.210					
Barbecued	Steak	BA	NA			0.00025		2006	CHARRED	US
	Steak	BA	NA			0.415		2006	CHARRED	US
	Steak	BA	NA			0.475		2006	CHARRED	US
	Steak	BA	NA			0.486		2006	CHARRED	US
	Beef	BA	NA			0.145	4.210	1995	Lodovici et al	IT

NA: not available; CA:canned; FR:fresh; RA: raw; SM: smoked; NE: cooked; BA: barbecued; ra: rare; me: medium; wd: well done; vwd: very well done.

b) Acrylamide

Details on how dietary acrylamide intake was calculated have been previously published [101]. Briefly, to calculate acrylamide intake a database was prepared containing values of acrylamide concentration reported from analyses of Norwegian food items [100,141–143] and the National Food Administration in Sweden [144]. However, when acrylamide values were not available from Norwegian or Swedish food samples, values from the European Union database were selected [145]. For food items with multiple analyses of acrylamide concentration the median concentration was used. Finally, daily acrylamide intake was estimated by multiplying food item concentrations of acrylamide by intake in grams for each woman. Total dietary intake of these compounds was assessed by summing the intake of all food items.

5 RESULTS

5 RESULTS

5.1 Paper I

Smoking during pregnancy is associated with higher dietary intake of polycyclic aromatic hydrocarbons and poor diet quality

Talita Duarte-Salles, Michelle A Mendez, Verónica Pessoa, Mònica Guxens, Inmaculada Aguilera, Manolis Kogevinas, Jordi Sunyer

Public Health Nutr 2010; 13(12): 2034-43.*

* This paper is reproduced according to the original print version. References of this paper are included in the references section of the thesis.

5.2 Paper II

Dietary benzo(a)pyrene and fetal growth: effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism

Talita Duarte-Salles, Michelle A Mendez, Eva Morales, Mariona Bustamante, Agueda Rodríguez-Vicente, Manolis Kogevinas, Jordi Sunyer

Environmental International 2012; 45C:1-8.*

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5.3 Paper III

Dietary benzo(a)pyrene and birth weight: associations modified by vitamin C intakes in The Norwegian Mother and Child Cohort study (MoBa)

Talita Duarte-Salles, Michelle A. Mendez , Helle Margrete Meltzer, Jan Alexander, Margaretha Haugen

Submitted to Environment International.*

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Dietary benzo(a)pyrene and birth weight: associations modified by vitamin C intakes in The Norwegian Mother and Child Cohort Study (MoBa)

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Running title: B(a)P intake during pregnancy and birth weight

Keywords: benzo(a)pyrene; diet; pregnancy; vitamin C; birth weight, MoBa

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Conflict of interest: The authors have no conflicts of interest to declare.

Abbreviations: FFQ, Food Frequency Questionnaire; MBRN, Medical Birth Registry of Norway; MoBa, the Norwegian Mother and Child Cohort Study; PAHs, polycyclic aromatic hydrocarbons; B(a)P, benzo(a)pyrene

ABSTRACT

Background: Maternal exposure to polycyclic aromatic hydrocarbons (PAH) during pregnancy has been associated with reduced fetal growth. However, the role of diet, the main source of PAH exposure among non-smokers, remains uncertain.
Objective: To assess associations between prenatal dietary exposure to the genotoxic PAH benzo(a)pyrene [B(a)P] and birth weight, exploring potential effect modification by maternal intakes of vitamin C, E and A, hypothesized to influence PAH metabolism.
Methods: This study included 50651 women in the Norwegian Mother and Child Cohort Study (MoBa). Dietary B(a)P and nutrient intakes were obtained from a food frequency questionnaire (FFQ) and estimated based on food composition data. Data on infant birth weight was obtained from the Medical Birth Registry of Norway (MBRN). Multivariable regression was used to assess associations between dietary B(a)P and birth weight, evaluating potential interactions with candidate nutrients.
Results: The multivariable-adjusted coefficient ± SE for birth weight associated with maternal B(a)P intake was -20.5±5.4g in women in the third compared with the first tertile of B(a)P intake. Results were similar after excluding smokers. Significant interactions were found between elevated intakes of vitamin C (above 85mg/day) and dietary B(a)P during pregnancy for birth weight ($P<0.05$), but no interactions were found with other vitamins. The multivariable-adjusted coefficients ± SE for birth weight in women in the third compared with the first tertile of B(a)P intake were -44.4 ± 16.4g in the group with low vitamin C intakes *vs.* -17.6 ± 5.8g in the high vitamin C intake group.
Conclusion: The results suggest that higher prenatal exposure to dietary B(a)P may reduce birth weight. Lowering maternal intake of B(a)P and increasing vitamin C intake during pregnancy may help to reduce any adverse effects of B(a)P on birth weight.

1. Introduction

Maternal diet during pregnancy is the main source of essential nutrients that are needed for optimal fetal and child development [1,114]. At the same time, however, it is also the main source of *in utero* exposure to contaminants, such as polycyclic aromatic hydrocarbons (PAH) [5–7], which have been demonstrated to be able to cross the placenta barrier, and may therefore compromise fetal development [76–78]. Benzo(a)pyrene [B(a)P] has been identified as human mutagen, carcinogen, and endocrine disruptor, and has, because it is the most potent one, been widely used as a marker of exposure to total carcinogenic PAH [5]. Oral exposure to B(a)P is known to induce developmental and reproductive toxicity in experimental studies in animals, including reduced fetal growth [2]. Food can be contaminated by environmental PAHs that are present in air, soil or water, by industrial food processing methods and by home food preparation [5]. Thus, PAHs are found widespread throughout the diet.

Previous epidemiological studies suggest prenatal exposure to airborne PAH (or levels of bulky DNA adducts; a marker of overall PAH exposure); may be associated with adverse reproductive or child health outcomes, including reduced fetal growth and lower scores on childhood tests of neurodevelopment [65–68]. Recently, we reported an association between dietary B(a)P intake during pregnancy and lower birth weight in a Spanish birth cohort study [160,182]. Although diet is the main source of PAH exposure among individuals not exposed to high levels of tobacco smoke or with occupational exposures [5–7,47–50], evidence on whether the dietary route of exposure may relate to birth outcomes is still limited.

Antioxidant nutrients such as vitamin C, E, and A, and carotenes, have been suggested to reduce DNA damage related to PAH exposure and could therefore help to protect against adverse health outcomes related to exposure to such contaminants, e.g. by inducing the activity of detoxifying enzymes such as the glutathione S-transferase (GSTs) [123–128]. Moreover, maternal

intake of antioxidant nutrients during pregnancy may also modify the formation of DNA adducts in cord blood [124]. To our knowledge only one previous study have examined whether antioxidant intakes modify associations between prenatal exposure to dietary PAH and fetal growth indicators [182]. That study was settled in a Spanish population in which the consumption of fruit and vegetables was above the recommendations for pregnant women and higher than the consumption previously reported in Nordic countries [183]. Additionally, different patterns of nutrient intakes have been previously reported between Mediterranean and Nordic countries [184].

The present study aimed to assess associations between maternal dietary intakes of B(a)P, as an indicator of PAH exposure, during pregnancy and birth weight in a large population-based birth cohort within The Norwegian Mother and Child Cohort Study (MoBa), focusing on whether associations varied depending on levels of maternal antioxidant vitamin intakes. It also aimed to describe population characteristics as well as dietary aspects associated with higher intakes of B(a)P.

2. Methods

2.1. Population and study design

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health [135]. Participants were recruited from all over Norway from 1999-2008, and 38.5% of invited women consented to participate. The cohort now includes 108000 children, 90700 mothers and 71500 fathers. Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth. Follow-up is conducted by questionnaires at regular intervals and by linkage to national health registries. Several sub-studies are conducting additional collections of data and biological materials. The current study is based on version 5 of the quality-assured data files

released for research on 2010. Informed consent was obtained from each MoBa participant upon recruitment. The study was approved by The Regional Committee for Medical Research Ethics in South-Eastern Norway.

When preparing the dataset, 62124 women had answered questionnaires 1 and 3 (in pregnancy week 17 and 30, respectively), the baseline MoBa questionnaires covering information on sociodemographic characteristics, exposure to tobacco smoke during pregnancy and general health, questionnaire 2 (in pregnancy weeks 23-24), which covered dietary information, and questionnaire 4 (when the child was 6 months of age), which includes information on maternal health at time of delivery, including gestational weight gain during pregnancy. In addition they were recorded in the Medical Birth Registry of Norway (MBRN) and had singleton births. We excluded multiple participation in MoBa only using the first participating child (n=6604), pregnancy duration <28 weeks or >42 weeks (n=385), missing data on birth weight (n=22), had an energy intake < 4500 kJ or > 20000 kJ (n=796) and no information about smoking during pregnancy (n=817). Lastly, we excluded women with improbable (<-30 or >50 kg) (n=463) or missing (n=2386) gestational weight gain, leaving a study sample of 50651 women. Since smoking is a known lifestyle factor associated with reduction of birth weight and a significant source of B(a)P exposure, additional analyses were done (n=46420) excluding women who reported any smoking during pregnancy.

2.2. Dietary assessment and B(a)P intakes

The MoBa FFQ (downloadable at http://www.fhi.no/dokumenter/011fbd699d.pdf) was used for calculation of B(a)P and nutrient intake. This FFQ is a semi-quantitative questionnaire designed to provide information on dietary habits and intake of dietary supplements during the first four to five months of pregnancy [185]. It has been answered by participating women in MoBa since February 2002, and has been thoroughly validated with regard to foods and nutrients [186]. For

each of the 255 food and beverage items, the frequency of consumption was reported by selecting one out of 8-10 frequencies, ranging from never to several times monthly, weekly or daily. The FFQ was read optically and energy intake was calculated using FoodCalc [187] and the Norwegian Food Composition table [188]. Plausibility of energy intake was identified using published equations to calculate total estimated energy requirements among pregnant women [189,190]; women reporting intakes below or above 2 SDs of the requirements were defined as under-reporters and over-reporters, respectively.

To calculate B(a)P intake a database was prepared containing values of B(a)P concentration for each food item in the questionnaire. First, a compilation of all available data on food concentrations of B(a)P was undertaken to construct a food composition table. Exclusion criteria for the selection of eligible values applicable to this study includes: (i) values published before 1990, (ii) values from potentially highly polluted settings (e.g.: foods locally produced in Kuwait) [137], (iii) extreme outliers, and (iv) values for heterogeneous food groups instead of food items. Examples of the values assigned for each food item have previously been published [160]. When a published value was below the limit of detection or not detectable, the value assigned was the half of the detection limit. Average concentrations for B(a)P in each food were calculated using all available data for that food item. When no data was available for a food item that was asked at the FFQ, concentrations of B(a)P were imputed from food items with similar characteristics (e.g.: values for skimmed milk were imputed from semi-skimmed milk). Finally, daily intake of B(a)P was estimated by multiplying food item concentrations B(a)P by intake in grams for each woman. Total dietary intake of B(a)P was assessed by summing intakes for all food items and expressed as nanograms (ng) per day. In order to identify the main food contributors to B(a)P intake, the 255 food items in the FFQ were grouped into food groups based on nutrient profiles, culinary usage or known B(a)P levels.

2.3. Birth outcomes and other variables

Birth weight was measured by the midwife who attended the birth and reported to the MBRN [136]. Gestational age was calculated from date of delivery on the basis of first trimester ultrasound. In the event of missing an ultrasound measure of gestational age this was calculated from last menstrual period. Preterm birth was defined as born before week 37. Parity was based on data from both MoBa and MBRN and categorized as primiparous or multiparous. Data on maternal education attainment (≤12, 13-16 and 17+ years), maternal age and smoking were collected from questionnaires. Smoking during pregnancy was categorized as non-smokers, occasional smokers and daily smokers. Participants with unknown/missing values for education or father's smoking were grouped in a "missing" category; results were not meaningfully different when these subjects were excluded from the analysis sample in a complete case analysis (not shown). Pre-pregnant weight and height were self-reported at week 17 in pregnancy and pre-pregnant body mass index (BMI) was calculated in kg/m^2. Pre-pregnant BMI was categorized according to the WHO classification as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2). Sex of the child and weight of the mother at time of delivery (kg) were collected from questionnaire 4. Gestational weight gain (kg) was calculated from weight reported at the start of pregnancy and at time of delivery registered at the birth clinic on the women's health card.

2.4. Statistical analyses

Dietary B(a)P intakes were adjusted for total energy intake and tertiles were created using these energy-adjusted estimates. The intake of B(a)P (ng/day), energy-adjusted B(a)P (ng/kcal/day), food groups, and vitamin C, E and A, were estimated and expressed as means and standard deviations. Data on B(a)P and food group intakes, newborn and maternal characteristics were expressed over tertiles of energy-adjusted B(a)P intakes. Statistical significance was evaluated using $\chi 2$ or Kruskal-Wallis tests.

To identify the main characteristics related to high intake of B(a)P, multiple linear regression models were used. Several potential covariates that were not significantly related to dietary intakes of B(a)P (marital status, alcohol intake, income, education and smoking of the partner) and were excluded from final models for parsimony. Additionally, adjusting for these variables had no meaningful impact (changes in measures of association were <10%; not shown). In separate models, associations between food group and B(a)P intake were estimated in order to determine which food groups were most predictive of high dietary intake of this compound among pregnant women.

Multiple linear regression models were used to examine the relationship between dietary B(a)P intakes during pregnancy and birth weight. Models were adjusted appropriately for confounders or modifiers, which were assessed from a wide array of variables available in the Norwegian study (e.g. gestational age, parity, parental income, type of delivery, tobacco use and second-hand exposure). Modification of associations with B(a)P by maternal intakes of candidate nutrients during pregnancy, such as vitamin C, E and A, as well as fruit and vegetable intakes, were tested by including interaction terms in the regression models. Interactions with vitamin C below or above 85mg/day were significant using B(a)P either continuously or in tertiles (interaction $P<0.05$). No significant interactions were found between dietary B(a)P and vitamin E, vitamin A, or fruit and vegetable intakes ($P>0.10$). Models were further stratified by vitamin C intakes.

Results are reported as coefficients with standard errors (SE) for tertiles of B(a)P intakes. Covariates in final models were gestational age, sex of the child, age of the mother, parity, smoking during pregnancy, pre-pregnancy BMI, maternal weight gain, plausibility of energy intake, and vitamin C intake. Other covariates (parental education, income, father's weight and height, marital status, type of delivery and exposure to passive smoking) were tested as potential confounders but were excluded from final models for parsimony, as they did not affect our estimates (change-in-estimate <10%). To test for possible confounding by other aspects

of the diet, we confirmed that results were comparable after adjusting for maternal intakes of food groups such as fruits and vegetables, snacks, sweets, shellfish and processed meat (the last two being main sources of B(a)P dietary intakes), as well as intakes of other micronutrients hypothesized to protect against the formation of PAH-adducts, namely vitamin E, vitamin A and beta-carotene [124]. Associations were also similar after excluding preterm births (<37 weeks of gestation), low birth weight children (birth weight <2500g), or those women with missing information for education or father smoking during pregnancy (results not shown). No meaningful changes were observed in the results when using the residual approach to adjust for energy intake. Data were analyzed using STATA 10.1 (Stata Corporation, College Station, Texas).

3. Results

Overall (n=50651), the mean ± SD birth weight was 3600.6 ± 539.9 g (Table 1). Statistically significant differences in birth weight across tertiles of dietary B(a)P intake were observed. The mean birth weight was significantly lower in those in the highest vs. the lowest tertile of B(a)P intake (3586.6 vs. 3607.2 g, respectively, p-value <0.001). Maternal characteristics, including age, parity, gestational weight gain, pre-pregnancy BMI, smoking, and education, were significantly related with B(a)P intake during pregnancy (Table 1).

Table 2 shows the intake of B(a)P, food groups and vitamins by tertiles of dietary B(a)P intake. The mean ± SD of B(a)P intake among all women was 148.9 ± 47.9 ng/day. Women in the highest tertile of B(a)P intake had higher intake of shellfish, fish spreads, snacks, fruits, vegetables, other meats (not processed/cured), and other cereals (not bread or potatoes). The intake of vitamin C, E and A differed significantly across dietary B(a)P intake. Women in the third tertile of B(a)P intake had higher intake of vitamin C (215.7 ± 103.6 mg/day) and lower intake of vitamin E and A (10.5 ± 3.9 and 727.5 ± 541.4 mg/day, respectively), compared with

women in the first tertile (116.9 ± 56.7, 10.7 ± 4.3, and 978.5 ± 738.2 mg/day, respectively).

Figure 1 shows the main food groups contributing to total B(a)P intake, which were milk and yogurt (14%), cereals (13%), fruits (12%), sweets (10%), and total meat (10%). However, regression model adjusted for all food group intakes showed that the strongest predictors of higher B(a)P intakes were shellfish and processed/cured meat; results not shown.

After multiple adjustment, several maternal characteristics were significantly related with B(a)P intake during pregnancy (Table 3). Lower age of the mother, multiparity, lower educational level, any smoking during pregnancy, pre-pregnancy BMI <18.5, and higher gestational weight gain were significantly associated with higher dietary intake of B(a)P.

Table 4 presents the crude and adjusted associations between B(a)P intake during pregnancy and birth weight among all women and non-smokers. Tertiles of energy-adjusted B(a)P intake during pregnancy were associated with significant reductions in birth weight. Among all women and among non-smokers only, multivariable-adjusted coefficients±SE for birth weight were -20.5 ± 5.4g and -21.2 ± 5.6g, respectively, in women in the highest tertile compared with women in the first tertile of B(a)P intake.

Significant interactions between dietary B(a)P and vitamin C above the recommended intake for pregnant women in Norway (85mg/day) were found (P for interaction 0.022) (Table 4). After adjustment for potential confounders, coefficients ± SE for birth weight in women in the third tertile compared with women in the first tertile of B(a)P intake were -44.4 ± 16.4g in the group with low vitamin C intakes *vs.* -17.6 ± 5.8g in the high vitamin C intake group. After excluding smokers during pregnancy, the P for interaction between vitamin C intake (above or below 85mg/day) and B(a)P intake used was 0.049. Results stratified by vitamin C intake were similar compared with all women; multivariable-adjusted coefficients ± SE for birth weight in women in the highest

tertile of B(a)P intake were -40.9 ± 17.4g and -19.0 ± 6.1g in women with low and high vitamin C intake, respectively.

4. Discussion

In this pregnancy cohort study conducted in Norway, higher maternal dietary B(a)P intake during pregnancy was associated with a significant reduction in birth weight, with similar results after excluding women who smoked during pregnancy. There were significant interactions between dietary B(a)P and vitamin C intakes for birth weight. The association of dietary B(a)P with birth weight was stronger among women with low vitamin C intake. The main food groups contributing to total B(a)P intake were milk and yogurt, cereals, fruits, sweets, and meat; while the food groups that most strongly predicted high intakes of B(a)P were shellfish and processed/cured meat. Maternal age, parity, education, smoking, and gestational weight gain, were found to be related to the intake of dietary B(a)P during pregnancy.

Our results are in agreement with findings from a recent, but smaller study (n = 586), examining the associations between prenatal exposure to dietary B(a)P and fetal growth indicators in the Spanish cohort study Environment and Childhood (INfancia y Medio Ambiente – INMA) [182]. Maternal B(a)P intakes during pregnancy; also estimated based on a FFQ; was found to be significantly associated with reduced birth weight and length, and increased risk of small for gestational age (SGA). Earlier studies have reported significant negative associations between maternal PAH exposure, which was estimated based on levels of bulky DNA adducts; a biomarker of PAH exposure from all sources [70]; or personal measures of atmospheric PAH exposure, with birth weight, birth length and SGA in populations from the United States [65,66,71,173], Poland [64,65] and the Czech Republic [69].

Although diet is recognized as the main source of PAH exposure for non-occupationally exposed individuals and non-smokers [5–7,49], most studies exploring the role of PAH in fetal growth have not specifically examined the role of exposure through diet to these

compounds. Besides the Spanish study already mentioned above, only two prior epidemiologic studies have examined the role of prenatal exposure to B(a)P specifically from diet on fetal growth [71,72]. However, these studies used the consumption of a limited number of food items (smoked, grilled or barbequed meat intakes) as indicators of total dietary exposure to PAH and reported very weak inverse associations with indicators of size at birth. In the present study, we found no association between the frequency of grilled meat consumption and birth weight. The multivariable-adjusted coefficients ± SE for birth weight in women who reported consuming grilled meat once or more times per week (23%) was -5.8 ± 4.4g, p-value=0.191. PAHs are widespread throughout all food groups, however, shellfish and cereals have been identified as especially important sources and determinants of B(a)P intakes [62,160]. A strength of our study is that we took into account all sources of dietary B(a)P intake to adequately estimate the exposure. We found that meat contributed only 8.6% for the total dietary B(a)P intake.

The average B(a)P intake estimated in this study was 0.15 μg/day, which is comparable to the intake previously reported among pregnant women in Spain (0.19 μg/day) [160], and is in the range of average intake that was estimated according to a survey conducted in adult population from sixteen European countries (0.18 to 0.25 μg/day) including Norway [3]. In that survey, the average of estimated B(a)P intake among adult population in Norway was 0.25 μg/day, which was higher than the estimated intake in our pregnancy cohort. Such differences could be explained by disparities in dietary habits by the populations studied (all adults *vs.* pregnant women), but may also be due to differences in methods used to assess dietary intakes or estimates of PAH concentrations.

The food groups milk and yogurt, cereals, fruits, sweets, and meat contributed to almost 60% to the total B(a)P intake. On the other hand, however, the food groups that most strongly predicted high intakes of B(a)P were shellfish and processed/cured meat, which is in agreement with the results previously reported in a population

of pregnant women in Spain [160]. Thus substantial reductions in B(a)P intakes could be achieved by reducing processed meats and shellfish intakes during pregnancy. In the Spanish study, milk and yogurt, cereals, fruits, and meat were also important contributors to the total B(a)P intake, in addition to shellfish and vegetables. Cereals have been reported as the main contributor of dietary B(a)P intake in several studies due to its high consumption [2,59].

We found that occasional or daily smoking during pregnancy was associated with higher dietary B(a)P intake in our population after multivariate adjustment for other women characteristics. These results are in agreement with what was previously reported in a population of Spanish pregnant women [160], in which the frequency of women who reported smoking during pregnancy was higher than in our population (16.5% *vs.* 8.3%, respectively). The main source of PAH exposure among active smokers is accepted to be tobacco smoke. However, our results also indicate that diet is a significant source of the PAH B(a)P among both smokers and non-smokers. The concentration of B(a)P in one cigarette has been estimated to be in the range of 8.5-11.6 ng/day [191]. The reported mean use of cigarettes in our population was of 5.7 cigarettes per day. Consequently, we estimate that the smoking-related B(a)P exposure may be approximately 48.5-66.1 ng/d, and the contribution of dietary B(a)P (mean 148.9 ng/day) on the order of 69-75% of the total exposure among smokers. Additionally, levels of B(a)P-DNA adducts have been associated with dietary PAH in both smokers and non-smokers [70], and dietary intake more strongly correlated than ambient air with urinary metabolites of PAH exposure in earlier studies [7].

Previous studies have shown that the formation of bulky DNA adducts related to PAH exposure can be reduced by antioxidant nutrients, including vitamin C [124–126,128]. In this population, we found that the association between maternal B(a)P intake and birth weight was stronger among those women who had a vitamin C intake below 85 mg/day, which is the recommended intake for pregnant women in Norway [192]. These results are consistent with what we have recently reported in a Spanish cohort study; a

significant interaction was found between maternal B(a)P intake and vitamin C intake below or above the mean intake of 189 mg/day in the association between B(a)P intake and birth weight [182]. The reason for the different cut-point for vitamin C on the interaction with B(a)P intake might be explained by the higher estimated mean of B(a)P intake in the Spanish cohort (27%) compared to this Norwegian population. Consequently, also higher vitamin C would be needed in the Spanish study to counter the effects of B(a)P in birth weight. In addition, it has previously been reported that only a small percentage of subjects from the Spanish population have intakes of vitamin C below the recommendations [193].

Other micronutrients with antioxidant properties, including vitamin E and A, have also been hypothesized to protect against the formation of PAH-related DNA adducts [124,128]. Sram and colleagues [128] found inverse associations between the levels of vitamin C, A and E in plasma and bulky DNA adducts. In our study we did not observe significant interactions with vitamin E or vitamin A intakes in associations between dietary B(a)P and birth weight. However, the associations between B(a)P intake and birth weight varied across intakes of these nutrients. For example, we found that multivariable-adjusted coefficients ± SE for birth weight in women in the third tertile compared with women in the first tertile of B(a)P intake were -26.2 ± 7.4g and -21.3 ± 6.9g in the group of women with low vitamin E (≤ the mean of 10mg/day) and vitamin A (≤ the mean of 800mg/day), respectively; and -12.3 ± 8.0g and -11.3 ± 8.7g in the high intake groups.

Laboratory experiments support the existence of a negative effect of prenatal exposure to B(a)P on fetal growth [5]. It has been shown that PAHs are capable of crossing the placental barrier [76–78]. However, the mechanisms through which these compounds may influence fetal growth are not fully known. A number of mechanisms linking PAHs to fetal growth have been postulated. For example, PAHs bind to receptors regulating the induction of P450 enzymes, which may decrease the uptake of oxygen and nutrients; similar consequences may also be related to binding of

these chemicals to receptors related to insulin and growth factor metabolism [81]. In *in vitro* studies, B(a)P exposure has been shown to affect early trophoblast proliferation due to the interaction with growth factor receptors [69,82].

Strengths of this study include the large sample size of this population-based mother and child cohort study (n = 50651), and the collection of detailed information on diet during pregnancy [185], which allowed a comprehensive assessment of dietary B(a)P intakes and the exploration of synergies with dietary antioxidants. This study also includes information on smoking habits and other life-style factors of parents, allowing identification of subgroups of women with higher dietary B(a)P intakes. In the analyses of association between B(a)P intake and birth weight, we were able to adjust for confounding from a wide array of socioeconomic and lifestyle factors. However, it is possible that uncontrolled confounding still remains since there are other factors that we were not able to consider, such as the exposure to atmospheric B(a)P or data on genetic polymorphisms involved in detoxification capacity of PAHs. Although the FFQ used to estimate dietary information was previously validated in the MoBa study, possible measurement error of B(a)P intake remains a limitation of this study. B(a)P formation in food is known to be affected by several parameters, such as cooking methods used or doneness levels of food, that were not included in the FFQ. The crude estimation of B(a)P intake using FFQs would in principle lead to a weaker association between the exposure and the outcome than the true association.

5. Conclusion

This study provides evidence that prenatal exposure to B(a)P from dietary sources is associated with reduced birth weight in a large population-based cohort study conducted in Norway, also after excluding smokers during pregnancy. Higher maternal vitamin C intake in pregnancy seems to play a beneficial role against adverse effects of prenatal exposure to dietary B(a)P on birth weight.

Table 1 Study population characteristics across tertiles of B(a)P intake during pregnancy in the MoBa cohort.

	All (n=50651)	**B(a)P-Tertile 1** (n=16884)	**B(a)P-Tertile 2** (n=16884)	**B(a)P-Tertile 3** (n=16883)	**p-value***
Newborn characteristics					
Birth weight (g), mean ± SD	3600.6 ± 539.9	3607.2 ± 544.3	3607.9 ± 530.3	3586.6 ± 542.3	<0.001
Sex (male), n (%)	25906 (51.1)	8709 (51.6)	8580 (50.8)	8617 (51.0)	0.352
Gestational age (weeks), mean ± SD	39.53 ± 1.68	39.52 ± 1.69	39.55 ± 1.67	39.51 ± 1.69	0.088
Maternal characteristics					
Mother's age (years), mean ± SD	30.1 ± 4.5	29.8 ± 4.5	30.2 ± 4.4	30.3 ± 4.5	<0.001
Nulliparous, n (%)	26320 (51.9)	8396 (49.7)	86871 (51.4)	9246 (54.8)	<0.001
Weight gain during pregnancy, mean ± SD	14.9 ± 6.0	15.1 ± 6.0	14.9 ± 6.0	14.7 ± 5.9	<0.001
Pre-pregnancy BMI (kg/m2), n (%)					<0.001
18.5-25	33405 (65.9)	10872 (64.4)	11174 (66.2)	11359 (67.3)	
< 18.5	1437 (2.8)	467 (2.8)	486 (2.9)	484 (2.9)	
25-30	11144 (22.0)	3826 (22.7)	3702 (21.9)	3616 (21.4)	
> 30	4665 (9.2)	1719 (10.2)	1522 (9.0)	1424 (8.4)	
Smoking during pregnancy, n (%)					<0.001
Non-smokers	46420 (91.6)	15166 (89.8)	15592 (92.3)	15662 (92.8)	
Occasionally	2261 (4.5)	833 (4.9)	697 (4.1)	731 (4.3)	
Daily	1970 (3.9)	885 (5.2)	596 (3.5)	490 (2.9)	

B(a)P, benzo(a)pyrene; SD, Standard Deviation; BMI, body mass index.

* p-value from χ^2 or Kruskal-Wallis tests.

Table 1 (continued) Study population characteristics across tertiles of B(a)P intake during pregnancy in the MoBa cohort.

	All (n=50651)	**B(a)P-Tertile 1** (n=16884)	**B(a)P-Tertile 2** (n=16884)	**B(a)P-Tertile 3** (n=16883)	**p-value***
Maternal education (years), n (%)					<0.001
<=12	15243 (30.1)	5705 (33.8)	4754 (28.2)	4784 (28.3)	
13-16	21847 (43.1)	7328 (43.4)	7484 (44.3)	7035 (41.7)	
17 +	12539 (24.8)	3515 (20.8)	4331 (25.6)	4693 (27.8)	
Missing/other	1022 (2.0)	336 (2.0)	315 (1.9)	371 (2.2)	
Plausibility of energy intake, n (%)					<0.001
Under-reporters	7709 (15.2)	1983 (11.7)	2448 (14.5)	3278 (19.4)	
Plausible reporters	38074 (75.2)	13076 (77.4)	12937 (76.6)	12061 (71.4)	
Over-reporters	4868 (9.6)	1825 (10.8)	1499 (8.9)	1544 (9.1)	

B(a)P, benzo(a)pyrene; SD, Standard Deviation; BMI, body mass index.
* p-value from χ^2 or Kruskal-Wallis tests.

Table 2 Mean estimated intake of B(a)P, and key food groups and nutrients by tertiles of B(a)P intake among pregnant women from the MoBa cohort

Dietary intakes [1], mean ± SD	**All** (n=50651)	**B(a)P-Tertile 1** (n=16884)	**B(a)P-Tertile 2** (n=16884)	**B(a)P-Tertile 3** (n=16883)	**p-value***
B(a)P					
B(a)P (ng/day)	148.9 ± 47.9	124.8 ± 32.7	146.8 ± 37.8	175.3 ± 53.0	<0.001
B(a)P adjusted for energy intake (ng/kcal/day)	0.065 ± 0.012	0.053 ± 0.005	0.064 ± 0.003	0.078 ± 0.009	<0.001
Food groups, (g/day)					
Seafood					
Shellfish	3.6 ± 5.3	2.9 ± 4.3	3.7 ± 4.9	4.3 ± 6.3	<0.001
Fish spreads	8.7 ± 13.4	7.6 ± 11.9	8.9 ± 13.2	9.6 ± 14.9	<0.001
Fresh fish	32.3 ± 19.8	32.5 ± 20.8	32.6 ± 19.5	31.9 ± 19.3	0.009
Meat					
Processed/cured meat	18.4 ± 10.5	18.5 ± 10.3	18.5 ± 10.2	18.2 ± 11.0	<0.001
Other	36.1 ± 15.9	35.0 ± 16.0	36.3 ± 15.5	37.1 ± 16.2	<0.001
Dairy products					
Milk and yogurt	435.9 ± 357.0	481.3 ± 380.2	419.5 ± 333.6	406.9 ± 351.3	<0.001
Cheese	23.2 ± 19.5	26.5 ± 21.9	23.3 ± 18.6	19.7 ± 17.1	<0.001

B(a)P, benzo(a)pyrene; SD, Standard Deviation.

* p-value from χ^2 or Kruskal-Wallis tests.

[1] Measured by Food Frequency Questionnaire.

Table 2 (continued) Mean estimated intake of B(a)P, and key food groups and nutrients by tertiles of B(a)P intake among pregnant women from the MoBa cohort

Dietary intakes [1], mean ± SD	**All** (n=50651)	**B(a)P-Tertile 1** (n=16884)	**B(a)P-Tertile 2** (n=16884)	**B(a)P-Tertile 3** (n=16883)	**p-value***
Cereals					
Potatoes	52.2 ± 32.1	52.1 ± 31.5	52.7 ± 31.6	51.8 ± 33.1	<0.001
Bread	216.3 ± 99.8	262.5 ± 103.8	214.6 ± 85.3	171.7 ± 87.6	<0.001
Other	95.5 ± 54.9	88.1 ± 45.2	96.7 ± 54.3	101.8 ± 63.1	<0.001
Snacks	14.1 ± 12.7	13.5 ± 12.3	14.6 ± 12.2	14.2 ± 13.5	<0.001
Vegetables	144.3 ± 90.8	114.5 ± 69.3	146.2 ± 83.8	172.3 ± 105.9	<0.001
Fruits	274.5 ± 196.3	209.9 ± 149.4	275.0 ± 179.3	338.7 ± 230.0	<0.001
Sweets	101.9 ± 56.8	104.3 ± 60.6	105.1 ± 57.0	96.6 ± 51.9	<0.001
Fats and oils	17.8 ± 15.5	19.2 ± 17.9	17.7 ± 14.6	16.5 ± 13.4	<0.001
Vitamin, (mg/day)					
Vitamin C	164.4 ± 89.3	116.9 ± 56.7	160.8 ± 71.4	215.7 ± 103.6	<0.001
Vitamin E	10.6 ± 4.0	10.7 ± 4.3	10.7 ± 3.9	10.5 ± 3.9	<0.001
Vitamin A	850.5 ± 639.1	978.5 ± 738.2	845.4 ± 596.6	727.5 ± 541.4	<0.001

B(a)P, benzo(a)pyrene; SD, Standard Deviation.
* p-value from χ^2 or Kruskal-Wallis tests.
[1] Measured by Food Frequency Questionnaire.

Table 3 Population characteristics associated with dietary B(a)P intake during pregnancy.

	All (n=50651)		
	β	SE	p-value
Mother's age (years)	0.04	0.01	0.001
Parity			
Nulliparous	Ref.		
Multiparous	-0.73	0.09	<0.001
Pre-pregnancy BMI (kg/m2)			
18.5-25	Ref.		
< 18.5	0.66	0.26	0.010
25-30	-0.06	0.10	0.533
> 30	-0.30	0.15	0.049
Maternal education (years)			
<=12	Ref.		
13-16	-0.54	0.10	<0.001
17 +	-0.23	0.12	0.063
Missing/other	0.41	0.31	0.181
Smoking 3rd trimester pregnancy			
Non-smokers	Ref.		
Occasionally	1.04	0.21	<0.001
Daily	0.65	0.22	0.003
Season of birth			
March-May	Ref.		
June-August	0.60	0.12	<0.001
September-November	0.95	0.12	<0.001
December-February	0.66	0.12	<0.001
Gestational weight gain (kg)	-0.02	0.01	0.008

B(a)P, benzo(a)pyrene; SE, standard error

Results from multivariate linear regression model predicting B(a)P adjusted simultaneously for total energy intake, vitamin C intake, and all variables showed in the table.

* B(a)P intake converted to picograms/kcal/day.

Table 4 Associations between birth weight and dietary B(a)P intakes during pregnancy*

	Crude			Adjusted		
	β	SE	p-value	β	SE	p-value
ALL WOMEN, (n=50651)						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	0.7	5.9	0.907	-9.1	4.8	0.056
B(a)P - Tertile 3	-20.6	5.9	<0.001	-20.5	5.4	<0.001
Vitamin C <85mg/day [1]						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	1.0	14.7	0.946	-14.2	11.7	0.225
B(a)P - Tertile 3	-69.8	20.5	0.001	-44.4	16.4	0.007
Vitamin C >=85mg/day						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	-3.1	6.6	0.640	-7.2	5.3	0.171
B(a)P - Tertile 3	-22.6	6.6	0.001	-17.6	5.8	0.003

*Multivariable models adjusted for gestational age, sex of the child, age of the mother, parity, pre-pregnancy BMI, maternal weight gain, smoking during pregnancy, plausibility of energy intake and vitamin C intake.

[1] p for interaction between dietary B(a)P and vitamin C above the recommended intake for pregnant women in Norway (85mg/day) in adjusted models: 3rd tertile of energy-adjusted B(a)P intake=0.022.

[2] p for interaction between dietary B(a)P and vitamin C above the recommended intake for pregnant women in Norway (85mg/day) in adjusted models: 3rd tertile of energy-adjusted B(a)P intake=0.049.

Table 4 (continued) Associations between birth weight and dietary B(a)P intakes during pregnancy*

	Crude			Adjusted		
	β	SE	p-value	β	SE	p-value
NON-SMOKERS, (n=46420)						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	-6.9	6.1	0.325	-11.2	5.0	0.025
B(a)P - Tertile 3	-28.2	6.1	<0.001	-21.2	5.6	<0.001
Vitamin C <85mg/day [2]						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	-7.8	15.6	0.617	-16.6	12.3	0.177
B(a)P - Tertile 3	-74.4	21.8	0.001	-40.9	17.4	0.019
Vitamin C >=85mg/day						
B(a)P - Tertile 1	Ref.			Ref.		
B(a)P - Tertile 2	-7.9	6.9	0.253	-9.4	5.5	0.087
B(a)P - Tertile 3	-28.2	6.8	<0.001	-19.0	6.1	0.002

*Multivariable models adjusted for gestational age, sex of the child, age of the mother, parity, pre-pregnancy BMI, maternal weight gain, smoking during pregnancy, plausibility of energy intake and vitamin C intake.

[1] p for interaction between dietary B(a)P and vitamin C above the recommended intake for pregnant women in Norway (85mg/day) in adjusted models: 3rd tertile of energy-adjusted B(a)P intake=0.022.

[2] p for interaction between dietary B(a)P and vitamin C above the recommended intake for pregnant women in Norway (85mg/day) in adjusted models: 3rd tertile of energy-adjusted B(a)P intake=0.049.

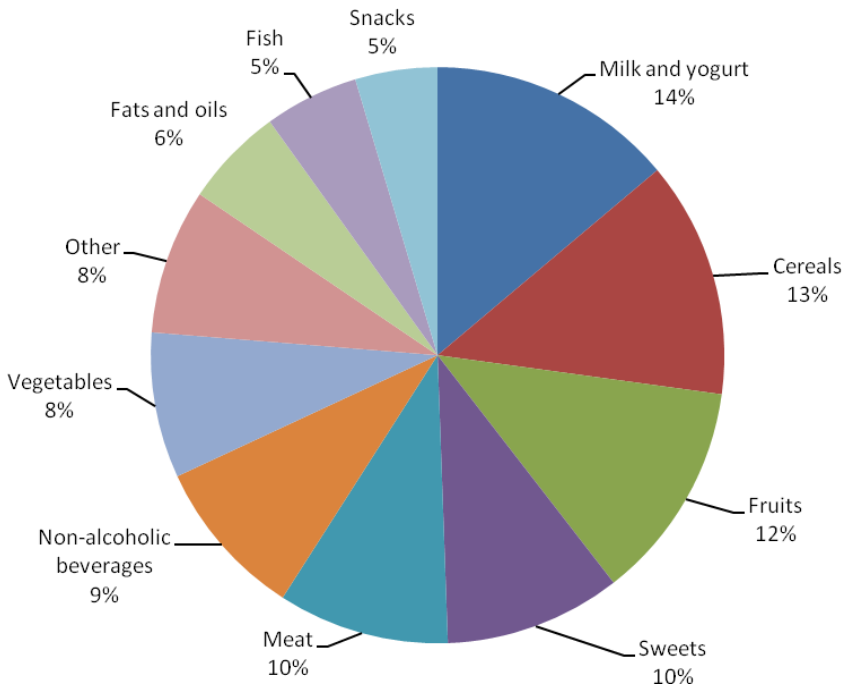

Figure 1 Contribution (%) of the most important food groups to dietary B(a)P intake in pregnant Norwegian women at the MoBa cohort study

5.4 Paper IV

Impact of acrylamide intake on fetal growth – results from the Norwegian Mother and Child Cohort study (MoBa)

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Impact of acrylamide intake on fetal growth – results from the Norwegian Mother and Child Cohort study (MoBa)

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Keywords: acrylamide; diet; small size for gestational age; birth weight; Hb adducts; MoBa, pregnancy

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Conflict of interest: H.S., P.R. and M.T. are shareholders in Adduct Analys AB, which

owns the patent for the applied analytical method for Hb adduct measurements.

Abbreviations: FFQ, Food Frequency Questionnaire; FIRE, Fluoresceine isothiocyanate *R* Edman; Hb, Hemoglobin; MBRN,

Medical Birth Registry of Norway; MoBa, the Norwegian Mother and Child Cohort Study; SGA, small for gestational age

ABSTRACT

Background: Acrylamide has shown developmental and reproductive toxicity in animals, as well as neurotoxic effects in humans. Since it is widespread in food and has been shown to pass the human placenta, concerns have been raised about potential developmental effects in humans.

Objectives: To assess associations between the risk of small for gestational age (SGA) and birth weight, with prenatal exposure to dietary acrylamide.

Methods: This study included 50651 women in the Norwegian Mother and Child Cohort Study (MoBa). Acrylamide exposure assessment was based on intake estimates obtained from a food frequency questionnaire (FFQ) which was validated with hemoglobin (Hb) adduct measurements reflecting acrylamide exposure in a subset of samples (n = 79). Data on infant birth weight and gestational age were obtained from the Medical Birth Registry. Multivariable regression was used to assess associations between prenatal acrylamide and birth outcomes.

Results: Acrylamide intake during pregnancy was associated with significant reductions in fetal growth. When women in the highest quartile of acrylamide intake were compared with women in the lowest quartile, the multivariable-adjusted odds ratio (OR) for SGA was 1.11 (95%CI, 1.02; 1.21) and the coefficient±SE for birth weight was -25.7±5.2 g. Results were similar after excluding mothers who smoked during pregnancy. Maternal acrylamide- and glycidamide-Hb adduct levels reflected estimated dietary acrylamide intakes (Spearman correlations = 0.24 (95%CI: 0.02; 0.44) and 0.48 (95%CI: 0.29; 0.63) respectively).

Conclusions: Lowering the dietary exposure to acrylamide may reduce the risk of acrylamide adversely affecting fetal growth.

INTRODUCTION

Indicators of intrauterine development, such as birth weight and small for gestational age (SGA), have been shown to predict infant survival and the prevalence of chronic diseases in adulthood [132,194]. Diet during pregnancy is a well recognized determinant of fetal growth [114]. A decade ago it was shown that acrylamide is formed during heating of food at high temperatures, and it is found in a variety of foods [8,91,92]. Acrylamide has been in industrial use since the 1950s and is also present in cigarette smoke [86,87]. Acrylamide has shown neurotoxic effects in humans and is classified as a probable human carcinogen [195]. Its metabolite glycidamide is assumed to be the genotoxic agent of acrylamide [84]. Acrylamide is also known to induce developmental and reproductive toxicity in animals including effects on fetal growth [4].

In humans, it has been shown that fetal exposure to acrylamide through the diet may start *in utero* since acrylamide has been found to cross the placenta barrier *in vitro* [108,109] as well as *in vivo* [110,111]. Recently, we showed that higher acrylamide exposure among non-smoking pregnant women was associated with a significant reduction in fetal growth, measured as birth weight and risk of SGA [107]. That study included 1101 women from five different European countries, and the exposure assessment was based on acrylamide Hb adduct levels measured in cord blood as well as food scores created from intake of acrylamide-rich foods that were obtained from food frequency questionnaires (FFQ).

Dietary acrylamide exposure estimated from FFQ data used in the The Norwegian Mother and Child Cohort Study (MoBa) has previously been validated using urine metabolites as biomarker of recent intakes [101]. N-terminal Hb adducts reflect a longer time window for exposure compared to urine metabolites, and have been used for biomonitoring acrylamide exposure in many studies [196]. The validity of Hb adducts as a marker of acrylamide exposure from food, has been demonstrated in animal studies as

well as in human intervention studies [197,198]. In other studies comparing FFQ based acrylamide intake estimates with measured acrylamide Hb adduct concentrations, low to moderate associations were observed [199–204].

In this study we have further explored the hypothesis that dietary acrylamide exposure during prenatal life might impair fetal growth measured as risk of small for gestational age (SGA) and birth weight in a very large population-based cohort study in Norway - The Norwegian Mother and Child Cohort Study (MoBa). We also aimed to assess population characteristics associated with higher intakes of acrylamide. The estimated acrylamide intake was further evaluated by means of measurements of acrylamide- and glycidamide-Hb adducts in a subset of the study participants.

METHODS

Population and study design

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health [135]. Participants were recruited from all over Norway from 1999-2008, and 38.5% of invited women consented to participate. The cohort now includes 108000 children, 90700 mothers and 71500 fathers. Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth. Follow-up is conducted by questionnaires at regular intervals and by linkage to national health registries. Several sub-studies are conducting additional collections of data and biological materials. The current study is based on version 5 of the quality-assured data files released for research on 2010. Informed consent was obtained from each MoBa participant upon recruitment. The study was approved by The Regional Committee for Medical Research Ethics in South-Eastern Norway.

When preparing the dataset, 62124 women had answered questionnaires 1 and 3 (in pregnancy week 17 and 30, respectively), the baseline MoBa questionnaires covering

information on sociodemographic characteristics, exposure to tobacco smoke during pregnancy and general health; questionnaire 2 (in pregnancy weeks 23-24), which covered dietary information; and questionnaire 4 (when the child was 6 months of age), which includes information on maternal health at time of delivery, including gestational weight gain during pregnancy. In addition they were recorded in MBRN and had singleton births. We excluded women with multiple participation in MoBa (n = 6604), pregnancy duration <28 weeks or >42 weeks (n = 385), missing data on birth weight (n = 22), an energy intake < 4500 kJ or > 20000 kJ (n=796) and no information about smoking during pregnancy (n=817). Lastly, we excluded women with improbable gestational weight gain (<-30 kilo or >50 kilo) (n = 463) or missing (n = 2386), leaving a study sample of 50651 women. Since smoking is a known lifestyle factor associated with reduction of birth weight and a significant source of acrylamide exposure, additional analyses were performed excluding women who reported any smoking during pregnancy, leaving n = 46420 non-smokers.

Dietary information

The MoBa FFQ (downloadable at http://www.fhi.no/dokumenter/011fbd699d.pdf) was used for calculation of acrylamide intake. This FFQ is a semi-quantitative questionnaire designed to provide information on dietary habits and intake of dietary supplements during the first four to five months of pregnancy [185]. It has been answered by participating women in MoBa since February 2002, and has been thoroughly validated with regard to foods and nutrients [186]. For each of the 255 food and beverage items, the frequency of consumption was reported by selecting one out of 8-10 frequencies, ranging from never to several times monthly, weekly or daily. The FFQ was read optically and energy intake was calculated using FoodCalc [187] and the Norwegian Food Composition table [188].

To calculate acrylamide intake a database was prepared containing values of acrylamide concentration reported from analyses of Norwegian food items [141–143] and the Swedish National Food Administration [144]. For foods not analysed in Norway or Sweden

we collected data from the European Union database [145]. For food items with multiple analyses of acrylamide concentration the median concentration was used. Examples of the values assigned for each food group have previously been published [101]. In order to identify food group predictors of higher acrylamide intake, the 255 food items in the FFQ were grouped into 19 food groups based on nutrient profiles, culinary usage or known acrylamide levels. For example, four food groups were defined for cereals and potatoes: fried potatoes; crisp bread; bread, which included dark and white bread; and other, including breakfast cereal, rice, couscous, pasta and pizza.

Hb adduct measurements

Blood samples were collected from mothers giving birth between 2007 and 2009, at Oslo University Hospital at Ullevål and Akershus University Hospital, and were enrolled in the MoBa sub-cohorts BraMiljö and BraMat [205,206]. A common protocol for the European Commission financed integrated project NewGeneris ('Newborns and Genotoxic exposure risks') was followed [207]. Maternal blood samples (non-smokers, n = 79) were analyzed for Hb adducts from acrylamide and glycidamide by application of the adduct FIRE procedure and analysis with LC/MS (Shimadzu prominence/AB Sciex 3200 qtrap), described by von Stedingk et al. [208]. The method performance of the adduct FIRE procedure for acrylamide and glycidamide Hb adduct measurements has previously been described [111,208].

Birth outcomes and other variables

Birth weight was measured by the midwife who attended the birth and reported to the MBRN [136]. Gestational age was calculated on the basis of the first trimester ultrasound until delivery. In the event of a missing ultrasound measure, the gestational age was calculated from last menstrual period. SGA was defined using MoBa data to calculate 10^{th} percentile for nulliparous and multiparous births for each week of gestation from 34 to 42 weeks; for children born during weeks 28-33 MBRN data published in 2000 was used [209].

Parity was based on data from both MoBa and MBRN and categorized into two categories, primiparous and multiparous. Data on maternal education attainment (≤12, 13-16 and 17+ years), maternal age and smoking were collected from questionnaires. Smoking during pregnancy was categorized as either non-smokers, occasional smokers, or daily smokers. Participants with unknown/missing values for education or father's smoking were grouped in a "missing" category. Pre-pregnant weight and height were self-reported at week 17 in pregnancy and pre-pregnant body mass index (BMI) was calculated (kg/m^2). Pre-pregnant BMI was categorized according to the WHO classification as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2). Sex of the child (boy and girl) and weight of the mother at the time of delivery (kg) were collected from questionnaire 4. Gestational weight gain (kg) was calculated from weight reported at the start of pregnancy and at the time of delivery as registered at the birth clinic on the women's health card.

Statistical analyses

Spearman correlations were used to examine the relationship between dietary acrylamide intakes and measured maternal Hb adduct concentrations of acrylamide and glycidamide. Dietary acrylamide intakes were divided by total energy intake and quartiles were created using these energy-adjusted estimates. The intake of acrylamide (ng/kcal/day) was estimated and is expressed as means with standard deviations. Data on acrylamide intakes and newborn characteristics were expressed over quartiles of energy-adjusted acrylamide intakes. Statistical significance was evaluated using $\chi 2$ or Kruskal-Wallis tests.

To identify the main characteristics related to high intake of acrylamide, multiple linear regression models were used. In separate models, associations between food group intake and intake of acrylamide were estimated in order to identify the food groups that were the strongest predictors of high dietary acrylamide intakes among pregnant women.

Multivariate logistic regression models were used to examine the relationship between dietary acrylamide intakes during pregnancy and the likelihood of SGA at birth; linear regression was used to assess associations with birth weight. Models were adjusted appropriately for confounders or modifiers, which were assessed from a wide array of variables available in the Norwegian study (e.g. gestational age, parity, parental income, type of delivery, tobacco use and second-hand exposure). Results are reported as odds ratio with 95% confidence intervals for SGA and as coefficients with standard errors (SE) for birth weight; results are reported for a 1-SD increase in continuous energy-adjusted acrylamide intake. Covariates in final models were gestational age, parity, sex of the child, age of the mother, maternal BMI, maternal gestational weight gain, and smoking during pregnancy. Other covariates (type of delivery, parental education, income, father's weight and height, marital status, and exposure to passive smoking) were tested as potential confounders, but were excluded from final models for parsimony, as they did not affect our estimates (change-in-estimate <10%). Possible confounding by other aspects of the diet were assessed by adjusting models for maternal intakes of food groups such as snacks, sweets, including cakes and chocolate, dairy products, alcohol and coffee; these variables were omitted as no meaningful changes were found in the associations.

Potential interactions between acrylamide intakes and active or passive smoking during pregnancy were tested by including interaction terms in the regression models; no significant interactions were observed. Similarly, we confirmed that results were comparable after excluding women who actively smoked during pregnancy (see Table 4), preterm births (<37 weeks of gestation), low birth weight children (birth weight <2500 g), or those women with missing information for education or partners' smoking during pregnancy (not shown). The analyses were repeated using acrylamide exposure relative to body weight (i.e., µg/kg body weight/day) as the exposure variable and similar results were found. No meaningful changes were observed in the results when using the residual approach to adjust for energy

intake or body weight. Data were analyzed using STATA 10.1 (Stata Corporation, College Station, Texas).

RESULTS

Validation of acrylamide intake estimates among non-smokers

Acrylamide and glycidamide Hb adducts were measured as markers of the internal dose of acrylamide. Mean maternal Hb adduct levels were 31 pmol/g Hb (min-max 9.9-72, n = 79) for acrylamide and 23 pmol/g Hb (min - max 8.8-44, n = 79) for glycidamide. A strong correlation between acrylamide- and glycidamide-Hb adduct levels (Spearman correlation = 0.62, $p < 0.001$, n = 79) was observed.

Maternal Hb adduct levels were compared with dietary acrylamide intake estimates obtained from FFQs. There were statistically significant correlations for maternal acrylamide- and glycidamide-Hb adduct levels versus dietary acrylamide intake estimated from FFQ (Spearman correlations=0.24 (95% CI: 0.02; 0.44) and 0.48 (95% CI: 0.29; 0.63) respectively) (Figures 1).

Acrylamide intake during pregnancy and fetal growth indicators

Overall (n = 50651), the mean±SD intake of dietary acrylamide among pregnant women was 27.1 ± 13.4 μg/day, 0.41 ± 0.22 μg/kg body weight/day, or 11.8 ± 4.7 ng/kcal/day (Table 1). Dietary acrylamide intakes were similar (26.7 ± 13.0 μg/day) after excluding smokers during pregnancy (see Supplemental Material, Table 1).

Table 2 shows the association between food groups and acrylamide intakes. The food groups that most strongly predicted high intake of acrylamide among pregnant women in the Norwegian MoBa study were snacks; which include potato chips, nuts, and popcorn; fried potatoes and crisp bread (coefficients±SE were 0.17±0.001, 0.15±0.001 and 0.13±0.001 respectively, vs. coefficients±SE ranging from -0.026±0.0007 to 0.009±0.0001 for other food groups).

After multivariate adjustment, increasing age, multiparity, lower educational level, season of birth, maternal smoking and paternal smoking during pregnancy, were significantly associated with higher dietary intake of acrylamide during pregnancy (Table 3). Among non-smoking women, the same population characteristics were found to determine acrylamide intake, in addition to overweight, (see Supplemental Material, Table 3).

Birth weight and the frequency of SGA differed significantly by quartiles of acrylamide intake during pregnancy (Table 1). The frequency of SGA was higher and the mean±SD of birth weight was lower in women in the 4^{th} quartile of acrylamide intake in comparison with that of women in the 1^{st} quartile (11.0% and 3591±542g vs. 9.6% and 3612±534g respectively, p-value<0.05). Table 4 presents the crude and adjusted associations between acrylamide intake during pregnancy and SGA and birth weight among all women and among non-smokers. Energy-adjusted acrylamide intake during pregnancy, used continuously or in quartiles, significantly increased the risk of SGA and was negatively associated with birth weight. The results persisted after exclusion of smokers. After adjusting for potential confounders, the odds ratio (OR) among all women for SGA was 1.11 (95%CI, 1.02; 1.21) for the highest quartile of acrylamide intake compared with the lowest quartile. Multivariable-adjusted coefficient±SE for birth weight for all women in the 2nd, 3rd and 4th quartile compared with women in the 1st quartile of acrylamide intake were -13.0±5.2 g, -20.8±5.2 g, and -25.7±5.2 g, respectively.

DISCUSSION

In this study, we found that higher maternal dietary acrylamide intakes during pregnancy were associated with a significant impairment of fetal growth measured as an increase in risk of SGA and a reduction in birth weight, with similar results after excluding women who smoked during pregnancy. A validation of estimated dietary acrylamide intakes from the MoBa FFQ was performed by measurements of Hb adduct levels in a subset of maternal samples. The three food groups that most strongly predicted high

intakes of acrylamide were snacks, which included potato chips, nuts, and popcorn; fried potatoes, and crisp bread. Maternal age, parity, education, season of birth and exposure to tobacco smoke, were found to be related to the intake of dietary acrylamide during pregnancy.

Our results are in agreement with findings in a recent, but smaller study, examining the associations between prenatal exposure to acrylamide as measured by Hb adducts, and fetal growth indicators in mother/child cohorts from five countries in Europe [107]. Significant negative associations between maternal acrylamide exposure and birth weight among non-smoking women were found. The estimation of acrylamide exposure was based on Hb adduct measurements in 1101 cord blood samples as well as by applying a food score approach based on the intake of acrylamide-rich foods collected by FFQs (*n* = 801).

An effect of prenatal exposure to acrylamide, with impaired fetal growth, has been observed in animal studies, with an effect seen at a few mg/kg/day, as reviewed by Manson et al. 2005 [4]. Although these results from animal experiments strongly suggest a negative effect of prenatal acrylamide exposure on fetal growth, the mechanisms responsible are not known. Perfusion studies have shown that acrylamide can cross the placental barrier in humans [108,109]. Measurements of Hb adducts from acrylamide in mother/cord blood samples have further shown that acrylamide is circulated in the body of the fetus [110,111]. Both acrylamide and its metabolite glycidamide are reactive electrophiles and thus have the potential to react with nucleophilic sites in biomacromolecules, which could affect cellular processes of importance for growth. It is known that during heat-processing of foods, in parallel with acrylamide formation, other Maillard products with potential toxic effects are formed [112]. It is likely that the observed effects on fetal growth associated with acrylamide exposure, might be a result of combined exposures to multiple compounds formed simultaneously with acrylamide during food processing. El-Sayyad and co-workers have shown that pregnant mice fed a diet containing 30% fried potato chips gave birth to offspring with

reduced birth weight. The reduction in birth weight was more pronounced for the fried potato chips diet compared to what could be expected from acrylamide alone, suggesting that there is a synergistic effect with other compounds [103].

Diet is recognized as a main source of acrylamide exposure among non-smokers without occupational exposure to acrylamide, as it is formed during cooking at high temperatures (e.g. frying, grilling, or roasting) of especially carbohydrate-rich foods containing the amino acid asparagine and reducing sugars [8]. The average acrylamide intake among pregnant women was 0.41 μg/kg body weight/day which is close to the intake found previously in a subsample of women from the MoBa study (0.52 and 0.44 μg/kg body weight/day for the estimation based in the FFQ and a 4-day food diary, respectively) [101], and to the median daily intake found in a group of non-pregnant Norwegian women aged 16-79 years (0.42 μg/kg body weight/day) [100]. Additionally, a FAO/WHO evaluation based on national survey data from 17 countries concluded that typical acrylamide intakes range from 0.3 to 0.8 μg/kg body weight/day [210].

The observed correlation between FFQ data and acrylamide Hb adduct levels are in agreement with results reported by other investigators while the observed correlation to glycidamide Hb adducts was higher than earlier data [200–204]. A higher correlation coefficient for glycidamide- compared to acrylamide-Hb adducts and food intake has also been reported by Tran and co-workers (2010), reporting data from analysis of more than 7000 individuals, with correlation coefficients between acrylamide intake and Hb adducts from glycidamide and acrylamide of 0.21 and 0.16 respectively [201]. It might have been expected that the glycidamide Hb adduct levels would be less strongly associated with acrylamide intake estimates compared to acrylamide Hb adducts since individual variations in the capacity to metabolize acrylamide to glycidamide are known to occur [211,212].

The observed significant associations between acrylamide intake estimates derived from FFQ and Hb adducts show that FFQ is valid

for exposure estimation. In this study, the FFQs were filled in during mid-pregnancy and the blood samples were collected at delivery. In other studies, a high with-in person correlation over time has been observed suggesting that even a single measurement is a good indicator of long term intake [203,212]. The FFQ data for acrylamide intake can thus be assumed to be representative for the whole pregnancy, which is in line with the associations observed in this study between FFQ data and Hb adduct levels.

Dietary information was collected using a FFQ validated in the Norwegian population of pregnant women. However, possible measurement error of acrylamide intake remains a limitation of this study since acrylamide formation in food is affected by several parameters, such as cooking methods or doneness. These questions were not included in the FFQ. Moreover, large variations in acrylamide content of a single food item have been reported [145]. The crude estimation of acrylamide intake using FFQs would in principle lead to a weaker association between the exposure and the outcome than the true association. The approach to use FFQ data can also be advantageous compared to biomarkers of exposure. Besides the cost effectiveness of using FFQ data, the obtained information is not necessarily biased from the same factors as those affecting the biomarkers. Relevant to this study is that FFQ acrylamide intake estimates are not biased from cigarette smoking to the same extent as the biomarkers. Cigarette smoking is highly associated with acrylamide Hb adduct levels [87,111], and misclassification of smokers could erroneously lead to the interpretation of an effect of acrylamide intake from food, which in fact could be related to smoking habits. It is well established that both active and passive smoking is associated with a reduction in birth weight and increased risk of SGA [213]. Misclassified smokers are likely to be evenly distributed for low and high consumers of acrylamide-rich foods.

One of the strengths of this study is that this is a large population-based mother and child cohort study (n = 50651) in which detailed information on diet during pregnancy was collected [185]. This

study also includes information on smoking habits and other life-style factors of parents, allowing identification of subgroups of women with higher dietary acrylamide exposure. In addition, the levels of acrylamide- and glycidamide-Hb adducts that were available in a subset of samples within the MoBa study also represent a strength of the present study since it allowed the validation of FFQ acrylamide estimates.

Our findings that prenatal exposure to dietary acrylamide is associated with decreased birth weight and increased risk of SGA might have implications for public health at earlier or later stages of life. SGA and low birth weight have been associated with neonatal morbidity and mortality risk [131,132], increased risk of delayed neurodevelopment [133] as well as risk of chronic diseases during adulthood including cardiovascular diseases, type 2 diabetes, and insulin resistance [114,194].

CONCLUSIONS

In this large population-based cohort study, higher prenatal exposure to dietary acrylamide was significantly associated with risk of SGA and reduction in birth weight, also after excluding smokers during pregnancy. The results suggest that prenatal exposure to dietary acrylamide may impair fetal growth. Reduced dietary acrylamide intake among pregnant women might be beneficial for fetal growth.

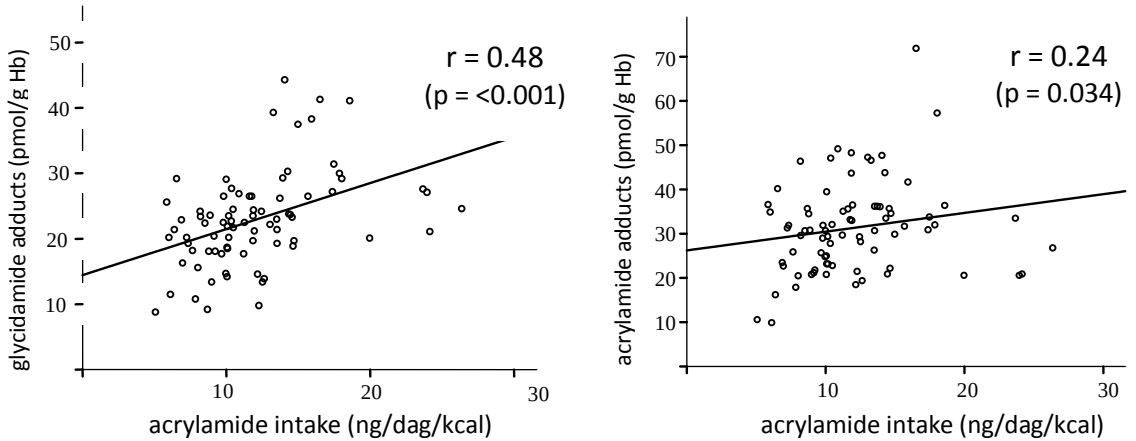


Figure 1 Relationships for acrylamide- and glycidamide-Hb adducts versus FFQ-based acrylamide estimated intake among non-smoking pregnant women (n=79).

Table 1 Acrylamide intake and newborn characteristics across quartiles of dietary acrylamide adjusted for energy intake during pregnancy in all women

	All women (n=50651)	**1st Quartile** (n=12663)	**2nd Quartile** (n=12663)	**3rd Quartile** (n=12663)	**4th Quartile** (n=12662)	**p-value***
Acrylamide intake (µg/day), mean±SD	27.11±13.36	15.12±5.18	22.71±6.03	29.15±7.84	41.48±14.70	<0.001
Acrylamide intake (µg/kg body weight/day), mean±SD	0.41 ±0.22	0.23±0.09	0.34±0.11	0.44±0.14	0.63±0.26	<0.001
Acrylamide adjusted for energy intake (ng/ kcal/day), mean±SD	11.75±4.65	6.62±1.42	9.81±0.74	12.58±0.90	17.98±3.70	<0.001
Energy intake (kcal/day), mean±SD	2304.79±604.06	2278.92±592.30	2314.56±585.52	2317.01±597.47	2308.69±638.80	
Newborn characteristics						
Gestational age (weeks), mean±SD	39.53±1.68	39.52±1.68	39.52±1.67	39.53±1.70	39.53±1.68	0.676
Birth weight (g), mean±SD	3600.58±538.98	3612.18±533.90	3602.11±538.83	3597.03±541.05	3591.02±541.94	0.014
SGA, n (%)	5188 (10.24)	1216 (9.60)	1270 (10.03)	1311 (10.35)	1391 (10.99)	0.003
Sex (male), n (%)	25906 (51.15)	6490 (51.25)	6393 (50.49)	6466 (51.06)	6557(51.78)	0.224

SD, Standard Deviation; SGA, Small for gestational age.

* p-value from $\chi 2$ or Kruskal-Wallis tests.

Table 2 Food groups predictors of dietary acrylamide intake during pregnancy in all women*

	All women (n=50651)		
	Food groups (grams/day), mean±SD	**β**	**SE**
Snacks	14.10±12.69	0.165	0.0008
Cereals and potatoes			
Fried potatoes	7.73±7.80	0.146	0.0013
Crisp bread	13.88±18.59	0.134	0.0006
Bread	216.28±99.76	-0.009	0.0001
Other	95.55±54.99	0.001	0.0002
Beverages			
Coffee	98.15±160.82	0.009	0.0001
Other	1540.80±765.96	0.000	0.0000
Alcoholic beverages	1.25±5.98	0.005	0.0017
Sweets	101.98±56.76	0.005	0.0002
Sweet spreads	34.79±25.49	0.005	0.0005
Dairy products			
Cheese	23.16±19.52	-0.002	0.0000
Milk and yogurt	435.93±357.02	-0.017	0.0006
Seafood	44.72±26.04	-0.005	0.0004
Meat	54.51±17.47	-0.007	0.0006
Fruits and vegetables	592.96±327.83	-0.002	0.0000
Eggs	11.05±12.07	-0.011	0.0008
Sauces	16.23±13.35	-0.012	0.0008
Fats and oils	17.80±15.47	-0.026	0.0007
Dried fruits	5.58±12.19	-0.006	0.0008

SD, Standard Deviation; SE, Standard Error.

* Results from multivariate linear regression model predicting energy-adjusted acrylamide intake (ng/kcal/day), adjusted simultaneously for all food groups shown in the table. p-value was 0.002 and 0.001 for the association between acrylamide and other cereals and alcoholic beverages respectively. p-value ≤ 0.001 for other food groups in the model.

Table 3 Population characteristics associated with dietary acrylamide intake during pregnancy in all women*

	n (%)	β	SE	p-value
Mother's age (years) , mean±SD	30.08 ± 4.51	0.025	0.005	<0.001
Parity				
Nulliparous	26320 (51.96)	Ref.		
Multiparous	24331 (48.04)	0.175	0.045	<0.001
Pre-pregnancy BMI (kg/m2)				
18.5-25	33405 (65.95)	Ref.		
< 18.5	1437 (2.84)	0.112	0.125	0.368
25-30	11144 (22.00)	0.062	0.051	0.219
> 30	4665 (9.21)	-0.082	0.073	0.260
Maternal education (years)				
<=12	15243 (30.09)	Ref.		
13-16	21847 (43.13)	-0.205	0.051	<0.001
17 +	12539 (24.76)	-0.534	0.060	<0.001
Missing	1022 (2.02)	-0.515	0.150	0.001
Season of birth				
March-May	12489 (24.66)	Ref.		
June-August	13363 (26.38)	0.180	0.058	0.002
September-November	12668 (24.99)	0.301	0.058	<0.001
December-February	12141 (23.97)	0.242	0.059	<0.001
Maternal smoking during pregnancy				
Non-smokers	46420 (91.65)	Ref.		
Occasional smoking	2261 (4.46)	1.170	0.102	<0.001
Daily smoking	1970 (3.89)	1.475	0.112	<0.001
Father smoking during pregnancy				
Non-smokers	38325 (75.66)	Ref.		
Occasional smoking	2656 (5.24)	0.189	0.093	0.042
Daily smoking	7430 (14.67)	0.154	0.062	0.013
Missing	2240 (4.42)	0.104	0.102	0.310

SE, Standard Error; SD, Standard Deviation; BMI, Body Mass Index.
* Results from multivariate linear regression model predicting acrylamide adjusted by energy intake (ng/day/kcal), adjusted simultaneously for all variables shown in the table.

Table 4 Associations between dietary acrylamide intakes during pregnancy and fetal growth indicators

	Crude			Adjusted *		
SGA	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
All, (n=50651) Acrylamide intake (ng/day/ kcal), 1-SD increase	1.055	(1.026; 1.085)	< 0.001	1.032	(1.003; 1.062)	0.029
Acrylamide - Quartile 1	Ref.					
Acrylamide - Quartile 2	1.049	(0.966; 1.140)	0.254	1.050	(0.965; 1.142)	0.255
Acrylamide - Quartile 3	1.087	(1.001; 1.180)	0.046	1.083	(0.995; 1.018)	0.061
Acrylamide - Quartile 4	1.162	(1.071; 1.261)	< 0.001	1.110	(1.021; 1.206)	0.014
Non-smokers, (n=46420) Acrylamide intake (ng/day/ kcal), 1-SD increase	1.030	(0.999; 1.062)	0.056	1.033	(1.001; 1.065)	0.041
Acrylamide - Quartile 1	Ref.			Ref.		
Acrylamide - Quartile 2	1.070	(0.979; 1.169)	0.132	1.080	(0.987; 1.180)	0.092
Acrylamide - Quartile 3	1.070	(0.979; 1.169)	0.132	1.090	(0.997; 1.192)	0.059
Acrylamide - Quartile 4	1.116	(1.022; 1.217)	0.014	1.128	(1.032; 1.233)	0.008

SE, Standard Error; SD, Standard Deviation; OR, Odds Ratio; CI, Confidence Interval; SGA, small for gestational age.

* Results from logistic regression model adjusted for gestational age, parity, sex of the child, age of the mother, maternal body mass index categorical, maternal gestational weight gain (kg), and smoking during pregnancy.

** Results from linear regression model adjusted for gestational age, parity, sex of the child, age of the mother, maternal body mass index categorical, maternal gestational weight gain (kg), and smoking during pregnancy.

Table 4 (continued) Associations between dietary acrylamide intakes during pregnancy and fetal growth indicators

	Crude			Adjusted **		
BIRTH WEIGHT	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**
All, (n=50651) Acrylamide intake (ng/day/kcal), 1-SD increase	-9.179	2.392	< 0.001	-9.888	1.845	< 0.001
Acrylamide - Quartile 1	Ref.			Ref.		
Acrylamide - Quartile 2	-10.075	6.773	0.137	-12.998	5.200	0.012
Acrylamide - Quartile 3	-15.158	6.773	0.025	-20.848	5.202	< 0.001
Acrylamide - Quartile 4	-21.168	6.773	0.002	-25.666	5.217	< 0.001
Non-smokers, (n=46420) Acrylamide intake (ng/day/kcal), 1-SD increase	-5.790	2.491	0.020	-9.464	1.916	< 0.001
Acrylamide - Quartile 1	Ref.			Ref.		
Acrylamide - Quartile 2	-10.995	7.052	0.119	-15.826	5.423	0.004
Acrylamide - Quartile 3	-9.674	7.052	0.170	-19.828	5.424	< 0.001
Acrylamide - Quartile 4	-14.405	7.052	0.041	-25.062	5.425	< 0.001

SE, Standard Error; SD, Standard Deviation; OR, Odds Ratio; CI, Confidence Interval; SGA, small for gestational age.

* Results from logistic regression model adjusted for gestational age, parity, sex of the child, age of the mother, maternal body mass index categorical, maternal gestational weight gain (kg), and smoking during pregnancy.

** Results from linear regression model adjusted for gestational age, parity, sex of the child, age of the mother, maternal body mass index categorical, maternal gestational weight gain (kg), and smoking during pregnancy.

Supplemental Material, Table 1 Acrylamide intake and newborn characteristics across quartiles of dietary acrylamide adjusted by energy intake during pregnancy among non-smokers

	Non-smokers (n=46420)	**1st Quartile** (n=11605)	**2nd Quartile** (n=11605)	**3rd Quartile** (n=11605)	**4th Quartile** (n=11605)	**p-value***
Acrylamide intake (µg/day), mean±SD	26.7 ± 13.0	14.9 ± 5.1	22.4 ± 5.9	28.7 ± 7.6	40.7 ± 14.3	<0.001
Acrylamide intake (µg/kg body weight/day), mean±SD	0.40 ± 0.21	0.23 ± 0.09	0.34 ± 0.11	0.43 ± 0.14	0.62 ± 0.25	<0.001
Acrylamide adjusted for energy intake (ng/ kcal/day), mean±SD	11.6 ± 4.6	6.6 ± 1.4	9.7 ± 0.7	12.5 ± 0.9	17.7 ± 3.6	<0.001
Energy intake (kcal/day), mean±SD	2292 ± 594	2265 ± 582	2302 ± 576	2306 ± 588	2293 ± 628	<0.001
Newborn characteristics						
Gestational age (weeks), mean±SD	39.5 ± 1.7	39.5 ± 1.7	39.5 ± 1.7	39.5 ± 1.7	39.5 ± 1.7	0.772
Birth weight (g), mean±SD	3610 ± 537	3618 ± 530	3607 ± 538	3609 ± 539	3604 ± 542	0.240
SGA, n (%)	4476 (9.6)	1058 (9.1)	1125 (9.7)	1125 (9.7)	1168 (10.1)	0.106
Sex (male), n (%)	23732 (51.1)	5945 (51.2)	5875 (50.6)	5921 (51.0)	5991 (51.6)	0.490

SD, Standard Deviation; SGA, Small size for gestational age.

* p-value from $\chi 2$ or Kruskal-Wallis tests.

Supplemental Material, Table 2 Food groups predictors of dietary acrylamide intake during pregnancy among non-smokers *

	Non-smokers (n=46420)		
	Food groups (grams/day), mean±SD	**β**	**SE**
Snacks	14 ± 12	0.169	0.0009
Cereals and potatoes			
Fried potatoes	7.5 ± 7.5	0.150	0.0014
Crisp bread	14 ± 19	0.126	0.0006
Bread	216 ± 99	-0.009	0.0001
Other	97 ± 55	0.001	0.0002
Beverages			
Coffee	87 ± 140	0.009	0.0001
Other	1547 ± 758	0.000	0.0000
Alcoholic beverages	1.1 ± 5.2	0.007	0.0020
Sweets	101 ± 56	0.005	0.0002
Sweet spreads	35 ± 25	0.005	0.0005
Dairy products			
Cheese	23 ± 19	-0.018	0.0006
Milk and yogurt	433 ± 353	-0.002	0.0000
Seafood	45 ± 26	-0.005	0.0004
Meat	54 ± 17	-0.007	0.0006
Fruits and vegetables (including juice)	598 ± 326	-0.002	0.0000
Eggs	11 ± 12	-0.011	0.0009
Sauces	16 ± 13	-0.011	0.0008
Fats and oils	18 ± 15	-0.026	0.0007
Dried fruits	5.8 ± 12	-0.006	0.0009

SE, Standard Error.

* Results from multivariate linear regression model predicting acrylamide adjusted by energy intake (ng/day/kcal), adjusted simultaneously for all food groups shown in the table. p-value ≤ 0.001 for the association between acrylamide intake and each food group in the model.

Supplemental Material, Table 3 Population characteristics associated with dietary acrylamide intake during pregnancy among non-smokers *

	Non-smokers (n=46420)			
	n (%)	**β**	**SE**	**p-value**
Mother's age (years) , mean±SD	30.2 ± 4.4	0.014	0.005	0.009
Parity				
Nulliparous	24307 (52.4)	Ref.		
Multiparous	22113 (47.6)	0.151	0.046	0.001
Pre-pregnancy BMI (kg/m2)				
18.5-25	30978 (66.7)	Ref.		
< 18.5	1227 (2.6)	0.066	0.133	0.619
25-30	10127 (21.8)	0.132	0.053	0.012
> 30	4088 (8.8)	0.002	0.077	0.980
Maternal education (years)				
<=12	12543 (27.0)	Ref.		
13-16	20733 (44.7)	-0.234	0.053	<0.001
17 +	12210 (26.3)	-0.536	0.062	<0.001
Missing	934 (2.0)	-0.526	0.155	0.001
Season of birth				
March-May	11608 (25.0)	Ref.		
June-August	12280 (26.5)	0.182	0.059	0.002
September-November	11504 (24.8)	0.330	0.060	<0.001
December-February	11028 (23.8)	0.264	0.061	<0.001
Father smoking during pregnancy				
Non-smokers	36920 (79.5)	Ref.		
Occasional smoking	2315 (5.0)	0.283	0.098	0.004
Daily smoking	5426 (11.7)	0.166	0.067	0.014
Missing	1759 (3.8)	0.043	0.112	0.704

SE, Standard Error; SD, Standard Deviation; BMI, Body Mass Index.
* Results from multivariate linear regression model predicting acrylamide adjusted by energy intake (ng/day/kcal), adjusted simultaneously for all variables shown in the table.

6 DISCUSSION

6 DISCUSSION

This thesis presents the first epidemiological studies to conduct comprehensive assessments of dietary exposure to food contaminants, B(a)P and acrylamide among two different European populations of pregnant women, and to assess the impact of prenatal exposure to these compounds specifically from the diet on fetal growth indicators.

This section provides a complementary and global discussion of the results presented in the thesis, aiming to provide a broader and more integrated interpretation of the entire study project. More detailed discussions are provided in the original papers presented in the results section of this thesis.

6.1 Prenatal exposure to contaminants through maternal diet and fetal growth indicators

Although evidence from animal studies suggests that PAHs and acrylamide may be associated with impaired fetal growth [2,103], few human studies examine levels of exposure during pregnancy, or whether there may be adverse health effects linked to prenatal exposure to these compounds specifically from the diet.

To date, epidemiological studies regarding the effects of prenatal exposure to PAHs and acrylamide on fetal and child outcomes have focused mainly on biomarkers to assess the levels of exposure [65–67,107], while the exposure to these compounds through maternal diet has been neglected. Moreover, while the beneficial and protective proprieties of diet have been widely investigated [214,215], research on the risk factors related to the exposure to contaminants through diet is still limited.

The results presented in this thesis showed that prenatal exposure to B(a)P and acrylamide specifically from maternal diet may reduce birth weight and increase the risk of SGA. Evidence on the protective capacity of vitamin C intakes against the adverse effects

of exposure to dietary B(a)P during pregnancy was also provided. These findings were consistent in two European populations from the INMA and the MoBa cohort studies. Given the lack of evidence on the effects of prenatal exposure to PAHs and acrylamide from maternal diet, which is the main source of fetal exposure to these compounds in the general population [2,6,7], and the previously suggested implications of reduced fetal growth for child and adult health [10], the work presented in this thesis is of substantial relevance. Further research is needed to fully understand the role of diet as a source of prenatal exposure to contaminants.

6.2 Dietary exposure to contaminants during pregnancy

Since PAHs and acrylamide are widespread throughout the diet [2,145], it is important to take into account all sources of dietary intake in order to adequately estimate the exposure. This thesis presents the first studies to assess and to characterize dietary exposure to these compounds among the population of pregnant women.

To our knowledge, there are only two prior epidemiologic studies that have examined the role of prenatal exposure to B(a)P specifically from diet on fetal growth [71,72]. However, these studies used the consumption of a limited number of food items (smoked, grilled or barbequed meat intakes) as indicators of total dietary exposure to PAHs. In this thesis, a food composition table containing all previously reported levels of B(a)P in food was created. As mentioned in the methods section, the estimation of B(a)P intake was performed based on the combination of data from FFQs and the levels of B(a)P in each food included in the composition table. Thus, the presented estimation of B(a)P intake had into account the exposure from the whole diet. This could explain why significant associations between maternal B(a)P intakes and fetal growth indicators were found, while very weak associations were reported in the studies mentioned above [71,72].

In the MoBa study, the methods used to estimate acrylamide intake were previously developed, described, and validated against levels of acrylamide metabolites excreted in urine, in a subset of sample [101]. In this thesis, a validation of dietary acrylamide estimates was further performed in the MoBa study, using levels of acrylamide- and glycidamide-Hb adducts measured in maternal blood from a subset of sample.

There are other challenges in measuring dietary exposure to contaminants that were not possible to account for in this thesis. Details about these challenges will be further discussed below in the limitations section.

6.3 Strengths

Both INMA and MoBa are population-based mother and child cohort studies. The cohort design, the prospective nature, and the long follow-up of these studies allowed the evaluation of the effects of prenatal exposures on birth outcomes and will allow the establishment of longitudinal associations between dietary exposure to contaminants during pregnancy and child development, including respiratory outcomes and neurodevelopment, in the near future.

The comprehensive assessment of diet in this thesis is a step forward compared to previous studies. The two FFQs administered to the mothers during pregnancy in the INMA and MoBa studies, were previously validated in the Spanish and the Norwegian population of pregnant women, respectively [149,186]. The use of extensive FFQs allowed us to perform a comprehensive estimation of dietary intakes of food contaminants based on concentrations in individual foods from the whole diet, and consequently, to quantify the relevance of specific foods to the total contaminant intake. The collection of detailed information of diet during pregnancy also enabled us to estimate nutrient intakes and the further exploration of synergies between contaminant and antioxidant intakes on birth outcomes.

The availability of relevant covariates in the two cohort studies, including socioeconomic and life-style factors from both parents, as well as other sources of exposure to the studied compounds, such as tobacco smoke, allowed the adjustment for potential confounders of association between prenatal exposure to dietary contaminants and fetal growth indicators. The thorough characterization of the population and the large sample size allowed the identification of subgroups of women with higher dietary B(a)P and acrylamide intakes during pregnancy. Moreover, data on the genetic polymorphism *GSTP1*, which is known to be involved in detoxification processes of B(a)P, was available for both mother and child in the INMA study. This allowed to evaluate the potential effect modification of the association between exposure to B(a)P and fetal growth indicators by such genetic polymorphism. In the MoBa study, levels of acrylamide- and glycidamide-Hb adducts were available in a subset of samples within the study population. This data allowed the validation of the acrylamide estimates based on the FFQ.

In a separate sub-sample of the INMA study, the relationship between estimated dietary intakes of B(a)P and levels of bulky DNA adducts was assessed. It was not possible to include the results of the performed validation of B(a)P estimates against biomarkers of exposure in the results section of the thesis, since the data used comes from a EU funded project, the Newgeneris, and a similar analysis is planned to be soon performed in the whole project. However, the results of this validation analysis are presented in the appendix 1. As shown in the appendix, dietary B(a)P estimates were related to levels of bulky DNA adducts measured in cord blood among non-smoking women with low dietary intakes of vitamin C. A detailed discussion about these findings can be found in the appendix 1.

Finally, as mentioned in the methods section, this thesis included results from two very different populations, one from the South and the other from the North of Europe. Testing the same hypothesis in these two populations with large geographical, economical, and cultural differences is an advantage of this thesis.

6.4 Limitations

The generation of B(a)P and acrylamide in food is affected by several parameters. The levels of these compounds in food can substantially vary depending on the food preparation processes. The first limitation of this study is that the FFQs used to estimate dietary contaminant intakes, did not collect data on the frequency of use of different cooking methods or the preferred doneness of food, which could lead to the underestimation of total B(a)P or acrylamide intake. In this case, the crude estimation of these compounds intake would in principle lead to a weaker association between exposure and outcome than the true. Moreover, it must be noted that to date, only a limited number of studies have measured the levels of these compounds in few food after applying different cooking methods and doneness levels.

Large variations in B(a)P and acrylamide content of a single food item have been reported [2,145]. To take this into account, several exclusion criteria were applied to select the published values of B(a)P in food for the calculation of the mean concentration of B(a)P in each food, as mentioned in details in the methods section. We also performed an estimation of B(a)P intake using only the values published in European countries, and another estimation excluding the lowest and the highest values. No meaningful difference in the results was observed under any of these alternatives.

Regarding the estimation of acrylamide intake, a previous study conducted in a subset sample of the MoBa cohort, compared the traditional assessment based on FFQ and food diary, with the probabilistic method in which the usual intake based on two non-consecutive days of food consumption was modeled. The different assessment methods resulted in comparable dietary estimates of acrylamide exposure in pregnant women.

6.5 Public health implications

The public health implications of the findings presented in this thesis are substantial. This thesis provide evidence about the adverse effects of prenatal exposure to B(a)P and acrylamide through maternal diet on birth weight and the risk of SGA. We have shown that maternal intake of B(a)P and acrylamide during pregnancy were associated with the reduction of birth weight and increased the risk of SGA. As shown in the introduction section, previous studies demonstrated that reductions in birth weight and being SGA are associated with child and adult morbidity, including increased risk of delayed neurodevelopment [133], cardiovascular diseases, type 2 diabetes, and insulin resistance [114,194]. Thus, our findings might have implications for public health at earlier or later stages of life. However, further research is needed to replicate our findings in other populations and to assess the long-term effects of prenatal exposure to B(a)P and acrylamide through diet.

Additionally, higher reduction in birth weight due to maternal B(a)P intake was found among women with lower vitamin C intake. Therefore, maternal vitamin C intake during pregnancy seems to play a beneficial role against adverse effects of prenatal exposure to dietary B(a)P on birth weight. These results highlight the importance of reinforcing the recommendations for a correct vitamin C intake during pregnancy.

The identification of refined food subgroups that contribute most to the total dietary intake of B(a)P and acrylamide, as well as the food subgroups that strongly predict high levels of intake of these compounds among the population of pregnant women, facilitate the development of specific recommendations to aid pregnant women to reduce their dietary intake of these compounds during pregnancy.

Finally, we found a significant association between smoking and higher dietary B(a)P and acrylamide intakes during pregnancy. Tobacco smoke is accepted to be the main source of exposure to

these compounds among smokers (ref). The additional contribution of diet to B(a)P and acrylamide exposure during pregnancy among smokers is of concern. Thus, more efforts are needed to reduce dietary intake to food contaminants among this group of women.

6.6 Future research

This thesis presents the first epidemiological studies on measuring and determining the role of prenatal exposure to contaminants, specifically from maternal diet, on birth outcomes. Our results suggest that there is indeed an association between prenatal exposure to B(a)P and acrylamide through maternal diet and fetal growth indicators. There is a clear need of future studies that confirm our results and elucidate on the potential pathways of the association between prenatal dietary exposure to these compounds and fetal and child development.

More accurate measurements of total exposure to B(a)P and acrylamide through diet are also needed. Thus, studies should measure the levels of B(a)P and acrylamide in food after using different cooking methods and doneness. The variables proven relevant for estimating the intake should thus be collected in future epidemiological studies.

Experimental and epidemiological studies have suggested that prenatal exposure to acrylamide or B(a)P could be related to child outcomes not included in this project, such as neurodevelopment and respiratory disorders. However, the effect of prenatal exposure through maternal diet has not been studied before. An ongoing study within the INMA cohort addressed this question. Preliminary results show that maternal intake of B(a)P during the first trimester of pregnancy is associated with lower scores on infant's mental development at the age of 14 months. This finding is consistent with studies showing lower cognitive development at age 3 associated with cord blood PAH adducts. Respiratory health as well as cognitive development have also been recently assessed in children aged 4 in the INMA-Sabadell cohort, thus, the analyses

on the associations between prenatal exposure to dietary B(a)P and these outcomes will be soon possible. Additionally, there is also need of more experimental studies to better understand the role of prenatal exposure to contaminants through maternal diet on fetal and child development.

Finally, further research is needed to replicate our findings in other populations and to explore later adverse effects of prenatal exposure to contaminants from maternal diet, as well as to explore the possible synergic effects of prenatal exposure to the combination of different food contaminants.

7 CONCLUSIONS

7 CONCLUSIONS

- Smoking during pregnancy is associated with higher dietary B(a)P intakes during pregnancy compared to non-smokers in both the Spanish and the Norwegian cohort studies; INMA and MoBa, respectively.

  -Since tobacco smoke is an important route of PAH exposure, the added dietary burden in these women is of concern.

- The food groups that most strongly predicted high intakes of B(a)P among pregnant women are shellfish and processed/cured meat.

- Higher maternal dietary B(a)P intakes during pregnancy is associated with reduction of birth weight in the two European birth cohort studies (INMA and MoBa), and also with birth length and SGA in the INMA study. These results persist after excluding smokers.

  -Since reduced fetal growth has been related to short and long-term health outcomes, prenatal exposure to dietary B(a)P might have implications for public health at earlier or later stages of life.
  -Reduced dietary B(a)P intake among pregnant women might be beneficial for fetal growth.

- There are significant interactions between dietary B(a)P and vitamin C intakes for fetal growth indicators in the two studied populations. The effect of dietary B(a)P intake during pregnancy on fetal growth is stronger among women with low vitamin C intake.

  -Increasing maternal intakes of vitamin C during pregnancy may help to attenuate any adverse effects of maternal dietary B(a)P on fetal growth.

- In the Spanish cohort study, the presence of the genetic polymorphism *GSTP1* in mothers or children, increase the susceptibility to effects of maternal B(a)P intakes on fetal growth indicators.

  -Although this genetic polymorphism, *GSTP1*, has been related to detoxification capacity of contaminants such as B(a)P, future studies are needed to confirm this finding in other populations.

- In the MoBa study, higher maternal dietary acrylamide intakes during pregnancy is associated with an increase in risk of SGA and a reduction in birth weight, also after excluding women who reported smoking during pregnancy.

  -Consequently, prenatal exposure to dietary acrylamide might have implications for public health at earlier or later stages of life.
  -Reduced dietary acrylamide intake among pregnant women might be beneficial for fetal growth.

- The estimated dietary acrylamide intakes from the MoBa FFQ was validated by measurements of Hb adduct levels in a subset of maternal samples.

- The food groups that most strongly predicted high intakes of acrylamide in the Norwegian population are snacks, including potato chips, nuts, and popcorn; fried potatoes, and crisp bread.

  -Future studies are needed to estimate dietary acrylamide intakes during pregnancy and to explore its association with birth outcomes in other populations.

APPENDIX 1. Dietary B(a)P estimates and bulky DNA adducts in the INMA-NewGeneris study

Background and objectives: In order to assess the validity of estimated dietary intakes of B(a)P, we assessed the relationship between intake estimates and levels of bulky DNA adducts in a separate sub-sample. DNA adducts are widely used as a biomarker of PAH exposure [76–78]. Among non-smokers without occupational exposure, diet is thought to contribute perhaps 90% of total PAH exposure [5–7,47–50], and previous studies have shown dietary exposure to PAHs to increase levels of these DNA adducts [216,217].

Methods: This validation analysis was conducted in a sample of the INMA cohort recruited as part of the EU-funded NewGeneris project [207]. Maternal blood samples and lifestyle questionnaires similar to those in the main study were obtained during the third trimester of pregnancy from pregnant women participating in this study. Cord blood was collected at delivery.
Dietary intakes: FFQ data were used to estimate intakes of B(a)P, vitamin C and total daily energy intake (kcals/day).
Questionnaire data: Gestational age at blood sample collection was estimated based on the date of the last menstrual period, and self-report was used to identify women who smoked during pregnancy, as well as those exposed to high levels of environmental tobacco smoke (ETS).
Laboratory measures: Lymphocytes were isolated in maternal and cord blood samples, and 32P-postlabelling was used to estimate the content of bulky DNA adducts as described elsewhere [218]. After excluding smokers, among whom adducts may largely reflect tobacco exposure, adduct levels were available for a total of n=34 maternal and n=45 cord blood samples.

Data analysis: Current smokers were excluded from the analysis. Multivariate linear regression was conducted to assess the association between dietary B(a)P intake and bulky DNA adducts. In addition to crude models, multivariate models were run

adjusting for vitamin C intake and gestational age; adjusting for ETS exposure did not meaningfully affect results (not shown). Interaction terms and stratification were used to assess whether the relationship between dietary B(a)P and DNA adducts was modified by elevated vitamin C intakes (> the median). Generalized additive models (GAMs) were also used to assess the shape of B(a)P-adduct relationships. Analyses were repeated excluding 2 extreme outliers for DNA adducts (>78 adducts/10^8 nucleotides).

Results: Mean (SD) cord blood adduct levels were 20.9 (11.4); the 25^{th} and 75^{th} percentiles were 13.2 and 26.9, respectively. Overall, there was a positive but non-linear relationship between B(a)P intakes and cord blood adduct levels (**Figure 1a**). However, as there was a significant interaction between elevated vitamin C intakes (> the median) and high dietary B(a)P (interaction $P<0.05$), associations were assessed stratifying by vitamin C. As shown (**Figures 1b and 1c; Table 1**), dietary B(a)P was positively associated with cord blood adducts only among women with low vitamin C intakes.

Discussion: Dietary B(a)P estimates were related to levels of bulky DNA adducts measured in cord blood among non-smoking women with low dietary intakes of vitamin C. These data support the validity of estimated dietary intakes of B(a)P. These results are consistent with findings reported among US women by Kelvin et al [124], in which antioxidant vitamins were found to modify the relationship between B(a)P DNA adducts and atmospheric PAH estimated using personal exposure monitors. Women with low concentrations of vitamins E and beta-carotene in maternal serum were found to have higher levels of adducts, and strong relationships between atmospheric PAH and adducts levels; the opposite was true for women with higher concentrations of these vitamins. However, vitamin C was not examined in that study, while in our study interactions with vitamin E and beta-carotene were not observed. Disparities may be partly explained by differences in dietary sources of these nutrients. Given the high intakes of olive oil in Spain, added oils—which also contain PAHs—are the predominant source of dietary vitamin E [175], in contrast

to the wide variety of food sources in the US, including ready-to-eat cereals, breads and fruits and vegetables [176].

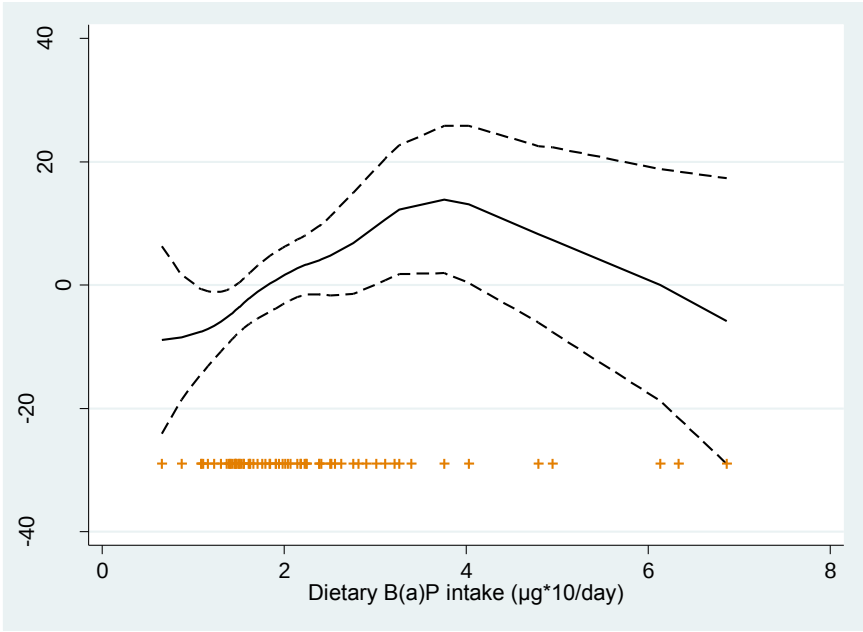
Table 1. Association between dietary B(a)P intake and bulky DNA adducts in cord blood: Results from linear regression models

	Vitamin C ≤ median		**Vitamin C > median**	
	Crude	**Multivariate**	**Crude**	**Multivariate**
CORD BLOOD (n=45)	Coeff±SE		Coeff±SE	
Dietary B(a)P *	**+15.1 ± 4.8‡**	**+21.0 ± 4.9‡**	-1.3 ± 1.1	-2.4 ± 1.6
Vitamin C (mg/day): log transformed	--	**-16.6 ± 8.4†**	--	+8.2 ± 8.2
Gestational age (days)	--	**-0.44 ± 0.2†**	--	+0.1 ± 0.1

Analysis conducted among non-smoking women.
Median vitamin C (cord blood sample) = 190.2 mg/day; Median vitamin C (maternal blood sample) = 183.9 mg/day.
B(a)P values converted to 10 µg/day.
‡ coefficient $p<0.05$. † $p<0.10$.
*p for interaction between > median of vitamin C intake and dietary B(a)P in cord blood sample < 0.001.

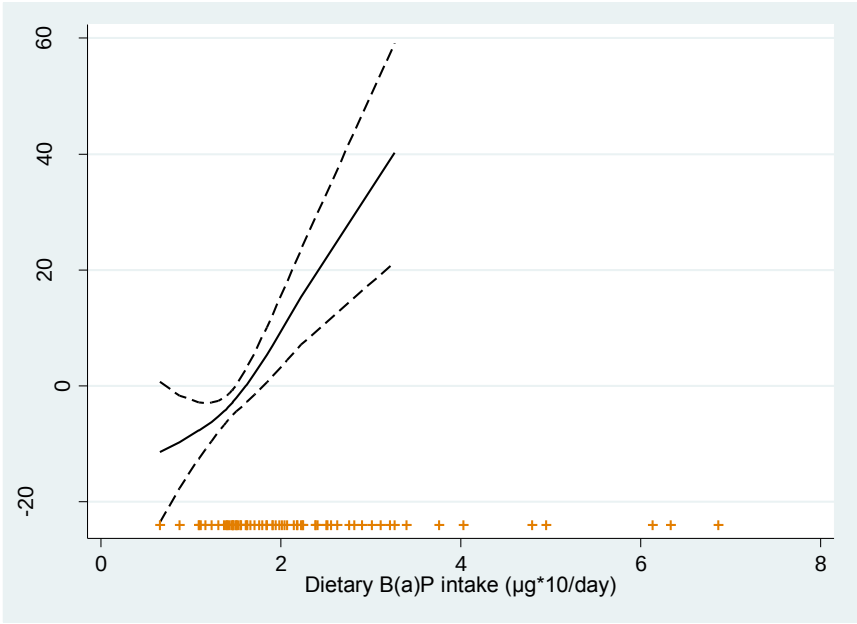
Figure 1. Generalized additive model plots (relationship and 95% confidence intervals) between dietary B(a)P intake estimates and bulky DNA adduct levels in cord blood samples among non smoking women.

a) Association between cord blood adducts and estimated B(a)P intakes: All women excluding smokers (n=45)



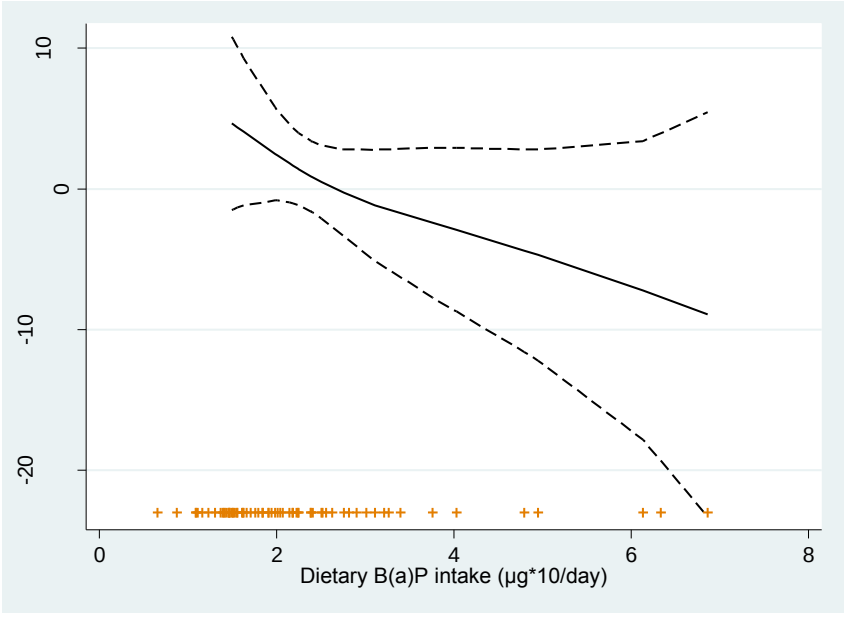
GAM plot for the non-linear model with lower AIC (degrees of freedom = 5), adjusted for log-transformed vitamin C intakes and gestational age.

b) Association between cord blood adducts and estimated B(a)P intakes: Non-smokers with dietary vitamin C <= median (n=23)



GAM plot adjusted for log-transformed vitamin C intakes and gestational age.

c) Association between cord blood adducts and estimated B(a)P intakes: Non-smokers with dietary vitamin C > median (n=22)



GAM plot adjusted for log-transformed vitamin C intakes and gestational age.

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