Thesis for doctoral degree (Ph.D.)
2022

Early-Life Metal Exposure and Child Growth and Development

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Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022

ISBN 978-91-8016-605-8
Cover illustration: adapted from a photograph by Nayeem Is. J Preenon.

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By

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To my family

POPULAR SCIENCE SUMMARY OF THE THESIS

Cadmium, lead and arsenic are toxic metals which occur naturally in the Earth's crust and are further spread by human activities such as mining. Vegetable crops absorb them from the soil and the water used to irrigate them, and people all over the world consume food and drinking water containing these metals. Whilst there is extensive evidence on how long-term exposure to these metals affects the health of adults, much less is known about their impact on children, especially regarding cadmium. It is suggested that early-life exposure to metals is linked to altered development in children which may play a role in their future health.

Therefore, the aim of this thesis was to clarify if exposure to cadmium, lead and arsenic early in life may affect child growth and pubertal development.

This was studied in around 2000 mothers and their children living in a rural area in southern Bangladesh. Pregnant women were asked to participate in the study, their background characteristics were recorded, and their exposure to metals was determined via measurements in urine and/or blood samples collected during pregnancy. After the children were born, they were examined by trained personnel and biological samples were collected on several occasions from birth until 15 years of age. The children's metal exposure was assessed via measurements in urine and blood at around 5 and 10 years, and, in a smaller group of these children, several biomarkers of bone health were measured in samples collected at 9 years of age. The children's weight and height were recorded several times, as well as how their puberty was developing.

We found that children with higher cadmium concentrations in urine and blood had lower vitamin D, which is a hormone that is important for the absorption of calcium from the diet and for growth and formation of bones. Children with higher cadmium exposure also had altered levels of biomarkers reflecting bone turnover. In particular, it seemed that in boys with higher cadmium exposure there was an imbalance between bone formation and bone degradation.

We also found that children with higher cadmium exposure were lighter and shorter around 10 years of age than children with lower exposure, and this difference was more evident in boys than in girls. Also, boys exposed to higher lead concentrations were lighter and shorter than those exposed to lower concentrations. Although many children were exposed to high levels of arsenic, we did not find any link between arsenic exposure and growth at 10 years.

As cadmium and lead are known to affect the body's hormone system, we studied if exposure to these metals during the mothers' pregnancy and during the daughters' childhood was linked to the girls' start of puberty. We discovered that the girls with the highest urinary cadmium concentrations got their first menstruation about 3 to 4 months later than the girls with the lowest concentrations. The relationship between the girls' urinary lead concentrations and their timing of puberty was less clear, but there was a suggestion of earlier puberty start in girls at the highest exposure levels at 10 years.

To conclude, this research provides important evidence that growth, bone health, and pubertal development of children can be negatively affected by cadmium from food at exposure levels relevant for millions of children around the world. As the recommended maximum exposure levels of cadmium are based on health effects in adults, these results emphasize the need to also focus on children's health in science and policy.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Kadmium, bly och arsenik är giftiga metaller som förekommer naturligt i jordskorpan och som dessutom sprids genom mänskliga aktiviteter, som exempelvis gruvdrift. Grödor absorberar metallerna från jorden och från vattnet som används för bevattning och människor över hela världen konsumerar mat och dricksvatten som innehåller dessa metaller. Medan det finns tydliga evidens för hur långtidsexponering påverkar vuxna människors hälsa så finns det avsevärt mycket mindre kunskap om hur dessa metaller påverkar barn, framförallt när det gäller kadmium. Man misstänker att exponering för dessa metaller tidigt i livet är kopplat till effekter hos barn som kan spela roll för deras framtida hälsa.

Därför är syftet med denna avhandling att klargöra huruvida exponering för framför allt kadmium, men även bly och arsenik, kan påverka barns tillväxt och pubertala utveckling.

Detta studerades i närmare 2000 mödrar och deras barn som lever på landsbygden i södra Bangladesh. Gravida kvinnor ombads delta i studien, deras bakgrund dokumenterades och deras exponering för dessa tre metaller mättes i urin- och/eller blodprover som togs under graviditetstiden. Efter att barnen fötts undersöktes de flertalet gånger av utbildad personal och biologiska prover samlades in från födsel till att de nådde ungefär 15 års ålder. Barnens exponering för metaller bedömdes via mätningar i urin och blod vid ungefär fem och tio års ålder och i en mindre grupp av dessa barn mättes även biomarkörer för benhälsa vid nio års ålder. Barnens vikt och längd mättes vid flera tillfällen, och deras pubertala utveckling dokumenterades.

Vi fann att barn som hade högre koncentrationer av kadmium i urin och blod hade lägre nivåer av D-vitamin, vilket är ett hormon som hjälper kroppen att absorbera kalcium från födan och som är viktigt för skelettets tillväxt och uppbyggnad. Barn med högre kadmiumexponering hade även förändrade nivåer av biomarkörer som reflekterade skelettets nedbrytning och uppbyggnad. Mer specifikt verkade det som att bland pojkar med högre kadmiumexponering saknades en balans mellan skelettets nedbrytning och uppbyggnad.

Vi fann även att barn som hade exponerats för högre nivåer av kadmium både vägde mindre och var kortare vid tio års ålder i jämförelse med barn som hade exponerats för lägre nivåer, och denna skillnad var mer uttalad bland pojkar än bland flickor. Liknande samband kunde även påvisas bland pojkar med högre blyexponering, då även dessa vägde mindre och var kortare i jämförelse med pojkar som hade lägre exponering. Trots att många av barnen hade exponerats för höga halter av arsenik så fann vi inte någon koppling mellan deras arsenikexponering och tillväxt vid tio års ålder.

Då både kadmium och bly påverkar kroppens hormonsystem så studerade vi om exponering för dessa metaller under mödrars graviditet och under döttrarnas barndom kunde kopplas till pubertetens start hos dessa flickor. Vi upptäckte att flickorna med de högsta urinhalterna av kadmium fick sin första menstruation mellan tre och fyra månader senare än flickorna med de

lägsta urinhalterna av kadmium. Det fanns även en indikation på att ökade urinhalter av bly vid tio års ålder var kopplade till en tidigare start av pubertet.

Sammanfattningsvis så har denna forskning medfört att man funnit tecken på att kadmium kan påverka barns tillväxt, skelettets hälsa och pubertal utveckling vid exponeringsnivåer som är relevanta för miljontals barn globalt. De rekommenderade högstanivåerna för kadmium är baserade på hälsoeffekter hos vuxna, och dessa resultat understryker behovet av att även fokusera på barns hälsa inom forskning och vid etablering av hälsoriskbedömningar.

ABSTRACT

Cadmium, lead and arsenic are toxic metals, the exposure to which occurs primarily through food and drinking water. While many studies are available about their health effects in adults, studies in children and adolescents are more limited, especially for cadmium.

The overall aim of this thesis was to assess if early-life metal exposure, especially cadmium, but also lead and arsenic, may affect children's growth and pubertal development at school-age.

This research was conducted in a large mother-child cohort in a rural area called Matlab, in southern Bangladesh. The cohort was nested in a randomized food and micronutrient supplementation trial called MINIMat (Maternal and Infant Intervention, Matlab), which was established in 2001-2003. Women were recruited during early pregnancy and their children were followed up repeatedly from birth up to the age of 15 years. Urine and/or blood samples were collected from the mothers during pregnancy and from the children at several time points to assess their exposure to metals and to measure various health-related biomarkers. The children's weight and height were measured during infancy, childhood and adolescence, and pubertal development in late childhood and adolescence.

In **Paper I**, we investigated if early-life cadmium exposure was associated with changes in bone-related biomarkers at 9 years of age (n=504), as cadmium has been linked to bone toxicity in adults. Using adjusted linear regression analyses, we found that both children's urinary cadmium (reflecting life-long exposure) and erythrocyte cadmium (reflecting the last few months) were associated with decreased levels of vitamin D3, a hormone involved in calcium homeostasis and with importance for bone mineralization. We also observed that childhood cadmium exposure was associated with changes in biomarkers of bone remodeling. Urinary cadmium was associated with an increase of both osteocalcin (biomarker of bone formation) and urinary deoxypyridinoline (DPD, biomarker of bone resorption). Interestingly, when stratifying the models by gender, we found that urinary cadmium was associated with an increase of osteocalcin in girls, but with a decrease of osteocalcin in boys, suggesting a dysregulation of the feedback mechanisms in bone remodeling in boys.

We also observed a tendency of an inverse association between urinary cadmium and weight-for-age Z-score (WAZ) at 9 years, but the confidence intervals were wide. Therefore, in order to ascertain this association, we investigated this in a larger subset of children in the MINIMat cohort in **Paper II** (n=1530). We also explored associations with the children's lead and arsenic exposure. In this larger sample, we found that concurrent urinary cadmium was inversely associated with WAZ and possibly also with height-for-age Z-score (HAZ) at 10 years, and that the associations were stronger in boys. We also found that short-term lead exposure, measured in urine, was associated with decreased WAZ and HAZ in boys, while neither maternal nor childhood arsenic exposure was associated with any measure of size, despite a very large variation in the arsenic exposure. In longitudinal analyses from birth to 10 years, we

found that maternal erythrocyte cadmium in early pregnancy was associated with decreased WAZ in boys.

As cadmium and lead are reported endocrine disruptors, we investigated if the exposure to these two metals was associated with changes of timing of menarche in girls in **Paper III** (n=935). Using survival analysis, we observed that an increased exposure to cadmium during childhood was associated with a delay in menarche. The potential impact of lead was less clear.

To conclude, this research provides important evidence that early-life exposure to dietary cadmium can adversely affect child growth and pubertal development at exposure levels relevant for millions of children around the world. This emphasizes the need for further research in children and adolescents and that research on children should be considered in updates of the international health risk assessment of cadmium.

LIST OF SCIENTIFIC PAPERS

This thesis is based on these three papers, which from here on will be referred to by their Roman numerals:

I. **Malin Igra A**, Vahter M, Raqib R, Kippler M. Early-Life Cadmium Exposure and Bone-Related Biomarkers: A Longitudinal Study in Children. *Environmental Health Perspectives.* 2019 Mar;127(3):37003.

II. **Malin Igra A**, Warnqvist A, Rahman SM, Ekström EC, Rahman A, Vahter M, Kippler M. Environmental metal exposure and growth to 10 years of age in a longitudinal mother-child cohort in rural Bangladesh. *Environment International.* 2021 Nov;156:106738.

III. **Malin Igra A**, Rahman A, Johansson ALV, Pervin J, Svefors P, El Arifeen S, Vahter M, Persson LÅ, Kippler M. Early-life environmental exposure to cadmium and lead and age at menarche: a longitudinal mother-child cohort study in Bangladesh. *Submitted.*

SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

- **Igra AM**, Harari F, Lu Y, Casimiro E, Vahter M. Boron exposure through drinking water during pregnancy and birth size. *Environment International.* 2016 Oct;95:54-60.

- Glynn A, **Igra AM**, Sand S, Ilbäck NG, Hellenäs KE, Rosén J, Aspenström-Fagerlund B. Are additive effects of dietary surfactants on intestinal tight junction integrity an overlooked human health risk? - A mixture study on Caco-2 monolayers. *Food and Chemical Toxicology*. 2017 Aug;106(Pt A):314-323.

- Herlin M, Broberg K, **Igra AM**, Li H, Harari F, Vahter M. Exploring telomere length in mother-newborn pairs in relation to exposure to multiple toxic metals and potential modifying effects by nutritional factors. *BMC Medicine*. 2019 Apr 11;17(1):77.

- Gliga A, **Malin Igra A**, Hellberg A, Engström K, Raqib R, Rahman A, Vahter M, Kippler M, Broberg K. Maternal exposure to cadmium during pregnancy is associated with changes in DNA methylation that are persistent at 9 years of age. *Environment International.* 2022 Mar 22;163:107188.

CONTENTS

LIST OF ABBREVIATIONS

As	Arsenic
As^{III}	Arsenite
As^{V}	Arsenate
BMD	Bone mineral density
BMDL	Benchmark dose level
b.w.	Body weight
Cd	Cadmium
CI	Confidence interval
DMA	Dimethylarsinic acid
DPD	Deoxypyridinoline
EFSA	European Food Safety Authority
Erα	Estrogen Receptor α
GFR	Glomerular filtration rate
GH	Growth hormone
GW	Gestational week
HAZ	Height-for-age Z-score
HDSS	Health and demographic surveillance system
HR	Hazard ratio
IARC	International Agency for Research on Cancer
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
ICP-MS	Inductively coupled plasma mass spectrometry
IGF-1	Insulin-like growth factor 1
IGFBP3	Insulin-like growth factor binding protein 3
LOD	Limit of detection
MMA	Monomethylarsonic acid
MT	Metallothionein
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
Pb	Lead

PTH	Parathyroid hormone
RANKL	Receptor activator of nuclear factor kappa-B ligand
SD	Standard deviation
TSH	Thyroid stimulating hormone
TWI	Tolerable weekly intake
U-Cd	Urinary cadmium
WAZ	Weight-for-age Z-score
WHO	World Health Organization

1 PREFACE

The Developmental Origin of Health and Disease (DOHaD) hypothesis proposes that unfavorable environmental conditions early in life play a role in the development of chronic disease in adulthood. This was originally suggested about the role of fetal malnutrition on cardiovascular disease at middle-age (Barker 1997), but it is an approach that can be applied to many different types of exposures and conditions, including the exposure to environmental contaminants.

The present thesis focuses on the toxic metal cadmium, which is ubiquitously present in the environment and contaminates food. Young children are particularly exposed, in part because they consume more food relatively to their body weight than adults. Nevertheless, until the last two decades, previous research had focused on the health effects of this metal in occupationally exposed workers and in elderly individuals after a lifetime of exposure, in whom it causes adverse health effects on especially kidney and bone.

Recently, it is becoming clear that cadmium exposure early in life may affect young children's growth and development. However, less is known about possible health effects later in childhood, and if they may be influential for children's future health decades later.

This thesis aims at contributing to fill the knowledge gap about the role of early-life exposure to cadmium in growth and development of children at peripubertal age. The toxicants lead and arsenic were also studied, as exposure to them through staple foods is common and, together with cadmium, they are considered by the World Health Organization (WHO) to be among the top ten chemicals of major public health concern (WHO).

2 BACKGROUND

2.1 CADMIUM

2.1.1 Exposure to cadmium

Cadmium (Cd) is a toxic metal ubiquitously found in the Earth's crust. It is released into the environment through both natural and anthropogenic activities. The environmental dispersion by natural activities may include volcanic activity, weathering of rocks, sea spray, and mobilization of previously deposited cadmium. Anthropogenic sources include industrial emissions (i.e. mining and smelting), pollution by use of cadmium-containing fertilizers, combustion of fossil fuels, waste incineration and releases from tailings and landfills. The dispersion of cadmium into the environment and widespread contamination of soil, especially on arable land, in many areas of the world is of concern, as cadmium is easily taken up by vegetable crops, such as rice, wheat, vegetables and potatoes, as well as the tobacco plant. In fact, it has been emphasized that the exposure via food in many areas is high enough to be of importance to human health (EFSA 2009).

Occupational cadmium exposures have generally decreased since the 1970's. Occupations in which exposure may occur include manufacturing and refining of cadmium and cadmium-containing products, like nickel-cadmium batteries and pigment, and also production of alloys, mechanical plating and zinc smelting. In the general population, food is the main source of cadmium exposure world-wide. It has been estimated that more than 80% of the dietary cadmium comes from vegetables crops (Amzal et al. 2009), especially cereals, root vegetables, potatoes and vegetables (Olsson et al. 2002). High concentrations can be found in shellfish and offal meat (EFSA 2009), but, as these food items are more rarely consumed than plant-based foods, they contribute less to the daily intake.

Drinking water is normally not a source of cadmium (Olsson et al. 2002). The daily cadmium intake in Sweden, where a varied diet is usually consumed, has been estimated to be around 15 µg, while populations relying on rice as the main staple food, for example in many parts of Asia, have a higher intake (Song et al. 2017). As the tobacco plant effectively absorbs cadmium from the soil, and 50% of inhaled cadmium is absorbed in the lung, smokers are additionally exposed (ATSDR 2012). A lifetime of regular tobacco smoking is estimated to contribute with as much cadmium as the diet (Barregard et al. 2010). Even second-hand smoke has been suggested to increase blood cadmium concentrations in women (Jung et al. 2015), while blood cadmium concentrations in 7-10-year old-children of smoking or non-smoking parents did not differ (Willers et al. 1992). Concentrations in ambient air are generally low (Vahter et al. 1992).

2.1.2 Toxicokinetics and biomarkers of exposure

Around 5% of the cadmium present in the diet is absorbed in the gastrointestinal tract in adults. However, the absorption may vary depending on the composition of the food and on the individual's nutritional status (Berglund et al. 1994). The gastrointestinal uptake of cadmium is mediated mainly through the same transporters involved in the absorption of iron, zinc and

calcium, such as the divalent metal transporter 1 (DMT1), the Zrt- and Irt-related protein (ZIP) of zinc transporter family (ZIP14), and the Ca^{2+}-selective channel Transient Receptor Potential Vanilloid subfamily member 6 (TRPV6) (Satarug 2018). This is supported by epidemiological studies, in which especially iron deficiency has resulted in increased cadmium concentrations in both blood and urine (Akesson et al. 2002; Berglund et al. 1994).

In addition, pregnant women, who have an increased need for iron, have been found to have higher cadmium concentrations in blood shortly after pregnancy than in early pregnancy (Kippler et al. 2009), and it was observed that urinary cadmium concentrations increased with the number of completed pregnancies (Akesson et al. 2002). Children may also have an unmet need for iron, and a study on 1 year old infants found that their gastrointestinal absorption of cadmium was approximately 18%, with large variations among the children, ranging from 4% to 37% (Crews et al. 2000). This is possibly due to that iron uptake is not regulated in small children as in adults (Lönnerdal 2017). In studies of suckling piglets, it was found that the expression of the iron transporters was not higher in iron-deficient piglets than in piglets with adequate iron stores (Ohrvik et al. 2007).

Once cadmium is absorbed by the enterocytes and it reaches the liver, it upregulates and binds to the low molecular protein metallothionein (MT) (Klaassen et al. 1999). Cadmium in the systemic circulation is concentrated almost exclusively in erythrocytes (Carlson and Friberg 1957). The small amounts in plasma are mainly transported bound to albumin or to MT or glutathione. As the majority of the circulating cadmium is bound to erythrocytes (life span around 3 months), the erythrocyte fraction or whole blood is considered to be a good biomarker of recent exposure.

Because of the small size of MT (6-7 kDa), the Cd-MT complex in plasma is filtered through the kidney's glomeruli, and subsequently reabsorbed by the renal proximal tubuli, resulting in accumulation in the cortex of the kidney, where it has a half –life of decades (Akerstrom et al. 2013). The cadmium concentration in urine is correlated to the concentration in the kidneys (Akerstrom et al. 2013). Therefore, urinary cadmium is considered to be a biomarker of chronic exposure. While some studies have used cadmium in hair as a biomarker of exposure, others have shown that it does not appear to reflect the internal dose (Skroder et al. 2017).

2.1.3 Current risk assessment and health effects in adults

As cadmium accumulates in the body over the lifetime, previous research has almost exclusively focused on health effects occurring later in life.

As mentioned above, cadmium accumulates in the proximal tubular cells of the kidneys where it causes tubular damage, resulting in renal dysfunction. The early sign of tubular damage is proteinuria, which is the presence of low-molecular weight proteins in urine due to the inability of the renal tubuli to reabsorb them after the primary filtration. Biomarkers of proteinuria include for example β2-microglobulin, the enzyme N-acetyl-β-glucosaminidase (NAG), and α1-microglobulin, among others (Satarug 2018). Tubular damage is the critical effect at the basis of the current health risk assessment of cadmium, which estimated the tolerable weekly

intake (TWI) of cadmium to be 2.5 µg/kg body weight (b.w.) (EFSA 2009). Vegetarians have an average intake of as much as 5.4 µg Cd/kg b.w. in Europe. Children's cadmium exposure is estimated to be 60% higher than that of adults, primarily because they consume more food relatively to their body weight (EFSA 2009). In the evaluation by the European Food Safety Authority (EFSA) it was estimated that the Swedish general population has an average intake of
2.3 µg Cd/kg b.w., which is very close to the TWI (EFSA 2009). In a Chinese study, the mean dietary cadmium intake of adults was estimated to be around 3.3 µg/kg b.w. per week, with high consumers being exposed to as much as 8.0 µg/kg b.w. per week due to the extensive intake of rice (Song et al. 2017). The mean total intake of cadmium from the diet was estimated to be 4.0 µg/kg b.w. per week in Bangladesh (assuming a body weight of 60 kg) (Al-Rmalli et al. 2012).

Dietary cadmium exposure has also been associated with increased risk of cardiovascular disease (Barregard et al. 2016; Fagerberg and Barregard 2021). In addition, cadmium is classified as a human carcinogen (group 1) by the International Agency for Research on Cancer (IARC), based on lung cancer in occupational studies (Faroon et al. 2012). Recently, there have been reports suggesting that cadmium may increase the risk of hormone-related cancers, such as endometrial and breast cancer (Akesson et al. 2008; Larsson et al. 2015), possibly related to that cadmium seems to be able to act as an endocrine disruptor (Johnson et al. 2003).

Cadmium has also been shown to affect bone health in elderly individuals, most dramatically in the case of Itai-Itai disease which occurred in certain endemic areas in Japan in the 1950's. This disease, mostly reported in elderly women and defined by osteomalacia, fractures as well as renal dysfunction, was caused by high cadmium exposure due to intake of rice from fields irrigated with cadmium polluted wastewater from nearby industries (Nordberg 2009). More recently, also low-to-moderate cadmium exposure has been associated with increased risk of osteoporosis and fractures (Akesson et al. 2014; Alfvén et al. 2000; Nawrot et al. 2010). However, the critical exposure level is still not defined.

2.1.4 Early-life cadmium exposure and child growth

During pregnancy, cadmium accumulates in the placenta (Esteban-Vasallo et al. 2012; Kippler et al. 2010a), and only a small portion reaches the fetus directly. Because of this, the possible toxicity of cadmium to unborn children was until the beginning of the 21st century largely unexplored. Since then, there has been emerging evidence that gestational cadmium exposure can nonetheless affect the fetus, especially fetal growth (Flannery et al. 2022). Among the first large scale epidemiological studies, a study of pregnant Bangladeshi women (median urinary cadmium concentration 0.63 µg/L, range 0.19-2.1 µg/L), found that maternal urinary cadmium concentrations over 1.5 µg/L were associated with decreased fetal growth, assessed through ultrasound (Kippler et al. 2012c). In a follow-up study in the same cohort, maternal urinary cadmium concentrations in early pregnancy were inversely associated with infant weight and head circumference at birth (Kippler et al. 2012a). Also, in both studies, there was an evident sex difference with more prominent associations in female fetuses and female newborns than

in males. In infant girls, an increase of 1 µg/L of cadmium in maternal urine resulted in a decrease of 45 g in birth weight (Kippler et al. 2012a). Interestingly, the observation of female susceptibility to gestational cadmium exposure has been repeated in several other populations since this initial finding (Table 1).

Table 1. Summary of studies exploring the association between maternal cadmium exposure during pregnancy and size at birth differed by infant sex.

Study location	n	Exposure	Outcome	Reference
South Africa	641	Maternal blood, mean 0.25 µg/L	Decreased birth weight in female newborns	Röllin et al. 2015
USA	396	Maternal urine GW15, mean 0.31 µg/g creatinine	Decreased birth length in female newborns	Romano et al. 2016
UK	4191	Maternal blood GW11, mean 0.56 µg/L, median 0.29 µg/L, range 0.14-6.3	Decreased birth weight, head circumference and crown-heel length in female newborns	Taylor et al. 2016
China	282	Maternal urine GW13 (mean 0.51 µg/g creatinine), GW24 (0.59 µg/g creatinine) and GW35 (0.61 µg/g creatinine)	Decreased birth weight in females (association with 1st trimester exposure)	Cheng et al. 2017
UK	275	Maternal blood at GW12, median 0.023 µg/L	Decreased birth weight in male newborns	Luo et al. 2017
China	237	Maternal urine at delivery, mean 1.48 µg/g creatinine	Decreased birth weight, length and head circumference in female newborns	Y Zhang et al. 2018

Abbreviation: GW, gestational week.

Of note, the inverse association between cadmium exposure and child growth was not only found to be evident at birth, but to persist into childhood. The Bangladeshi children studied by Kippler and colleagues (Kippler et al. 2012a; Kippler et al. 2012c) were followed up at 5 years of age and it was found that the children's concurrent cadmium exposure was inversely associated with weight and height, and once again more markedly in girls (Gardner et al. 2013). On the other hand, the effect from the prenatal exposure was no longer present at 5 years, suggesting that different mechanisms of cadmium toxicity are involved during different stages of growth. Other longitudinal studies have observed an association between maternal urinary cadmium concentrations during pregnancy or cadmium concentrations in cord blood and decreased growth during childhood (Chatzi et al. 2018; Delvaux et al. 2014; Lin et al. 2011).

Studies investigating the association of early-life cadmium exposure and growth at school-age are scarce.

2.2 LEAD

2.2.1 Exposure to lead

The metal lead (Pb) has been used for millennia for a large variety of applications due to its ductility, low melting point, and resistance to oxidation. Among others, common uses have been in battery manufacturing, paint, water pipes, ammunition, and as an additive in petrol.

The anthropogenic activities that have mostly contributed to the spread of lead in the environment have been mining, smelting, and especially the combustion of leaded petrol, the latter which is now banned almost worldwide.

Environmental exposure to lead occurs largely through food and drinking water. Crops absorb lead from the soil, and cereal products and vegetables have been shown to be a major dietary contributor (EFSA 2013). Game meat can be contaminated through the use of lead ammunition, and it can therefore be an important exposure source in the individuals who consume it regularly. Drinking water can be contaminated from water pipes and faucets containing lead, particularly if the water is acidic or soft (EFSA 2013). Inhalation of indoor dust can also be an exposure source depending on the type of housing materials and wall paint, as is ingestion of dust and soil in young children due to their frequent hand-to-mouth behavior. Children can also ingest lead by chewing on toys (EFSA 2013), in which the use of lead has been banned in Europe and the U.S. but is still ongoing in other parts of the world.

Mean lead dietary exposure in European adults was estimated to be in the range of 0.36 to 1.24 μg/kg b.w. per day (EFSA 2013).

2.2.2 Toxicokinetics, biomarkers of exposure and health effects

Approximately 5-10% of ingested lead is absorbed in the gastrointestinal tract of adults. Absorption in children has been reported to be higher than in adults, and deficient iron status in children is associated with an increased lead uptake (EFSA 2013). Lower dietary calcium intake also seems to increase gastrointestinal lead uptake, both in adults and in children (EFSA 2013).

In blood, 96% to 99% of the lead is found in erythrocytes (EFSA 2013). As for cadmium, the half-time of lead in blood is determined by the life cycle of erythrocytes, and therefore blood or erythrocyte lead concentrations reflect recent exposure of the past few months. Lead accumulates in bone tissue, which contains around 90% of the body burden. It accumulates both in cortical bone, where it is inert and has a half-life of several decades, and trabecular bone (EFSA 2013). Therefore, lead concentrations in bone are considered to be a biomarker of long-term exposure. Lead in trabecular bone can be dislodged during periods of intense bone remodeling, such as during pregnancy (Gulson et al. 2016), or bone breakdown as in osteoporosis (Gulson et al. 2002). It is not known if lead is released from the bone during periods of rapid growth in children. Excretion of lead occurs primarily in urine and in feces. Urinary lead is a short-term exposure biomarker, with day-to-day variability (EFSA 2013).

In adults, the risk assessment is based on chronic kidney disease and effects on systolic blood pressure (EFSA 2013). Lead passes both through the placenta and the blood-brain barrier. The critical health effect at the basis of the current risk assessment of lead exposure in children is developmental neurotoxicity, for which the benchmark dose lower confidence limit ($BMDL_{01}$) was estimated to be 12 μg/L in whole blood, corresponding to 0.50 μg/kg b.w. per day from food (EFSA 2013). Early-life lead exposure has also been associated with stunting and delayed

puberty in children (Gleason et al. 2016; Raihan et al. 2018; Nkomo et al. 2018; Wu et al. 2003).

2.3 ARSENIC

2.3.1 Exposure to arsenic

Arsenic (As) is a metalloid found in the environment in inorganic and organic form. The inorganic forms are arsenite (As^{III}) and arsenate (As^{V}), which are found in bedrock and from there they can leach into ground water. Rice also contains inorganic arsenic. Organic arsenic forms, such as arsenobetaine and arsenosugars, are less toxic and are found in seafood. Other organic forms found in food, including rice, are the metabolites of the biotransformation of inorganic arsenic, monomethylarsonic acid (MMA) and especially dimethylarsinic acid (DMA) (EFSA 2010; WHO 2017).

Humans are primarily exposed to inorganic arsenic through drinking water. More than 140 million people around the world are reported to be exposed to drinking water with arsenic concentrations that exceed the WHO guideline value of 10 µg/L (Ravenscroft 2009). Countries with a known problem of high arsenic concentrations in the drinking water are Bangladesh, India, Taiwan, as well as parts of the U.S., South America and China. Populations with a rice-based diet are also prone to elevated arsenic exposure; a study reported a median arsenic concentration of 142 µg/kg dry weight in rice from Bangladesh (Gardner et al. 2011a).

2.3.2 Toxicokinetics, biomarkers of exposure and health effects

Once ingested, 80-90% of inorganic arsenic is absorbed by the gastrointestinal tract. It is transported to the liver, where it is metabolized into MMA and then further into DMA largely by the enzyme arsenite methyltransferase (AS3MT).

In the blood, arsenic is distributed between erythrocytes and plasma; the proportion between the compartments varies depending on dose and valency of arsenic. In a study of 9-year-old children in Bangladesh, the median concentration in plasma was 27% of the median concentration in erythrocytes (Skröder et al. 2018). As with cadmium and lead, the arsenic concentration in erythrocytes is a biomarker of recent exposure. Arsenic is predominantly excreted in urine, where it has a half-life of around a few days and consists of both inorganic forms and the organic metabolites MMA and DMA (Vahter 2002). Nails and hair can also be useful biomarkers reflecting the internal dose of arsenic because it binds to sulphur-rich proteins in these matrices (Skroder et al. 2017).

Arsenic is classified as a human carcinogen (group 1) by IARC. Exposure to inorganic arsenic is associated with lung, bladder, and skin cancer (IARC 2012). The current provisional tolerable weekly intake (PTWI) of 15 µg/kg b.w. by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is deemed by EFSA to not be low enough to protect from cancer in these organs, as well as skin lesions, which can be early signs of chronic arsenic toxicity. EFSA identified a range of $BMDL_{01}$ values between 0.3 and 8 µg/kg b.w. per day (EFSA 2010).

The estimated upper and lower bounds for the mean exposure to total arsenic in food in European adults are 0.94 and 1.2 µg/kg b.w. per day (EFSA 2010), and thus there is little or no margin of exposure. In 1993, the WHO established a guideline value of 10 µg/L for arsenic in drinking water (WHO 2017). It has been estimated that the WHO guideline entails a risk of 1-3 cancer cases per 1,000 individuals consuming 1 liter per day of water with 10 µg As/L, which is a higher risk than what is usually accepted (1 case in 100,000 individuals) (National Research Council Subcommittee to Update the Arsenic in Drinking Water 2001). Other epidemiological studies have also found associations of exposure to inorganic arsenic with cardiovascular and respiratory diseases and diabetes (Nardone et al. 2017; Navas-Acien et al. 2005; Navas-Acien et al. 2006).

Inorganic arsenic passes through the placenta, exposing the growing fetus (Concha et al. 1998). Maternal exposure to arsenic during pregnancy has been found to be associated with mortality and morbidity outcomes in the offspring (Quansah et al. 2015; Rahman et al. 2017), including impaired immune function (Ahmed et al. 2014), and increased risk of respiratory tract infections and diarrhea (Rahman et al. 2017).

The relationship between arsenic exposure early in life, both measured in mothers during pregnancy and during the first years of life of the offspring, and child growth and morbidity was recently reviewed (Rahman et al. 2017). Many studies originated from Bangladesh, where arsenic exposure was associated with smaller size at birth (Rahman et al. 2009), at 2 years (Saha et al. 2012), and at 5 years old (Gardner et al. 2013). However, findings of other studies investigating early-life arsenic exposure and infant and child growth were heterogeneous (Rahman et al. 2017). Longitudinal studies investigating the association between arsenic exposure and growth later in childhood are lacking.

2.4 GROWTH AND DEVELOPMENT OF CHILDREN

All the metals introduced above have been shown to be associated with impaired fetal growth, and cadmium and lead have been reported to act as endocrine disruptors.

Here is a short overview of child growth, bone health and puberty onset, which are processes that are hypothesized to be affected by these metals.

2.4.1 Growth

Linear growth is important for the attainment of adult height. Disruption of linear growth is also associated with future negative health outcomes (Victora et al. 2008). Impaired cognitive development, cardiovascular disease, metabolic syndrome, and unfavorable maternal reproductive outcomes have been reported to be associated with stunting or catch-up growth (De Sanctis et al. 2021; Victora et al. 2008).

The fastest growth period happens *in utero*, between gestational weeks (GW) 20 and 24, when the fetus grows 2.5 cm per week. The rate of growth is still high during the first year of life, increasing by on average 25 cm in a year, and slows down from approximately 18 months.

Growth in infants is largely influenced by nutritional status, while hormonal regulators are more important than nutrition after 2 years of age (Benyi and Sävendahl 2017). The growth velocity is then slower until the start of the pubertal growth spurt, which leads to an increase in height of 20-25 cm in girls and 25-30 cm in boys. After reaching peak high velocity in later stages of puberty, growth velocity declines steeply, and adult stature is obtained (Benyi and Sävendahl 2017).

Several hormone systems are involved in the regulation of linear growth, as summarized in a recent review (Benyi and Sävendahl 2017). The growth hormone (GH) is secreted by the pituitary gland and stimulates the secretion of insulin-like growth factor 1 (IGF-1) from the liver (Kanbur et al. 2005). They are potent anabolic hormones and they both act directly on the growth plate, i.e., the area at the end of long bones where elongation happens. IGF-1 acts on the growth plate by stimulating proliferation and hypertrophy of chondrocytes and by promoting ossification through osteoblasts. IGF-1 has an anabolic effect on every tissue of the body, and apart from its role in the growth plate, it also promotes bone mineralization and increase in muscle mass (Benyi and Sävendahl 2017). During puberty, testosterone activity leads to higher IGF-1 levels (Wood et al. 2019). Thyroid hormones are also important for linear growth, regulating chondrocyte maturation, mineralization and secretion of cartilage matrix (Combs et al. 2011). Estrogens are the determinant in closing the growth plate in both girls and boys at the end of the pubertal growth spurt (Benyi and Sävendahl 2017). Leptin, a hormone produced by adipose tissue as well as muscle, placenta, and the pituitary gland, promotes chondrocyte proliferation and differentiation, and stimulates GH secretion.

A pathway through which malnutrition during childhood affects linear growth is by lower IGF-1 levels, which are increased with higher protein intake, as well as through lower leptin and insulin concentrations (Benyi and Sävendahl 2017). Other factors affecting linear growth are socioeconomic conditions, genetics, and the exposure to toxic chemicals (Bellinger 2012; Black et al. 2013).

2.4.2 Bone health

The skeleton and teeth contain 99% of the calcium in the body. Bones mainly consist of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] combined with collagen and mineral salts. Bone is a dynamic tissue that is first modeled during fetal and infant growth, and then remodeled throughout life to maintain the shape of the bone during growth, to repair fractures, and to regulate the calcium concentration in plasma. The most abundant cells in bone tissue are osteocytes (90-95%) (Schaffler and Kennedy 2012). Chondrocytes are found at the end of long bones in the growth plate, where they proliferate during linear growth and are finally replaced by osteoblasts in the process of endochondral ossification (Hinton et al. 2017). Bone remodeling is finely tuned by feedback mechanisms (Raggatt and Partridge 2010) and involves the process of bone resorption by osteoclasts and bone formation by osteoblasts. Osteoblasts produce collagen type 1, osteocalcin (the most abundant non-collagenous protein) and alkaline phosphatase (an enzyme for the incorporation of inorganic phosphate to form hydroxyapatite). Parathyroid hormone (PTH) and vitamin D are two hormones involved in plasma calcium and

phosphate homeostasis to enable adequate bone formation. PTH acts to induce the concentration of calcium in plasma by upregulating its reabsorption in the kidneys and by stimulating bone resorption. Vitamin D acts mostly on the small intestine to absorb calcium and phosphate, but also directly on bone tissue to stimulate osteoblasts to produce osteocalcin (Anderson et al. 2013; Morris et al. 2012).

There are two types of bone tissue: cortical bone, which is dense and compact and constitutes 80% of the total bone mass, and trabecular bone, which is light and porous and makes up the remaining 20%. Cortical bone is mainly found in long bones, while trabecular bone is present in the vertebrae, the pelvis, and at the ends of long bones. Trabecular bone is perfused with blood vessels and has a faster rate of metabolism, and it is the primary location within the skeleton from which calcium is obtained in periods of increased demand. Bone mineral density (BMD) is the amount of mineral (mainly calcium) stored in bone, and it can be measured through dual energy X-ray absorptiometry. Lower BMD is predictive of an increased risk of fractures (Cummings et al. 1993). Osteoporosis is a disease characterized by low bone mass and deterioration of the structure of bone which result in fractures. In osteoporosis, there is an imbalance between the bone remodeling processes, where bone resorption dominates over bone formation. Known risk factors for developing osteoporosis include heredity, older age, female sex, smoking, low body mass index, early menopause, low intake of vitamin D and calcium, among others (Pouresmaeili et al. 2018).

Osteoporosis has been described as a “pediatric disease with geriatric consequences” (Hightower 2000). A key factor for future skeletal health is the acquisition of an adequate peak bone mass. Although peak bone mass is reached between 20 and 30 years of age (Berger et al. 2010), most of bone acquisition is obtained during growth and development (Baxter-Jones et al. 2011), and nutritional and lifestyle factors are influential in gaining bone mass (Weaver et al. 2016) (Figure 1). Simulations have shown that a 10% increase in peak bone mass would delay osteoporosis by 13 years (Hernandez et al. 2003).

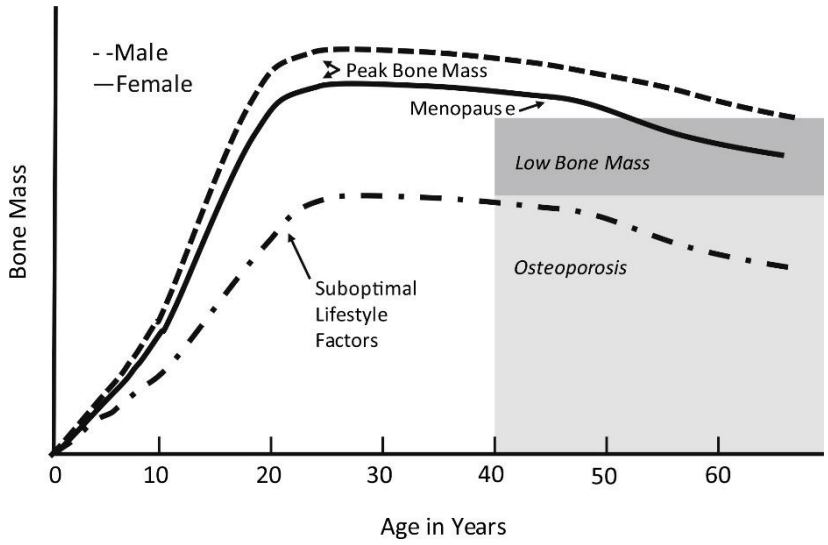

Figure 1. Changes in bone mineral mass over the lifetime, and impact on peak bone mass by suboptimal lifestyle factors, including diet and nutritional status. Reproduced from Weaver et al. (2016).

The cost of osteoporosis to society and healthcare is enormous, both monetarily and in loss of quality of life. In the U.S., it was estimated that fractures caused by osteoporosis lead to more hospitalizations than heart attacks, strokes and breast cancer combined (Kralick and Zemel 2020). A recent meta-analysis estimated the prevalence of osteoporosis to be 21.7% among the whole world's elders, reaching 24.3% in Asia (Salari et al. 2021).

2.4.3 Puberty onset

Puberty is the transitional phase between childhood and reproductive maturity. The onset of puberty is started by the activation of the hypothalamic-pituitary-gonadal (HPG) axis, triggering the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. GnRH then leads to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. These in turn act on the gonads to produce and secrete sex steroids and peptide hormones leading to the development of both primary and secondary sexual characteristics (Pinilla et al. 2012).

In girls, the first sign of puberty is breast budding, thelarche, which occurs approximately 2 years before the first menstruation, menarche (Wood et al. 2019). Menarche is induced by the fluctuating and increasing circulating levels of the sex hormone estradiol (Pinilla et al. 2012). After menarche, it takes around a year for the menstrual cycle to become regular. In boys, the marker of puberty onset is a testicular volume of 4 ml (Wood et al. 2019). The progress of puberty is commonly characterized with the help of Tanner stages, which describe the physical characteristics of pubertal development. The Tanner scale goes from 1 to 5; it categorizes the development of pubic hair and genitals in boys, and the development of pubic hair and breasts in girls (Marshall and Tanner 1969; Wood et al. 2019). The growth spurt associated with puberty always occurs before menarche in girls, usually at Tanner developmental stage 2. Instead, boys experience their growth spurt later into puberty, at around stage 3-4 (Wood et al. 2019).

Around half of the variability in the timing of puberty onset is explained by heredity (Towne et al. 2005). Nutrition can also play a role, where consumption of animal protein is associated with earlier menarche, and consumption of more vegetables is associated with later menarche (Canelón and Boland 2020). Stunting during childhood has been reported be associated with later puberty onset (Svefors et al. 2020). Exposure to different endocrine disrupting compounds have been found to be associated with both earlier and later onset of puberty (Lopez-Rodriguez et al. 2021; Schoeters et al. 2008). Epigenetic mechanisms are thought to be one way in which timing of menarche can be affected (Toro et al. 2018)

The timing of puberty onset has been found to be associated with various health conditions later in life. Earlier puberty is associated with an increased risk of cardiovascular disease, type 2 diabetes, and cancer (Day et al. 2015; Kim and Je 2019; Lee et al. 2019; Werneck et al. 2018). Instead, delayed puberty has been reported to be associated with an increased risk of osteoporosis both in men and in women (Vandenput et al. 2019; Bonjour and Chevalley 2014).

3 RESEARCH AIMS

The overall purpose of the studies included in this thesis was to elucidate the role of early-life environmental metal exposure on growth and development in school-aged children, with focus on cadmium and gender differences.

Specifically, this thesis aimed to clarify:

- The impact of cadmium exposure during the mother's pregnancy and during childhood on bone-related biomarkers at 9 years of age (**Paper I**),

- If exposure to cadmium, lead and arsenic during the mother's pregnancy and during childhood was associated with growth up to 10 years of age (**Paper II**),

- If exposure to cadmium and lead during the mother's pregnancy and during childhood had any impact on age at menarche, as a measure of timing of puberty onset in girls (**Paper III**).

4 MATERIALS AND METHODS

4.1 STUDY AREA AND PARTICIPANTS

4.1.1 Matlab and the arsenic problem

The studies included in this thesis were conducted in a mother-child cohort in Matlab, a rural region in Bangladesh located approximately 53 km south-east of the capital Dhaka (Figure 2). The climate of this area is sub-tropical and the year is divided into three seasons: monsoon, post-monsoon (cool-dry), and pre-monsoon (hot-dry). The Dhonagoda river flows through Matlab and it annually floods the surrounding areas because of the heavy precipitations during the monsoon season. Most of the population lives in poor socioeconomic conditions, in small single room houses with a dirt floor, bamboo walls and a tin roof. Agriculture is the primary occupation. The diet is heavily reliant on rice, and on vegetables and pulses, and some freshwater fish and very little meat. The most frequently consumed drinking water is obtained from tube wells that were installed in the 1970's. Since 1966, the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) has also been running a health and demographic surveillance system (HDSS) in Matlab. The HDSS provides regular visits by community health workers and collects data about births, deaths, marriages and in- and out-migration in the area. The mother-child cohort was established in half of the HDSS area (block A, B, C and D, Figure 2; population of about 110,000), where icddr,b provides health services to women of reproductive age and child health care through four health care facilities which are linked to the hospital in Matlab.

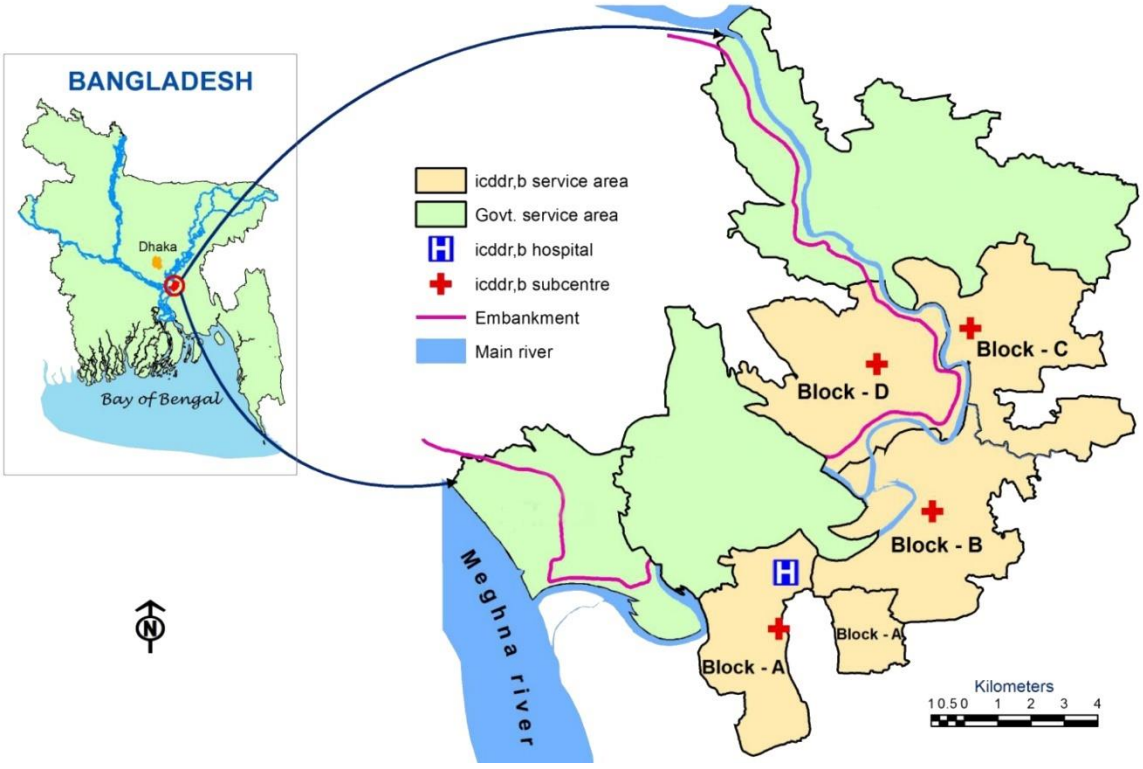

Figure 2. Map of the region of Matlab in Bangladesh, including the location of the hospital and the four health care facilities. Adapted from (icddr,b 2017).

The mother-child cohort was nested in a randomized food and micronutrient supplementation trial called MINIMat (the Maternal and Infant Nutrition Intervention, Matlab), with the aim to assess if a combination of food and micronutrient supplementation would improve pregnancy outcomes and decrease infant and child mortality (Persson et al. 2012).

The MINIMat trial enrolled women in the early stage of pregnancy between November 2001 and October 2003. The eligibility criteria were a viable fetus with a gestational age less than 14 weeks (assessed via ultrasound), no severe illness, and a written consent for participation. In total, 4436 pregnant women were enrolled, and they were randomly assigned to one of three different supplementation groups [30 mg iron and 400 µg folate (standard treatment), or 60 mg iron and 400 µg folate, or a capsule containing 15 recommended micronutrients including 30 mg iron and 400 µg folate; starting at gestational week (GW) 14] and to food supplementation which was either provided early (around GW9) or at the usual timing (GW20) (Persson et al. 2012). The food supplementation was provided 6 days per week and consisted of a paste to mix with water, containing 80 g of roasted rice powder, 40 g of roasted pulse powder, 20 g of molasses, and 12 mL (6 g) of soybean oil, amounting to 608 kcal.

Out of the 4436 pregnant women, 845 were lost to follow up, mainly due to pregnancy loss, withdrawal of consent or out-migration from the study area. There was a total of 3625 live births between April 2002 and June 2004, of which 3560 were born as singletons and 65 from twin pregnancies (Persson et al. 2012). Birth anthropometry was available for 3267 of these singleton births.

It was soon found that the drinking water consumed in the area commonly had high arsenic concentrations, 40% over the WHO limit of 10 µg/L (Vahter et al. 2006). This spurred the need for a long-term longitudinal research project to evaluate the potential health effects of arsenic exposure and other environmental contaminants, focusing on outcomes related to child growth and development. For this purpose, the nested mother-child cohort was established. For exposure assessment in early pregnancy, the metal concentrations were measured in an aliquot of the urine sample collected for pregnancy testing.

4.1.2 Participant selection in the present studies

The children were followed up multiple times during infancy, childhood, and adolescence, but not all children were invited to every follow up in order to limit the burden of blood sampling and testing on each child, thereby generating different branches in the mother-child cohort.

A branch consisting of 1303 children born between May 2003 and April 2004 was followed up at 4.5 years of age with the primary aim to investigate asthma and allergy in relation to arsenic exposure, thereby called the “immune cohort”. A subsample of 640 of these children was again followed up at 9 years, donating urine and blood in which several immune markers were assessed (Ahmed et al. 2013). The sample of **paper I** was the 551 children who had been followed up at 9 years and who had provided urine and/or blood samples for metal analysis. In complete subject analysis, 504 children had urinary metal exposure data and 487 had

erythrocyte metal data, as well as outcome (bone-related biomarkers and anthropometry) and covariate data at 9 years of age.

For **paper II**, we included 1530 children from another branch of the mother-child cohort, called the "developmental cohort". These children were born between October 2002 and November 2003, and they were followed up at 5 and 10 years of age for developmental and behavioral outcomes as well as anthropometry. This branch of the cohort was chosen for **paper II** due to its large size. However, the metal exposure assessment was based on concentrations in urine and not in blood for this subsample of children.

In total, 2307 children (1132 boys and 1175 girls) born between May 2002 and June 2004 participated in a puberty follow-up between the ages of 12 and 15 years. As **paper III** investigated the relationship between metal exposure and age at menarche, the study sample consisted only of girls. Out of the 1175 girls in the puberty follow-up, we included all girls who had metal exposure data either from the mother's pregnancy (in erythrocytes), or in urine at 5 or 10 years of age (n=935).

The Venn diagram below shows the overlap in participant mother-child dyads in **papers I-III** (Figure 3).

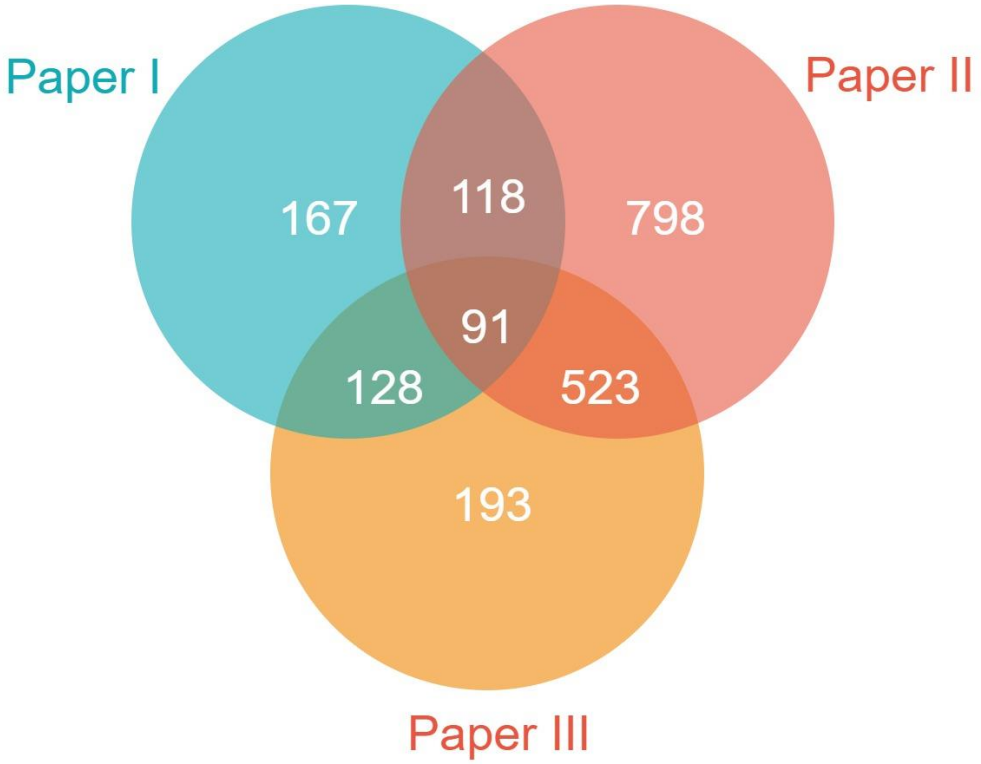

Figure 3. Venn diagram showing the overlap in the 2018 participating mother-child dyads between **papers I-III**.

4.2 SAMPLING AND DATA COLLECTION

4.2.1 Exposure biomarkers

The present studies included metal concentrations measured in urine and erythrocytes at different time points. A summary of the elements and in which biological matrix they were measured in for each paper is presented in Table 2. We studied the maternal metal exposure measured both in urine and in erythrocytes in **paper I**, but in **papers II-III** we decided to only include the erythrocyte metal concentrations. The erythrocyte concentrations at GW14 were chosen to reflect the exposure during early pregnancy as, between the available concentrations in urine and erythrocytes, erythrocyte concentrations were deemed to be the biomarker that best reflects what can be transferred from plasma to the fetus (for arsenic and lead) (Chen et al. 2014; Concha et al. 1998) or accumulated in placenta (for cadmium) (Osman et al. 2000).

As mentioned in section 2.1.2, urinary concentrations of cadmium reflect long-term exposure, as cadmium accumulates in the renal cortex (Akerstrom et al. 2013), while urinary concentrations of arsenic and lead have a short half-life and reflect the exposure of the past few days. Urinary arsenic concentrations contain both organic and inorganic species of arsenic, and, as the organic species are considered less toxic than the inorganic forms, inorganic arsenic and its metabolites were speciated through high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (ICP-MS) analysis, as previously described (Gardner et al. 2011b). Therefore, in **paper I** we adjusted the models for urinary arsenic concentrations measured as the sum of inorganic arsenic (As^{III}, As^{V}) and its methylated metabolites [monomethylarsonic acid (MMA), dimethylarsinic acid (DMA)]. The speciated urinary arsenic data was not available for all the children included in **papers II-III**, where we therefore used total arsenic concentrations. However, it was previously shown that in this population with low fish intake the sum of arsenic metabolites and total arsenic concentrations were very highly correlated both in the mothers (Gardner et al. 2011b) and in the 9-year-old children (Skröder Löveborn et al. 2016).

Urinary metal concentrations were adjusted for the average specific gravity (1.012 for both child and maternal urine), which was measured by a digital refractometer (EUROMEX RD712 Clinical Refractometer, EUROMEX Holland, Anhem, the Netherlands), to compensate for variation in urine dilution (Nermell et al. 2008). This method of compensation for urine dilution is preferable to creatinine adjustment especially in growing adolescents, as urinary creatinine varies with muscle mass as well as meat consumption (De Craemer et al. 2017). Moreover, adjustment for specific gravity was also found to be more appropriate for a population where malnutrition is widespread (Nermell et al. 2008).

Table 2. Summary of the exposure biomarkers included in **papers I-III**, and at which time point they were measured.

Paper	Sample	Time point	Metals studied	Metals as covariates
I	Urine	GW8, 4.5y, and 9y	Cd	As (sum of metabolites)
	Erythrocytes	GW14, 4.5y, and 9y	Cd	-
II	Erythrocytes	GW14	Cd, Pb and As	-
	Urine	10y	Cd, Pb and As (total)	-
III	Erythrocytes	GW14	Cd and Pb	As
	Urine	5y and 10y	Cd and Pb	As (total)

Abbreviations: GW, gestational week; Cd, cadmium; Pb, lead; As, arsenic.

4.2.2 Covariates

Maternal characteristics were collected either at the enrollment into MINIMat (maternal age, parity, weight, height, education years) or from the HDSS (e.g. the household's assets). The socioeconomic asset score was generated through principle component analysis and was based on information about the household's dwelling characteristics and ownership of assets (Gwatkin 2000). An updated asset score was generated at the follow-ups at 9 and 10 years of age, and it was used in **paper I** and in **paper II**, respectively. The asset score was categorized in quintiles in **papers I-II**, and in tertiles in **paper III**.

Season of conception was calculated from the gestational age at birth and the gestational age at the ultrasound around GW14. In **paper I**, the season of blood sampling at 9 years was categorized as pre-monsoon season spanning from January to May, monsoon from June to September, and post-monsoon from October to December. However, as we later learned that another categorization is more appropriate for the weather conditions in Bangladesh, the season of conception in **paper II** was categorized as pre-monsoon lasting from March to May, monsoon from June to October, and post-monsoon from November to February.

The micronutrient and food supplementation, described in section 4.1.1, was included as a covariate in **paper I** and in sensitivity analysis in **papers II-III**. There were three micronutrient supplementation groups, and two food supplementation groups, generating six different combinations. In **papers I-II** we considered supplementation as a variable with six categories, while in **papers III** we included micronutrient supplementation (three categories) and food supplementation (two categories) as two separate variables.

Hemoglobin concentrations were measured in whole blood with a HemoCue photometer (HemoCue AB). They were used as a measure of nutritional status, but they were only available for the children included in **paper I**.

We did not adjust for maternal smoking habits or alcohol consumption during pregnancy because all the pregnant women were non-smokers and alcohol is not consumed in this population. Unfortunately, we did not have information about environmental tobacco smoking.

4.3 ICP-MS

In all samples, the metal concentrations were measured using ICP-MS (Agilent 7500ce or 7700ce, Agilent Technologies, Tokyo, Japan) at the Unit of Metals and Health, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. The principle of this technology is that the liquid samples are first nebulized, becoming an aerosol, and then the molecules are atomized and positively ionized by a torch of ionized argon gas. The ions are then separated based on their mass-to-charge ratio in the mass spectrometer, generating an electric signal that is measured and quantified with the help of calibration standards. The advantages of this technique are that it is high throughput, it is possible to achieve very low limits of detection, many elements can be measured simultaneously in the same run, and it requires a smaller sample volume (which is then diluted before analysis) in comparison with, for example, atomic absorption (Nageswara Rao and Talluri 2007). Cadmium (*m/z* 111) and arsenic (*m/z* 75) were measured in helium mode, while lead (*m/z* 208) was measured in no gas mode to minimize interferences.

4.3.1 Sample preparation

Urine samples were collected in trace-element-free bottles and kept at -70℃ until analysis, when they were brought to room temperature, shaken, and diluted 1:10 with 1% nitric acid (Scharlau, Scharlab, Sentmenat, Spain or Ultrapure Normatom, VWR Chemicals).

After blood sampling, erythrocyte and plasma were separated by centrifugation within a couple of hours, and the plasma was transferred to other vials while the erythrocytes were left in the blood collection tube and thereafter both fractions were stored at -70℃. Before analysis, the erythrocyte samples were diluted 1:25 with an alkali solution [2% (wt:vol) 1-butanol, 0.05% (wt:vol) EDTA, 0.05% (wt:vol) Triton X-100, 1% (wt:vol) ammonium hydroxide (NH_4OH) and 20 µg/L internal standard; Sigma-Aldrich], and then they were vortex mixed, sonicated for 5 min, and centrifuged at 179 × g for 2 min [MSE centrifuge, Super Minor; MSE (UK) Ltd.]. Approximately one third of the maternal erythrocyte samples included in the present studies were measured following acid digestion instead (Kippler et al. 2009). The two methods gave highly correlated results (Lu et al. 2015), but it was found that the alkali method consistently gave concentrations that were lower than the acidic method (about 9% for cadmium, 10% for lead, and 5% for arsenic). Therefore, the values obtained following acid digestion were multiplied by 0.91, 0.90, and 0.95 for cadmium, lead, and arsenic, respectively, before pooling the data.

4.4 OUTCOMES

4.4.1 Bone-related biomarkers

In **paper I**, we measured several bone-related biomarkers in plasma and urine at 9 years of age. Concentrations of parathyroid hormone (PTH), vitamin D3 (25-hydroxyvitamin D), and osteocalcin were measured in plasma through chemiluminescence using Cobas e601 (Roche Diagnostics). Plasma levels of insulin-like growth factor 1 (IGF-1) and insulin-like growth

factor binding protein 3 (IGFBP3) were measured using ELISA (Quantakine ELISA, R&D Systems, Inc.). All these measurements were performed at the Nutritional Biochemistry laboratory at icddr,b in Bangladesh.

Deoxypyridinoline (DPD) was measured in urine using MicroVue Bone DPD EIA (Quidel). Concentrations of calcium in urine (not acidified) were measured with ICP-MS (Agilent 7700ce; Agilent Technologies). Both measurements were performed at the Unit of Metals and Health, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, and the urinary concentrations of DPD and calcium were adjusted for specific gravity to account for the variation in dilution of the urine samples.

The concentration of thyroid stimulating hormone (TSH) was measured in the plasma of the children who had enough sample left (299 out of total 504). TSH was measured through chemiluminescence with Cobas (Roche Diagnostics) at the Centre for Clinical Laboratory Studies at Karolinska University Hospital, Stockholm. As the children's blood samples had been collected in sodium heparin tubes (Vacuette; Greiner Bio-One International AG), instead of lithium heparin, it was not possible to measure thyroxine (T4) and triiodothyronine (T3) due to incompatibility with the analytical method.

Details of the analytical performance of these measurements can be found in **paper I**.

4.4.2 Growth anthropometry

At birth, the infants' weight and length were measured by trained nurses. The weight and length/height were then measured monthly until 12 months of age, and later every three months until 2 years. Thereafter, for a part of the children (the so-called "immune cohort") the weight and height were measured at 4.5 and 9 years of age, while for the children belonging to the "developmental cohort" this was done at 5 and 10 years. Details of the weight and length/height measurements can be found in **paper II**.

The weight and length/height measurements were converted into weight-for-age Z-scores (WAZ) and height-for-age Z-scores (HAZ) using child growth international reference values developed by the WHO (de Onis et al. 2007; WHO 2006). Underweight was classified as WAZ below -2 and stunting as HAZ below -2.

In **paper I**, we looked at WAZ and HAZ cross-sectionally at 9 years of age. In **paper II**, in addition to analyses on the Z-scores at 10 years, we included all WAZ and HAZ values from birth until 10 years of age in a longitudinal analysis, in total 19 measurements.

For the sake of interpreting the clinical relevance of our findings in **paper II,** we also repeated some of the analyses using weight (in kilos) and height (in centimeters) as outcomes instead of WAZ and HAZ.

4.4.3 Age at menarche and Tanner developmental stages

The main outcome of **paper III** was age at menarche. Between 12 and 15 years of age, the girls were interviewed twice with an interval of six months, asking if they had reached menarche, and, if they had, the date of the event was recorded. A calendar with local events was used to help the girls to remember, and the mothers were asked to assist if it was needed. The six-month interval between interviews was chosen to minimize recall bias. Age at menarche was calculated by subtracting the date of birth from the recalled date of menarche.

At the two interviews, the girls were also asked to self-assess their breast and pubic hair development according to Tanner stages of pubertal development, with the help of pictures (Marshall and Tanner 1969). However, as the validity of self-assessed puberty developmental stages may be low (Rasmussen et al. 2015), we chose to not include these outcomes in the main analyses of **paper III**. Instead, we primarily used this data for descriptive purposes, and to observe if associations between metal exposure and puberty developmental stages were consistent with the results of the analyses on age at menarche.

4.5 STATISTICAL ANALYSES

All statistical analyses were performed using Stata/IC (version 12 for **paper I** and version 16 for **papers II-III**; StataCorp). The statistical significance level was set to $p<0.05$, but we also evaluated the consistency of data.

To evaluate bivariate associations, we used Spearman's rank correlation between continuous variables and Kruskal-Wallis, Mann-Whitney *U*-test or Pearson's chi-square test between continuous and categorical variables, and Pearson's chi-square test between two categorical variables. In **papers I-II**, metal concentrations were $\log_2$-transformed as the exposure data was right-skewed. In **papers III**, the metal exposure was categorized into quartiles, as a categorical exposure variable was necessary to conduct Kaplan-Meier analysis. We also categorized the metal exposure into tertiles in a minor analysis in **paper II** to evaluate the relevance of the findings using weight and height as outcomes instead of WAZ and HAZ.

The associations between exposure and outcome were assessed through multivariable-adjusted linear regression analyses in **papers I-II.** Before linear regression was applied, we checked if linearity between the $\log_2$-transformed exposure and the studied outcomes could be assumed with the help of scatter plots fitted with lowess lines. The fit of the models was visually assessed with q-q plots and residual versus fitted plots. The results were presented as non-standardized B-coefficients with 95% confidence intervals (CI). In **paper III**, linear regression could not be employed as one quarter of the girls had not yet reached menarche. Therefore, we evaluated the associations between metal exposures and age at menarche via survival analysis, with the Kaplan-Meier estimator of survival and Cox proportional hazards regression. The analyses of associations between metal exposures and Tanner stages in **paper III** were performed with ordered logistic regression. The results were presented as hazard ratios (HR) for the Cox regression and odds ratios for the ordered logistic regression, both with 95% CI.

We conducted complete subject analyses in all the studies included in this thesis. Individuals with missing covariate data were only included if the covariate in question was just used in sensitivity analyses and not in the main analyses. The covariates included in the models were chosen based on being variables known to affect the outcomes, or because they correlated with both the metal exposure and the outcome. When several covariates were highly correlated, we chose the variable that led to the highest R^2 in the regression model. In **paper III**, we employed a directed acyclic graph (DAG) to identify which covariates should be included in the models.

As we were particularly interested in differences between boys and girls, multiplicative interaction terms between exposure and gender were included in **paper I**. All analyses in **papers I-II** were stratified by gender. Various sensitivity analyses were conducted to assess potential confounders or mediators, such as adjusting for the supplementation group and combining the exposure to the three studied metals in the same model. A detailed description of all the analyses performed in each study can be found in **papers I-III**.

4.6 ETHICAL CONSIDERATIONS

All the studies were in concordance with the Helsinki Declaration and were approved by the Ethical Review Committee at icddr,b in Bangladesh and the Ethical Review Board in Sweden.

Written and oral consent was obtained from the mothers before enrolling in the MINIMat trial in Bangladesh. Parents or guardians gave their written consent before the children participated at each follow-up.

As many participants originate from low socioeconomic strata, it was important to inform the mothers and the children that they could withdraw their consent to participation in the study at any time point, without it affecting their access to routine healthcare service. The reward for participation was something small, for example pencils and a coloring book for the child or a snack, in order not to offer a too valuable reward which could have made the mothers feel obligated to participate.

Urine collection is non-invasive and does not involve any risks; the blood sampling was performed by trained personnel, and there was only a small risk of bleeding or bruising. Measurements of anthropometry and other health outcomes were collected using well-established methodologies. Only female personnel conducted the interviews to collect data about menarche and pubertal developmental stage to respect the integrity of the adolescent girls as much as possible.

All work at Karolinska Institutet was conducted on coded data, and the key is stored at icddr,b in Bangladesh. Therefore, the identity of the study subjects was impossible to know from the study ID. The data was only stored on secure servers.

In the early phase of the mother-child cohort, once it became clear that many water sources contained high arsenic levels, the water pumps where families collect drinking water were painted red if the water contained more than 50 µg/L (the national cut-off in Bangladesh) or

green if it contained less. The community was informed about the risks involved with arsenic in drinking water and about the color coding so they could choose safe drinking water sources, and it was recommended that especially pregnant women and children should drink from green painted wells. At the 10-year follow-up, the median arsenic concentrations in drinking water had indeed decreased. However, the children's exposure to arsenic was still elevated, mainly from food (Kippler et al. 2016). Most of the metal analyses were performed long after the actual sample collection, but in case of abnormally high concentrations the responsible PI at icddr,b was informed.

This research is not associated with any risks to society that we are aware of. As mentioned above, it entailed minimal risks to the individual participants, about which they were informed before the start of the study. It could also result in benefits to the participants if abnormally high exposure levels were discovered, which could be acted upon to reduce them. The purpose of this research is to gain knowledge about if and how common environmental contaminants may affect children's health. Ideally, the reported results will contribute to the body of evidence used to perform future health risk assessments and in the implementation of policies to reduce the exposure to toxic metals, thereby improving public health and indirectly benefitting the participants and future generations.

5 RESULTS AND DISCUSSION

Here follow the main results of the studies included in this thesis and associated discussion, as well as some unpublished data. Further details can be found in the individual papers (**papers I-III**).

5.1 EXPOSURE ASSESSMENT

An important strength of the studies included in this thesis is that we had measures of individual exposure to metals at several time points, both during the mother's pregnancy and twice during childhood. Moreover, for the children included in **paper I**, the exposure assessment was available through concentrations in both urine (long-term exposure for cadmium, short-term for lead and arsenic) and erythrocytes (exposure from the last 3-4 months) at all time points.

In order to compare the levels of cadmium, lead, and arsenic exposure of the mothers and children included in these studies (**papers I-III**) to other literature, the median and 5^{th}-95^{th} percentiles of the exposure biomarkers of all the included mother-child dyads (n=2018 in total) are presented in Table 3. As erythrocyte metal concentrations are seldom reported in the literature, the erythrocyte concentrations have been converted into an approximate blood metal concentration using the median hemoglobin values at each time point (11.6 g/dL in mothers at GW14, 12.7 g/dL in the children at 4.5 years, and 12.5 g/dL at 9 years), based on the formula reported by Gustin and colleagues (Gustin et al. 2020). For cadmium and lead, the estimated whole blood concentrations were calculated disregarding their concentrations in plasma, as more than 96% of cadmium and lead in blood are found in the erythrocytes. For arsenic, we performed the calculations by estimating the arsenic plasma concentration to be 27% of the concentration in the erythrocytes. This was based on the ratio between median plasma and erythrocyte concentrations measured when a subset of the children included in **paper I** were 9 years old (Skröder et al. 2018).

Urinary cadmium concentrations increased with increasing age, as expected for an exposure biomarker with a half-life of decades and were therefore the lowest in the children at 5 years of age and highest in the pregnant mothers. Instead, the current exposure, represented by erythrocyte concentrations, appeared to be quite constant over time. The urinary cadmium concentrations of the children were comparable to those observed in children and adolescents in Mexico (mean 0.22 µg/L and 0.10 µg/L) (Zamoiski et al. 2014; Ashrap et al. 2019) and the U.S. (0.26 µg/L) (Reynolds et al. 2020), and lower than those reported in a study in Pakistan (0.56 µg/L) (Sughis et al. 2011). The diet in Bangladesh is heavily reliant on rice, which is a known source of cadmium. Rice samples from the study area have been reported to contain a median concentration of 47 µg Cd/kg dry weight, with considerable variation (Kippler et al. 2010b).

The children's erythrocyte and urinary lead concentrations were markedly lower at 9 years than at 4.5 years of age, possibly because of less exposure from soil and dust through hand-to-mouth behavior as children grew up. The estimated whole blood lead concentrations at 9 years were

comparable to those that have been reported in studies of children of various ages in China (mean 46 µg/L) (Zhou et al. 2020), Uruguay (42 µg/L) (Donangelo et al. 2021), Russia (30 µg/L) (Burns et al. 2017), while the estimated concentrations at 4.5 years were more similar to those of children living near contaminated sites in Poland (77 µg/L) and China (73 µg/L) (Yang et al. 2013). We have previously observed that the study population appears to be exposed to lead through rice, cooking pots and metal sheet roof material (Bergkvist et al. 2010).

The participants of this mother-child cohort live in arsenic-endemic region with high arsenic concentrations in the drinking water, which is reflected in the arsenic concentrations in urine and erythrocytes. After mitigation efforts, the arsenic concentrations in drinking water decreased from the time of enrolment until the 10-year follow-up, but the children were still found to have elevated exposure, probably through rice (Kippler et al. 2016). Indeed, the median urinary concentrations in children at 5 and 10 years was 10 times higher than the median concentration reported in 6-11-year-old children of an NHANES cohort in the U.S. (6.4 µg/L) (Jain 2021). There are other arsenic-endemic regions apart from Bangladesh and South-East Asia, for example some areas in the Bolivian Andes, where the reported median urinary concentrations in adults (65 µg/L) (De Loma et al. 2019) were similar to what was observed in Bangladesh.

Table 3. Median concentrations (5^{th}-95^{th} percentile) of the available exposure biomarkers for all mother-child dyads included in **papers I-III**.

Metal exposure biomarkers	n	Median (5^{th}-95^{th} percentile)
Cadmium		
Urine (µg/L)		
Mothers at GW8	1874	0.60 (0.17; 1.9)
Children at 5y	1477	0.22 (0.083; 0.65)
Children at 10y	1523	0.24 (0.083; 0.64)
Erythrocytes (µg/kg)		
Mothers at GW14	1660	0.93 (0.29; 2.3)
Children at 4.5y	363	0.94 (0.42; 2.4)
Children at 9y	507	0.90 (0.42; 1.9)
Whole blood (estimated, µg/L)		
Mothers at GW14	1660	0.36 (0.11; 0.89)
Children at 4.5y	363	0.39 (0.18; 0.98)
Children at 9y	507	0.37 (0.17; 0.77)
Lead		
Urine (µg/L)		
Mothers at GW8	1588	2.3 (0.34; 6.5)
Children at 5y	1477	3.7 (1.6; 10)
Children at 10y	1523	1.6 (0.65; 4.1)
Erythrocytes (µg/kg)		
Mothers at GW14	1660	72 (23; 155)
Children at 4.5y	363	177 (98; 320)
Children at 9y	507	115 (71; 225)
Whole blood (estimated, µg/L)		
Mothers at GW14	1660	27 (8.9; 59)
Children at 4.5y	363	74 (41; 134)
Children at 9y	507	47 (29; 92)
Arsenic		
Urine (total, µg/L)		
Mothers at GW8	1933	82 (19; 560)
Children at 5y	1477	56 (17; 346)
Children at 10y	1523	57 (19; 375)
Erythrocytes (µg/kg)		
Mothers at GW14	1660	4.4 (1.2; 23)
Children at 4.5y	363	3.6 (1.3; 19)
Children at 9y	507	3.3 (1.3; 20)
Whole blood (estimated, µg/L)		
Mothers at GW14	1660	2.0 (0.54; 10)
Children at 4.5y	363	1.8 (0.63; 9.3)
Children at 9y	507	1.6 (0.62; 9.6)

Abbreviation: GW, gestational week.

5.2 BONE-RELATED BIOMARKERS

Long-term cadmium exposure has been linked to bone toxicity in middle-age and elderly individuals, manifesting in an increased risk of osteoporosis and fractures (Akesson et al. 2006; Alfvén et al. 2000; Nawrot et al. 2010). However, several knowledge gaps remain concerning the modes of action underlying the cadmium-related bone effects. Animal studies with high cadmium doses and epidemiological studies from contaminated areas have found an indirect effect via kidney damage, leading to altered vitamin D activation and/or decreased calcium reabsorption in the kidneys (Brzoska and Moniuszko-Jakoniuk 2005; Jin et al. 2004). On the other hand, epidemiological studies conducted in populations with lower exposure levels also suggest a direct effect on bone cells, leading to increased bone resorption (Akesson et al. 2014; Nawrot et al. 2010; Schutte et al. 2008). Little is known concerning the initiation of the cadmium-related bone toxicity and whether there are windows of susceptibility.

In **paper I**, we evaluated the associations between cadmium exposure during the mother's pregnancy and during childhood and bone-related biomarkers at 9 years of age.

5.2.1 Biomarkers of bone remodeling

Bone resorption and formation are highly coupled processes regulated through feedback mechanisms (Raggatt and Partridge 2010). Deoxypyridinoline (DPD) is a crosslink of type 1 collagen in bone which provides stiffness; its concentrations excreted in urine are a marker of bone resorption. Osteocalcin is a calcium-binding peptide and the most abundant non-collagenous protein in bone. It is secreted by osteoblasts and it therefore serves as a marker of bone formation (Greenblatt et al. 2017).

We found that concurrent urinary cadmium was positively associated with urinary DPD at 9 years of age (Figure 4). A doubling of concurrent urinary cadmium was associated with an increase in DPD corresponding to 22% of its standard deviation (SD).

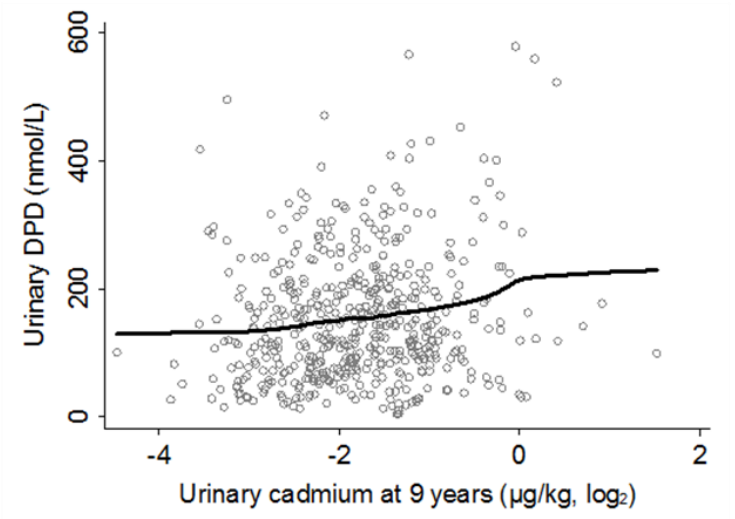

Figure 4. Scatter plot with lowess line of children's concurrent urinary cadmium concentrations (log_2-transformed) and urinary deoxypyridinoline (DPD) at 9 years.

We also found that the children's concurrent cadmium exposure, measured by concentrations both in urine and in erythrocytes, was positively associated with osteocalcin at 9 years of age. In sensitivity analysis, we adjusted the model of children's concurrent urinary cadmium and DPD for osteocalcin, and *vice versa*, to assess if one of these two outcomes mediated the association of urinary cadmium and the other outcome. We observed that the association between urinary cadmium and DPD was not affected by the adjustment for osteocalcin, while urinary cadmium was no longer associated with osteocalcin after adjusting for DPD. These results point towards the hypothesis that cadmium exposure induces bone resorption, which in turn elicits an increase in bone formation through feedback mechanisms.

Studies on bone effects of cadmium in children are scarce. The finding of a positive association between urinary cadmium and DPD was consistent with a previous cross-sectional study of 8- to 12-year-old Pakistani children (n=155), which, however, did not assess any markers of bone formation (Sughis et al. 2011). The only other available study which assessed children's cadmium exposure and markers of bone remodeling was conducted in 3- to 8-year-old children (n=246) living in an area with electronic waste-recycling industries in China (Yang et al. 2013). They did not observe any association between blood cadmium concentrations (about twice as high as in **paper I**) and either markers of bone resorption or bone formation.

Experimental studies of early-life cadmium exposure and its impact on bone are also scarce, but a study on young rats exposed to cadmium in drinking water from weaning to maturity reported that low-level cadmium exposure affected the mineralization of the tibia, resulting in weakened mechanical properties at maturity (Brzoska et al. 2005). Both *in vitro* and *in vivo* experimental studies have shown that cadmium promotes bone resorption by stimulating osteoclast formation (Rodriguez and Mandalunis 2016; Wilson et al. 1996).

In order to exclude the possibility that the association between urinary cadmium and urinary DPD was due to co-excretion, it would have been valuable to have measured a biomarker of bone resorption in another matrix than urine, such as Receptor activator of nuclear factor kappa-B ligand (RANKL) in plasma. However, the fact that urinary DPD was correlated with plasma osteocalcin (r_S=0.23; p-value<0.05) indicates that it was unlikely that co-excretion would be the entire explanation for the observed association between urinary cadmium and DPD.

5.2.2 Vitamin D3

Vitamin D is a hormone important for correct bone mineralization and severe deficiency can lead to rickets in children. It is involved in calcium and phosphate homeostasis and can act directly on bone tissue to regulate osteoblast and osteoclast activity (Anderson et al. 2013; Morris et al. 2012).

We found a robust inverse association between the children's cadmium exposure and concentrations of vitamin D3 (25-hydroxyvitamin D, the inactive form) at 9 years (Figure 5). This inverse association with vitamin D3 was observed in models of cadmium exposure measured both in urine and in erythrocytes, both concurrently at 9 years and at 4.5 years of age.

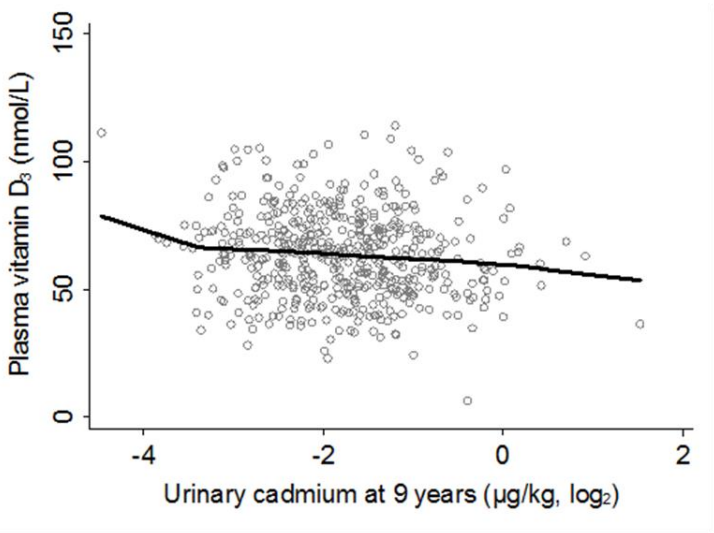

Figure 5. Scatter plot with lowess line of children's concurrent urinary cadmium concentrations (log_2-transformed) and plasma vitamin D3 at 9 years.

While the mechanism is not known, since we measured the inactive form of vitamin D3 in **paper I**, it seems unlikely that the observed inverse association between childhood cadmium exposure and vitamin D3 is due to an indirect toxic effect on the kidney, preventing its transformation into the active form. On the other hand, it has previously been reported that the children's urinary cadmium concentrations were inversely associated with their estimated glomerular filtration rate (eGFR) at 4.5 years and at 9 years (Skroder et al. 2015; Akhtar et al. 2021). However, the exposure levels were low and the effect estimates were modest. Moreover, the accuracy of eGFR in estimating the true glomerular filtration rate has been questioned when values are in the normal GFR range (Barregard et al. 2022).

We could only find one other study which had investigated the relationship between cadmium exposure and vitamin D in children (Zamoiski et al. 2014). It included Mexican adolescents (n=512) with similar urinary cadmium and 25-hydroxyvitamin D concentrations as the children in **paper I**. In contrast to **paper I**, they observed no associations between concurrent urinary cadmium concentrations and either 1,25-dihydroxyvitamin D (the active form) or 25-hydroxyvitamin D. Thus, more research is warranted on the consequences of a possible cadmium-related decrease in vitamin D levels in children.

5.2.3 Lead and bone-related biomarkers

Lead is known to accumulate in bone and to interfere with calcium metabolism (EFSA 2013). While all models in **paper I** were adjusted for arsenic exposure, as it is an important potential confounder to take into consideration in the study area, we did not include lead in the adjustments.

Therefore, the effect coefficients of linear regression models of erythrocyte lead concentrations at 9 years and the studied bone-related biomarkers are reported here in Table 4. The erythrocyte lead concentrations were log_2-transformed, as they were right-skewed as the cadmium and arsenic concentrations, and the models were adjusted as in Table 2 of **paper I** (see footnote of

Table 4 below). No association was found between the children's concurrent erythrocyte lead concentrations and any of the studied bone-related biomarkers, and the effect estimates of cadmium remained unchanged with lead in the models (less than 5% change in the effect estimates of all models). No association was found between maternal erythrocyte lead concentrations during pregnancy, or the children's erythrocyte lead concentrations at 5 years of age, and any of the bone-related biomarkers either.

Table 4. Results of multivariable-adjusted regression models of the children's concurrent erythrocyte lead concentrations (log_2-transformed) with bone-related biomarkers at 9 years of age.

Bone-related biomarker[1]	B (95% CI)[2]	p-value
PTH (pg/mL)	0.34 (-2.0; 2.7)	0.78
Osteocalcin (ng/mL)	2.5 (-2.4; 7.5)	0.31
DPD (nmol/L)	-1.5 (-19; 16)	0.86
Urinary calcium (mg/L; log_2)	0.029 (-0.23; 0.29)	0.83
Vitamin D3	-1.4 (-4.3; 1.6)	0.36
IGF-1 (ng/mL)	1.6 (-4.0; 7.1)	0.58
IGFBP3 (ng/mL)	0.63 (-152; 153)	0.99
TSH (mE/L)	0.12 (-0.28; 0.53)	0.55

Abbreviations: PTH, parathyroid hormone; DPD, deoxypyridinoline; IGF-1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor binding protein 3; TSH, thyroid stimulating hormone.
[1]N=487 children for all outcomes except TSH (n=297).
[2]Models adjusted for child gender, maternal education, household's socioeconomic status, child hemoglobin, and log_2-transformed concentrations of urinary arsenic and urinary cadmium at 9 years.

To our knowledge, associations of lead exposure and bone-related biomarkers in children have only been reported in one previous study. This was done in the study of children living in an electronic waste recycling area in China, earlier mentioned in section 5.2.1, in which blood lead concentrations (mean 73 µg/L) were positively associated with urinary DPD (Yang et al. 2013). However, as they lived in a contaminated area, it is possible that these associations were confounded by other exposures. More studies of children living in non-contaminated areas are needed to clarify the relationship between lead and cadmium exposure and bone health during childhood.

5.3 GROWTH ANTHROPOMETRY AT 10 YEARS OF AGE

Apart from lower peak bone mass and increased fragility, a possible consequence of toxic insults to bones can be decreased longitudinal growth. In the MINIMat cohort, it has previously been shown that maternal exposure to cadmium during pregnancy was associated with decreased birth size (Kippler et al. 2012a), and that the children's concurrent cadmium exposure was associated with lower WAZ and HAZ at 5 years (Gardner et al. 2013). No association between lead exposure and growth has previously been reported in the MINIMat cohort (Gardner et al. 2013). However, exposure to lead both prenatally and during childhood has been linked to decreased growth in infancy and childhood in other populations (Burns et al. 2017; Signes-Pastor et al. 2019; Zhou et al. 2020). Moreover, earlier studies in the MINIMat cohort have reported that maternal urinary arsenic concentrations during pregnancy were

inversely associated with size at birth (Rahman et al. 2009), and the children's urinary arsenic concentrations at 18 months and at 5 years were associated with decreased weight and height at 2 and 5 years, respectively (Saha et al. 2012; Gardner et al. 2013).

Therefore, we wanted to investigate if the associations between cadmium exposure, as well as lead and arsenic, persisted in later childhood, when a possible impact on bone remodeling by cadmium could be observed.

5.3.1 Cadmium

In **paper I**, there was an indication of an association between the children's concurrent urinary cadmium concentrations and decreased WAZ scores at 9 years. However, the confidence intervals were wide, and we could not ascertain an association with the sample size in **paper I** (n=504).

Hence, in **paper II** we aimed at elucidating the same question with the help of a much larger sample size (n=1530). Here, we could indeed observe that the children's concurrent urinary cadmium was associated with WAZ, and possibly also HAZ, at 10 years of age, while we did not observe any association between maternal cadmium exposure during early pregnancy and the children's WAZ and HAZ at 10 years. After stratification by gender, we found that the inverse association between the children's urinary cadmium and WAZ and HAZ was stronger in boys, as discussed below in section 5.4.

The findings of the non-stratified analyses were similar to those previously observed in these children at 5 years of age: even then, it was the children's own cadmium exposure that was associated with decreased WAZ and HAZ, and not the maternal exposure measured in urine during pregnancy (Gardner et al. 2013). Other studies have indicated that prenatal cadmium exposure may affect growth well into childhood. In a Greek study (n=515), elevated prenatal cadmium exposure (third tertile of maternal urinary cadmium compared to the two other tertiles) was associated with slower weight trajectories in all children and slower height trajectories in girls up to 4 years of age (Chatzi et al. 2018). However, confounding by maternal smoking could not be excluded. A study of Taiwanese children (n= 289) observed that cord blood cadmium concentrations were inversely associated with child weight, height and head circumference at 3 years of age (Lin et al. 2011). Another study in Belgian children (n=114) found that cord blood cadmium was associated with decreased weight and body mass index in girls at 7-9 years of age (Delvaux et al. 2014).

However, when we looked at the whole growth curve from birth until 10 years, we found that maternal erythrocyte cadmium was associated with decreased WAZ in boys. The effect estimate was modest, corresponding approximately to a difference in 0.1 kg when comparing the boys born by the mothers in the highest and lowest tertiles of cadmium exposure during pregnancy. As there was no association between maternal exposure and WAZ or HAZ at 10 years, it appeared as if the impact of the maternal exposure during pregnancy on child growth declined over time.

A major driver of systemic growth in virtually every tissue in the body, including bone, is the anabolic hormone insulin-like growth factor 1 (IGF-1), which is secreted from the liver after stimulation by the growth hormone (GH) from the anterior pituitary gland (Kanbur et al. 2005). Early-life cadmium exposure has been reported to affect the IGF-1 pathway. An experimental study found that young rats administered with 50 mg Cd/L through the drinking water had lower plasma levels of IGF-1 and insulin-like growth factor binding protein 3 (IGFBP3) (Turgut et al. 2005).

Unfortunately, we did not have data on plasma levels of IGF-1 for the children included in **paper II**, as we did for the smaller sample of children included in **paper I**. In the latter, while we could not ascertain an association, we observed a trend of an inverse association between the children's concurrent urinary cadmium and IGF-1 at 9 years. In addition, the suggested association between urinary cadmium and WAZ at 9 years was attenuated by the adjustment for IGF-1, adding evidence towards the hypothesis that lower IGF-1 levels may be one of the mechanisms by which cadmium exposure affects growth.

We found stronger associations between cadmium exposure during childhood and WAZ than with HAZ. When we adjusted the models of children's concurrent urinary cadmium and WAZ and HAZ at 9 years for urinary DPD, plasma osteocalcin or vitamin D3, these adjustments did not weaken the effect estimates of urinary cadmium with WAZ (**paper I**). This suggests that the possible effect of cadmium on growth is mediated through a different mode of action than its effects on bone remodeling. Nevertheless, we cannot exclude that these observed associations are because of a common causal pathway, especially as we did not have the necessary power to elucidate the association between children's concurrent urinary cadmium and WAZ at 9 years, when concentrations of bone-related biomarkers were available.

5.3.2 Lead and arsenic

In addition to cadmium, we also investigated if lead and arsenic exposure were associated with WAZ or HAZ at 10 years in **paper II**. Maternal exposure was assessed by concentrations in erythrocytes, and just as for cadmium, lead and arsenic in erythrocytes reflect the exposure of the past few months. In the children we measured urinary lead and arsenic, as blood samples were not collected from this part of the cohort. However, while urinary cadmium is a long-term exposure biomarker, it should be noted that urinary concentrations of lead and arsenic are susceptible to day-to-day variations, as their half-life is only a couple of days, thus increasing the uncertainty.

We found no association between gestational erythrocyte lead or the children's concurrent urinary lead and WAZ or HAZ at 10 years. However, after stratification by gender, we observed an inverse association between concurrent lead exposure and both WAZ and HAZ only in boys. The effect coefficients of the inverse associations between lead exposure during childhood and growth at 10 years were modest, boys in the highest exposure tertile were approximately 0.4 kg lighter and 0.7 cm shorter than the boys in the lowest tertile, and therefore

do not appear to be as critical as the neurodevelopmental effects which are the basis of the current risk assessment of lead in children (EFSA 2013).

The biomarkers of cadmium and lead exposure were weakly correlated (urinary cadmium and lead at 10 years: r_S=0.13, p-value<0.001; erythrocyte cadmium and lead at GW14: r_S=0.37, p-value<0.001) (**paper II**). Moreover, both exposures at 10 years were associated with decreased size in boys. Therefore, we conducted a sensitivity analysis, creating a combined exposure model with all three studied metals at the same time points. There, we found that mutual adjustment made the effect coefficients of cadmium and lead on WAZ and HAZ only slightly weaker (less than 15% change).

This was the first time that an association between lead exposure and growth was observed in this mother-child cohort. No association was found between prenatal or childhood exposure to lead and growth anthropometry at 5 years of age (Gardner et al. 2013). We do not know if this depends on an increased susceptibility with increasing age and longer exposure time, or if this age (around 10 years) is a window of susceptibility because of the impending start of puberty. An association of childhood blood lead with WAZ/weight or HAZ/height has previously been reported in other study populations living both in contaminated areas (Ignasiak et al. 2006; Yang et al. 2013) and in non-contaminated areas (Burns et al. 2017; Zhou et al. 2020). In a longitudinal study in Russian boys from 8 to 18 years of age, boys with blood lead concentrations at baseline above 50 µg/L were at adulthood 2.6 cm shorter than boys with concentrations lower than 50 µg/L (Burns et al. 2017). This threshold value was very close to the median value of estimated whole blood lead concentrations at 9 years of age of 47 µg/L in the children of the MINIMat cohort (Table 3).

We could not find any association between either prenatal or childhood exposure to arsenic and anthropometry at 10 years. It appears as if child growth becomes less susceptible to arsenic exposure with increasing age, as an inverse association was observed at birth (Rahman et al. 2009), at 2 years (Saha et al. 2012), and 5 years (Gardner et al. 2013), with smaller effect sizes over time. The above-mentioned follow-up studies at 2 and 5 years were the only two studies concerning childhood exposure to arsenic and growth which were mentioned in a review from 2017 (Rahman et al. 2017). Since then, another study of Bangladeshi children (not from the present mother-child cohort) was published, which found that children younger than 5 years of age with higher urinary arsenic concentrations had higher odds of being underweight (Alao et al. 2021). We could not find any studies investigating the relationship between arsenic exposure during childhood and growth past 5 years of age.

It was surprising that no associations were observed between arsenic exposure and growth at 10 years, as the mitigation efforts to reduce arsenic in drinking water have not been successful, and many families still consume drinking water with arsenic concentrations well above the WHO guideline value of 10 µg/L (range <0.01-675 µg/L) and eat arsenic-contaminated rice (Kippler et al. 2016).

5.4 GENDER DIFFERENCES

One of the aims of **papers I-II** was to elucidate any differences between boys and girls in the associations between metal exposure and outcomes related to bone health and growth. This was of particular interest because gestational cadmium exposure has previously been found to be associated with decreased birth size in girls but not in boys, a finding that was initially reported in this cohort (Kippler et al. 2012a; Kippler et al. 2012c) and later replicated in several other populations, as reviewed by Flannery and colleagues (Flannery et al. 2022). Moreover, in the MINIMat cohort, girls still seemed to be more susceptible when the association between cadmium exposure and growth anthropometry was investigated at 5 years of age, even if the gender difference was weaker then (Gardner et al. 2013). Therefore, we wanted to investigate if these gender differences persisted later in childhood.

As mentioned in section 5.2.1, urinary and erythrocyte cadmium were positively associated with plasma osteocalcin in all children at 9 years of age (**paper I**). However, when we stratified the analyses by child gender, we found that the association between urinary cadmium and osteocalcin went in different directions (Figure 6). While a positive association was observed in girls, where a doubling of urinary cadmium corresponded to an increase in osteocalcin of around one third of its SD, in boys a doubling of urinary cadmium was associated with a decrease in osteocalcin of 14% of its SD.

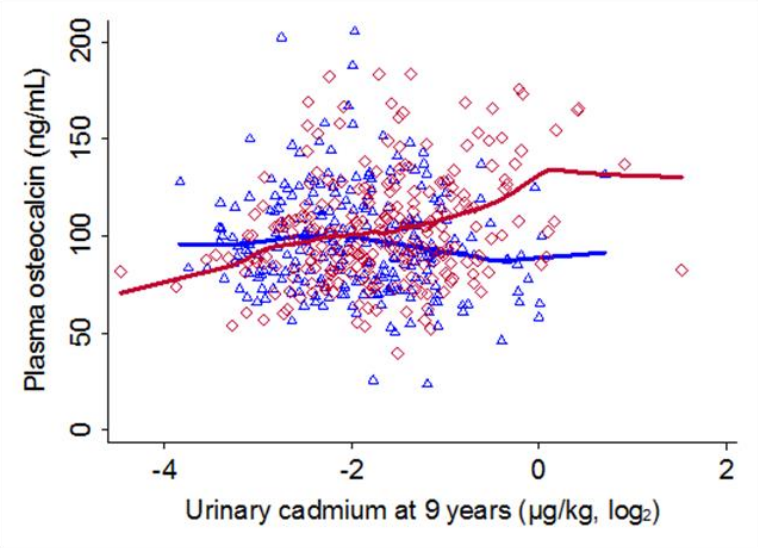

Figure 6. Scatter plot with lowess lines of children's concurrent urinary cadmium concentrations (log_2-transformed) in boys (blue triangles and lowess line) and girls (red diamonds and lowess line) and plasma osteocalcin at 9 years.

These results suggested that there was a dysregulation between bone resorption and bone formation at 9 years that was evident only in the boys, in whom the upregulated bone resorption did not seem to be compensated by an upregulation of bone formation. This observation was surprising, as it appeared that there was a switch in susceptibility between genders between the ages of 5 and 9 years.

When we explored the association between the children's concurrent urinary cadmium exposure and growth anthropometry at 9 and 10 years (**papers I-II**) we observed an inverse association with WAZ in all children. Once we stratified the models by gender, we found that the association with WAZ at 10 years of age was twice as strong in the boys as in the girls. Boys belonging to the highest tertile of urinary cadmium were about 0.6 kg lighter than the boys in the lowest tertile (**paper II**).

The potentially dysregulated bone remodeling in boys suggested in **paper I** could be a mechanism by which growth in boys might be more susceptible to cadmium at prepubertal age. In section 5.3.1, it was suggested that lower IGF-1 levels may be a way through which the association between urinary cadmium and decreased WAZ is mediated. Indeed, in **paper I** we found that boys had lower IGF-1 levels than girls at 9 years, and urinary cadmium concentrations were inversely associated with IGF-1 in boys, but not in girls. For reference, a multicenter study with ethnically diverse healthy participants reported that at 9 years of age the median IGF-1 concentrations were higher in boys than in girls (Bidlingmaier et al. 2014).

However, the higher IGF-1 levels in the girls could be, at least in part, because girls enter puberty earlier than boys, and a few of them were on the verge of entering puberty at this age. A year later, at 10 years, 11% of the girls had entered breast development stage 2 or higher (Svefors et al. 2016).

While it was surprising that there was a change in susceptibility to cadmium from girls to boys around 10 years of age, it seems as if growth is not the only outcome that displays this tendency. At 5 years of age, in the children included in **paper II**, an inverse association was reported between maternal as well as children's urinary cadmium concentrations and IQ, which was slightly stronger in the girls than in the boys (Kippler et al. 2012b). Instead, when these children were followed-up at 10 years, the inverse association between the children's concurrent urinary cadmium and IQ was mainly found in the boys (Gustin et al. 2018). Likewise, in a U.S. study, boys appeared to be more susceptible than girls in the association between cadmium exposure and neurodevelopmental outcomes at school age (Ciesielski et al. 2012).

The multitude of putative effects and the change in susceptibility between boys and girls over time suggests that cadmium adversely affects human health through different modes of action during different life stages such as during gestation, infancy, and childhood, as well as that boys and girls are more or less susceptible to chemical insults over the years.

In the most recent years, it has been hypothesized that cadmium may affect growth and development of fetuses and children through epigenetic reprogramming. An epigenetic mechanism could be hypothesized to be a reason for the differences in the associations observed between boys and girls. In fact, cadmium exposure appears to affect the epigenetic pattern in placenta (Everson et al. 2018) and cord blood (Kippler et al. 2013) in a sexually dimorphic way. It was seen that prenatal cadmium exposure affects DNA methylation of imprinted genes (Paternally Expressed Gene 3 or *PEG3* in girls and Maternally Expressed Gene 3 or *MEG3* in boys) in cord blood (Cowley et al. 2018; Vidal et al. 2015). Recently, it

was observed in a subset of this mother-child cohort that maternal erythrocyte cadmium concentrations during pregnancy were associated with changes in DNA methylation, especially hypomethylation, which persisted from birth to 9 years of age. However, gender differences could not be assessed because of lack of statistical power. The children's urinary cadmium concentrations were not found to be associated with the changes in DNA methylation in the same regions (Gliga et al. 2022). Other modes of action can be relevant for the children's current exposure, as those discussed above.

5.5 TIMING OF PUBERTY ONSET

Cadmium and lead have been reported to have endocrine disrupting properties (Johnson et al. 2003; Nkomo et al. 2018; Wu et al. 2003).

Thus, in **paper III** we aimed at elucidating if cadmium and lead exposure were associated with age at menarche, as a measure of timing of puberty onset in girls.

Girls between 12 and 15 years old were interviewed twice about their pubertal development, with six months between follow-ups. At the first follow-up, 61% of the girls had reached menarche, and by the second follow-up that proportion had increased to 77%. The median age at menarche of all girls was 13.0 years (25^{th}-75^{th} percentile 12.4, 13.7 years) as obtained from Kaplan-Meier analysis. Most girls assessed themselves as belonging to Tanner stage 3 of breast development and to stage 2 or 3 of pubic hair development at the second follow-up, where stage 1 represents the absence of any physical signs of puberty and stage 5 represents full pubertal maturation.

5.5.1 Cadmium

We observed that the girls in the highest quartile of urinary cadmium, both at 5 and at 10 years, reached menarche on average 2.9 and 3.8 months later, respectively, than the girls belonging to the lowest quartile of exposure (Figure 7). However, these estimates were unadjusted, and therefore we continued to evaluate the relationship with multivariable-adjusted Cox regression models.

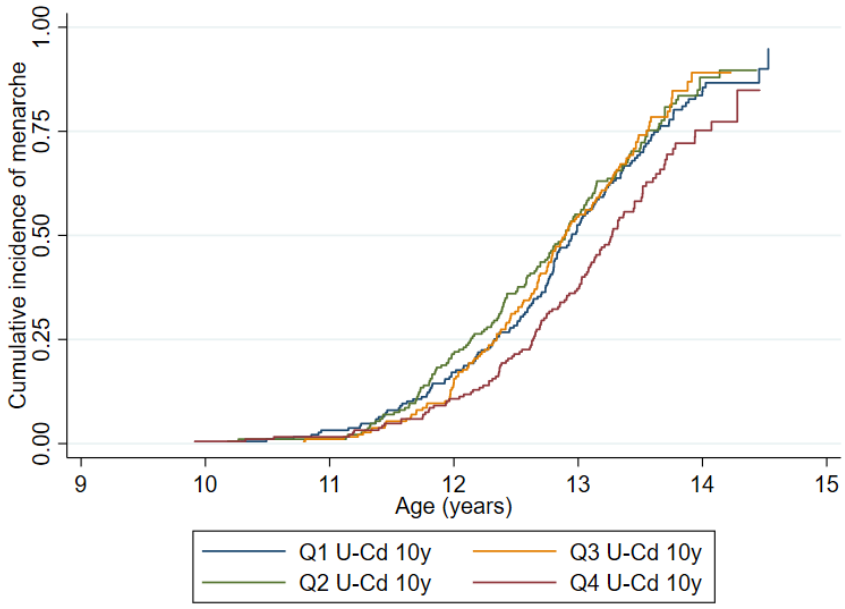

Figure 7. Cumulative incidence of menarche by quartiles of urinary cadmium at 10 years of age. Abbreviations: Q, quartile; U-Cd, urinary cadmium.

In Cox regression models, we found that the association between elevated urinary cadmium during childhood and delayed menarche was statistically significant even after adjustment for possible confounders. The girls belonging to the highest quartile of urinary cadmium at 10 years had 23% lower rate of menarche at a given age than the girls in the lowest quartile, and very similar associations were found with urinary cadmium at 5 years. We also observed that the girls belonging to the highest quartile of urinary cadmium at 10 years displayed less advanced breast development at the second puberty follow-up than the girls belonging to the lowest quartile.

The finding of delayed menarche in girls more highly exposed to cadmium went against what we hypothesized, as cadmium has been found to have estrogen-mimicking properties both *in vivo* and *in vitro* (Ali et al. 2010; Garcia-Morales et al. 1994; Johnson et al. 2003; Stoica et al. 2000) and, therefore, the opposite trend could be expected. However, in accordance with our findings, an association between urinary cadmium and delayed menarche was recently observed in two other smaller studies. A study from Mexico (n=132), in which the girls had approximately half the urinary cadmium concentrations as in **paper III**, found an association between the girls' peripubertal urinary cadmium and delayed menarche (Ashrap et al. 2019). They did not find any association between maternal exposure to cadmium during pregnancy and age at menarche, which is consistent with our own findings. The other study included girls in the U.S. (n=211) who had similar urinary cadmium concentrations as the girls in the MINIMat cohort at 5 or 10 years, and they also found an association with delayed menarche, as well as with decreased pubic hair development (Reynolds et al. 2020).

While several animal studies focusing on cadmium and reproductive maturity are available in the literature, only a few studies have investigated the hormonal changes that are behind the findings of either earlier or later reproductive maturity, and the results have been inconsistent.

This could at least in part be due to the difference in doses, some have used environmentally relevant doses while other have used very high doses, and how they administered the exposure, either orally or through intraperitoneal injection (Johnson et al. 2003; Li et al. 2018; Saedi et al. 2020; Salvatori et al. 2004; Samuel et al. 2011). While some experimental studies have observed a decrease in sex steroids in rodents exposed to cadmium (Saedi et al. 2020; Samuel et al. 2011; Zhang et al. 2008), another study found an activation of steroidogenesis and increased serum estradiol and progesterone (Li et al. 2018).

At the onset of puberty, the hormonal cascade which is started by the activation of the HPG axis leads to a surge in circulating estrogen concentrations, of which estradiol is the main one (Pinilla et al. 2012). The hormone estrogen is important for both the formation of bone tissue during puberty and the maintenance of bone mass during adulthood. Studies in rodents have shown that the estrogen receptor α (ERα), which is expressed in bone tissue and activated by estrogen, promotes the apoptosis of osteoclasts and acts as antiapoptotic on osteoblasts (Almeida et al. 2013; Nakamura et al. 2007). ERα modulates osteoclastic activity by suppressing the expression of RANKL in bone lining cells, where bone remodeling occurs (Streicher et al. 2017).

Thus, it could be hypothesized that a delay in the estrogen surge during adolescence, when most adult bone mass is achieved (Kralick and Zemel 2020), could be detrimental to bone accrual and could lead to a lower peak bone mass. Indeed, a large longitudinal study in U.K. following girls (n=3196) from 10 to 25 years of age found that later onset of puberty was associated with lower bone mineral density and bone mineral content during the entire follow-up time. While girls who entered puberty later did have a catch-up period of gain in bone mineral content, they still had lower bone mineral density and bone mineral content at 25 years than girls who entered puberty earlier (Elhakeem et al. 2019). Indeed, later age at menarche has been found to be associated with an increased risk of osteoporosis (Bonjour and Chevalley 2014), and a study using Mendelian randomization demonstrated that it may have a causal role in its etiology (Q Zhang et al. 2018).

Therefore, endocrine disruption leading to later puberty onset may be a mode of action through which cadmium exerts its toxic effect on bone observed in adulthood, independently from its possible effects on bone remodeling. In fact, it could be speculated that the association between girls' cadmium exposure at 10 years and later menarche is independent from the associations observed between children's concurrent cadmium exposure on bone-related biomarkers and growth reported in **papers I-II**. The latter outcomes were found to occur predominantly in boys, and while stunting during childhood has been linked to delayed pubertal development in this cohort (Svefors et al. 2020), we did not see any evidence of stunting by cadmium in the girls in **paper II**. Also, we found that the results were unchanged (only 1% change in the hazard ratio) when we adjusted the Cox regression models of girls' cadmium exposure at 10 years and age at menarche for stunting at 4.5 years in sensitivity analysis.

In **paper III**, we only studied the onset of female puberty. However, there are also studies which have indicated that cadmium exposure could affect male pubertal development, and act

in an anti-androgenic manner. A study of Flemish male adolescents found that urinary cadmium concentrations were inversely associated with testosterone levels (Dhooge et al. 2011). Another study of Italian boys between 12 and 14 years old found an association between urinary cadmium and lower testicular volume and testosterone levels (Interdonato et al. 2015). Unfortunately, we did not have data on testicular volume or testosterone levels for the boys of the mother-child cohort at the puberty follow-up between 12 and 15 years of age. We had data on the timing of their growth spurt, which is a measure of puberty onset, but it was available for only a portion of the boys (n=420) (Svefors et al. 2020). Of the boys who had data on pubertal growth spurt, only about 250 had data on metal exposure assessment at 10 years of age, and it was deemed to be a too small sample size to assess this outcome.

5.5.2 Lead and arsenic

Previous literature, consisting mainly of cross-sectional studies, has repeatedly reported an association between prenatal and childhood lead exposure and delayed menarche (Jansen et al. 2018; Liu et al. 2019; Selevan et al. 2003; Wu et al. 2003). The only large (n=918) longitudinal study available, conducted in the U.K., found no association between prenatal lead exposure and age at menarche (Maisonet et al. 2014). Elevated blood lead concentrations at birth and during childhood have also been associated with delayed puberty in boys in longitudinal studies from South Africa (n=732 boys) (Nkomo et al. 2018) and Russia (n=481) (Williams et al. 2019).

In the Kaplan-Meier analyses, we observed that girls belonging to the highest quartile of urinary lead at 5 and 10 years reached menarche 2.2 and 3.0 months earlier, respectively, than the girls belonging to the lowest exposure quartile.

When we used Cox regression models, we could not observe an association with urinary lead at 5 years, but we did find an association between urinary lead at 10 years and earlier menarche. After adjustments, although the confidence interval included 1.00, the hazard ratio remained similar to the one in unadjusted models. Girls in the highest exposure quartile had a 23% higher rate of menarche than the girls in the lowest quartile of urinary lead at 10 years. The same girls also had more advanced breast development at the second puberty follow-up.

This finding should, however, be interpreted with caution as we cannot exclude that the association of increased urinary lead and earlier menarche could be a result of reverse causality. Rapid growth is upregulated in puberty, and it happens also before the event of menarche (Wood et al. 2019). The process of bone remodeling during growth might cause the displacement of lead which has been stored in bone tissue, thus increasing its urinary concentrations. It can be hypothesized that the girls who reached menarche earlier and had a more advance pubertal development between 12 and 15 years had already entered the pubertal growth spurt at the time of the latest exposure assessment at 10 years. This hypothesis is consistent with the fact that there was no association between urinary lead at 5 years and age at menarche.

As previously mentioned, the concentration of lead in urine is not an optimal biomarker of lead exposure due to its rapid excretion from plasma. However, higher uncertainty in the exposure assessment should have biased the results towards the null. More longitudinal studies with exposure assessment in blood or erythrocytes are needed to further evaluate the association between lead exposure and timing of puberty onset.

In the girls included in **paper III**, it was recently discovered that elevated arsenic concentrations in drinking water consumed by mothers during pregnancy were associated with delayed menarche in the daughters (Rahman et al. 2021). Therefore, we adjusted all models for arsenic exposure, measured in the mothers' erythrocytes during pregnancy in models of prenatal exposure and in urine at 5 and 10 years in models of childhood exposure. In accordance with the previous results (Rahman et al. 2021), maternal erythrocyte arsenic concentrations in early pregnancy were associated with delayed menarche (Table 5). Urinary arsenic concentrations at 5 or 10 years were not associated with age at menarche. This could possibly be the consequence of a programming effect by arsenic during fetal life, independent of the children's exposure later in childhood.

Table 5. Results of Cox regression models of girls' early-life arsenic exposure (maternal erythrocytes during pregnancy and girls' urinary concentrations during childhood) and age at menarche.

Arsenic exposure biomarker	HR (95% CI)[1]	p-value
Erythrocyte As GW14 (n=771)		
Quartile 1	1.00	
Quartile 2	0.93 (0.74; 1.17)	0.55
Quartile 3	0.95 (0.76; 1.19)	0.66
Quartile 4	0.79 (0.62; 0.99)	0.043
Urinary As 5y (n=750)		
Quartile 1	1.00	
Quartile 2	0.89 (0.70; 1.13)	0.33
Quartile 3	0.68 (1.10)	0.23
Quartile 4	0.89 (0.70; 1.12)	0.31
Urinary As 10y (n=745)		
Quartile 1	1.00	
Quartile 2	1.14 (0.90; 1.44)	0.29
Quartile 3	1.16 (0.91; 1.48)	0.22
Quartile 4	1.07 (0.84; 1.36)	0.58

Abbreviations: HR, hazard ratio; CI, confidence interval; As, arsenic; GW, gestational week.
[1]Models adjusted for age, household's socioeconomic status, maternal body mass index, and maternal education at enrollment, and quartiles of cadmium and lead exposure at the respective time points.

5.6 METHODOLOGICAL CONSIDERATIONS

A known limitation of observational studies is that it is hard to infer causality, as it is difficult to control for all confounders, that are often unmeasured. However, an important strength of the studies included in this thesis was the prospective design, which is especially valuable when trying to assess causality. While outcomes included in **papers I-II** were measured at the same

time as the most recent exposure assessment, we also assessed maternal exposure during pregnancy and the children's exposure at 4.5 years (**paper I**). The outcome in **paper III** was measured a few years after the most recent exposure assessment at 10 years. However, we speculated that the association between urinary lead and earlier menarche may be explained by reverse causation. This was due to the specific timing of the exposure assessment at 10 years, and the fact that urinary lead is known to increase at times of increased bone remodeling.

Another advantage was the availability of individual exposure assessment with biomarkers reflecting the internal dose, measured through a sensitive analytical method. This limited the risk for exposure misclassification, especially for urinary cadmium, due to its long half-life. Nevertheless, while we are confident about the analytical quality of the measurements of urinary lead and arsenic, the biomarkers are prone to day-to-day variation, as previously mentioned. However, if the exposure assessment of lead and arsenic in urine does not accurately reflect the internal dose, it should be expected that the error would be non-differential, which would make it harder to reject the null hypothesis.

The outcomes which were included in **papers I-II** were measured by trained personnel (weight and height) and bone-related biomarkers analyzed in blood and urine with good analytical performance (details found in **paper I**). The main outcome of **paper III** was age at menarche, which was calculated from the recalled date at the event and the girl's birthday. Menarche is a significant milestone for girls, who were also helped by their mothers and a calendar to point out the date, and the median recall time was short, 0.8 years. Therefore, we are confident about the minimal risk for outcome misclassification. However, the Tanner developmental stages in **paper III** were self-assessed, and therefore their validity may be lower. For this reason, we decided to interpret those results more cautiously, and to mainly compare them to the associations found between metal exposure and age at menarche.

A way to reduce random errors and to increase sensitivity is to have a large sample size. The papers included in this thesis had a large sample size, especially **paper II** (n=1530) and **paper III** (n=935). Indeed, it could be observed how increasing the sample size from **paper I** (n=504) to **paper II** resulted in narrower confidence intervals in the association between the children's concurrent urinary cadmium and WAZ at 9 and 10 years of age.

Selection bias was not deemed to be a large problem in these studies. The eligibility criteria to participate in MINIMat were few, so the study sample reflected the population in Matlab. The participation rate was high and the lost-to-follow-up low, especially for studies with such a long follow-up time. In each paper, a comparison was made between the mother-child dyads that were included and excluded, considering background characteristics, and exposure levels and outcomes when possible. The differences that were found were very small and unlikely to be of biological relevance.

Although we performed many statistical tests, we did not adjust for multiple testing in any of the papers. A reason for this is that the outcomes and the exposure biomarkers were not independent from each other. Correction for multiple testing would have introduced an

increased risk of type II error, i.e. an incorrect acceptance of the null hypothesis. The associations observed throughout the papers were also assessed for consistency and robustness, for example if they were consistent over time, if they were similar when using different exposure biomarkers of the same metal (if available), and if they displayed a biologically reasonable dose-response curve.

As in all observational studies, we cannot exclude that there was some unmeasured confounding by factors associated with both the metal exposure and the outcomes. While all pregnant mothers enrolled in MINIMat were non-smokers and did not drink alcohol, we did not have information about the smoking habits of other members of the household, or about indoor cooking. Another potential confounder is the nutritional status and the dietary intake of the children. In **paper I,** we adjusted all models for the children's hemoglobin concentration. However, this was not available in **papers II-III**, and we could not adjust the models for WAZ or HAZ, which are otherwise used as markers of current and chronic nutrition status, as they were the outcomes of interest (**paper II**) or a consequence of the outcome (**paper III,** as pubertal development is a driver of growth).

5.7 GENERALIZABILITY

The studies included in this thesis were conducted in a study area where malnutrition is very common. Indeed, at 10 years of age, 42% of the children were underweight and 28% were stunted, while less than 1% were overweight according to international growth standards from the WHO (**paper II**), thus having a very different nutritional status compared to children living in high-income societies. Moreover, many of the participating mothers and children consumed a diet which was mainly based on rice, and they were exposed to elevated arsenic concentrations in the drinking water.

However, millions of people rely on a rice-based diet, especially in Asia, and 140 million people around the world are exposed to arsenic in drinking water at higher concentrations than the WHO criteria of 10 µg/L (Ravenscroft 2009). Therefore, the metal exposure reported in this study population is a reality for many. In addition, the ranges of cadmium, lead and arsenic exposure described in these studies are wide and span the environmental exposure levels reported by many other studies, both in populations living in background level exposure areas and in contaminated sites.

6 CONCLUSIONS

This thesis provides evidence that early-life cadmium exposure can play a role in children's growth and pubertal development, which has the potential to affect their future health. Boys and girls seem to have different windows of susceptibility for different health effects. The variety of indicated toxic effects of cadmium and the change in susceptibility between genders over time suggest that several different modes of action are involved.

Specifically, we can conclude that:

- Cadmium, linked to bone toxicity in adults, appears to affect bone remodeling already in pre-pubertal children, possibly by disrupting feedback mechanisms between bone resorption and formation especially in boys.

- Childhood exposure to cadmium was associated with lower vitamin D levels, a hormone important for calcium homeostasis, bone mineralization as well as for immune function.

- Exposure to cadmium during childhood was associated with lower weight and stature at 10 years, possibly mediated through IGF-1. It appeared as if the inverse association between childhood cadmium exposure and WAZ was independent of the suggested effects on bone.

- Boys appeared to be more susceptible to cadmium exposure at school-age in regards to growth, while girls have been found to be more susceptible in infancy and early childhood.

- Cadmium exposure was associated with delayed puberty in girls, likely through endocrine disruption and independently from the putative effect of cadmium on bone remodeling and growth.

- In boys, increasing exposure to lead was associated with lower weight and stature at 10 years.

- Despite the elevated exposure levels from drinking water and rice, arsenic exposure was not associated with child anthropometry at 10 years.

The effect coefficients of the reported associations were small. However, many millions of children are exposed to these metals at similar levels. This makes even small effects a public health concern, especially for children growing up with malnutrition and unfavorable conditions, who may be more susceptible to toxic insults.

7 FUTURE PERSPECTIVES

The studies in this thesis add evidence for early-life cadmium exposure being an influential factor in children's growth and pubertal development. However, many questions remain, and these aspects should be elucidated in particular:

- Whether the changes in bone-related biomarkers we observed here in pre-pubertal children actually result in functional changes in bone health at maturity, for example peak bone mass, which could be predictive of future disease.

- If the associations with decreased growth during infancy and childhood persist also during adolescence, and if they result in a lower final weight and height at adulthood.

- Gender differences in susceptibility to cadmium and lead during adolescence.

- The molecular mechanisms of the gender differences in susceptibility, and how they may change over the course of childhood, possibly through epigenetic effects.

- How hormones related to growth, bone health and puberty are affected by cadmium and lead exposure during puberty, in both boys and girls.

- If cadmium, lead, and arsenic impact the timing of onset of male puberty.

- If the associations found in these studies are observed also in other populations where malnutrition is not as widespread.

- An update to the risk assessment of cadmium, taking possible effects on children's health into account.

8 ACKNOWLEDGEMENTS

This work was conducted at the Unit of Metals and Health at the Institute of Environmental Medicine, Karolinska Institutet. It was funded by Formas, the Swedish Research Council, the Swedish International Development Cooperation Agency (Sida), and Karolinska Institutet.

I would like to thank everyone who has contributed to this thesis, and especially:

All the mothers and the children who participated in the studies in Bangladesh and all the field workers involved in the data collection from icddr,b, without whom this work could not have happened.

Maria Kippler, my main supervisor, thank you for giving me the opportunity to be a PhD student at Karolinska Institutet. I remember attending one of your lectures (about the health effects of cadmium!) during my bachelor program, and I can safely say that you are one of the reasons why I became a toxicologist. During these seven long years as my supervisor, you have constantly shown me your diligence, resourcefulness, knowledge, scientific curiosity, patience, and humanity. Thank you for believing in me.

Marie Vahter, my co-supervisor, for your enthusiasm for science, incredible knowledge, your patience with my drafts, and for encouraging and reminding me why what we do is important.

Karin Broberg, my co-supervisor, for your intellectual curiosity and knowledge about so many different fields, and for finding beauty in nature in an inspiring way.

Annika Hanberg, my mentor, for your encouragement and for having had such a pivotal role in my training as a toxicologist.

All my co-authors, and in particular *Anna Warnqvist* and *Anna Johansson*, for answering my many questions about the statistical methods we used, *Moshfiqur Rahman* and *Anisur Rahman*, for explaining things about Bangladesh that I could not have known from here, and *Pernilla Svefors* for helping me with your insight into child growth.

My colleagues at the Unit of Metals and Health, past and present. Thank you very much to *Michael Levi*, *Helena Nordqvist* and *Ying Lu* for your expertise in the lab. *Barbro Nermell*, for your helpfulness, thoroughness, and support. *Sultan Ahmed (Emon)*, for your good company at the office. *Nadia Vilahur*, for your help in the lab at the beginning of my PhD. Thank you to *Florencia Harari*, for guiding me in writing my first research article. *Ulrike Dauter*, for our interesting discussions at the office and your support, I am sorry that the pandemic has cut on our time together so much. *Mariza Kampouri*, for your enthusiasm and encouragement. *Anda Gliga*, thank you for your encouragement, honesty, practicality, humor, and your valued friendship.

The Metalligans, the best group of PhD student colleagues I could have asked for. I'm the last one to finish, but we stay Metalligans forever!

Helena Skröder Löveborn, thank you for your helpfulness and endless generosity with your time, energy, competence, your support and your friendship.

Klara Gustin Mossegård, your humor has made everything better from the moment you joined the group. Our mutual support during this past half a year has meant so much to me.

Jessica De Loma, for your enthusiasm, helpfulness, optimism, resourcefulness, and honesty.

Ayman Alhamdow, for your patience and humor, your help with pyrosequencing and for putting things in perspective.

My best friend *Barbara*, for being so incredibly close and present for all these years, even if I moved so far away. Ti voglio bene! My dear friend *Kristina*, for being one of the kindest people I know. You were my first Swedish friend, and I am so happy that you are in my life.

My Italian friends in Stockholm, for your friendship and support.

My extended family in Italy and in Sweden, for being caring and supportive in all situations.

Mamma e Papà, for instilling in me the love of knowledge, for always believing in me, and for being proud of me. Vi voglio bene!

My husband *David*, for comforting me when everything felt too hard and not doubting that I could do this. Jag älskar dig!

My children *Jacob* and *Livia*. In all honesty, you did not help with this thesis. Quite the opposite, in fact! But being a mother to you helped me motivate myself to work for the health and wellbeing of all children. Vi voglio bene, tesori miei!

Annachiara

9 REFERENCES

Ahmed S, Rekha RS, Ahsan KB, Doi M, Grander M, Roy AK, et al. 2013. Arsenic exposure affects plasma insulin-like growth factor 1 (igf-1) in children in rural bangladesh. PloS one 8:e81530.

Ahmed S, Moore SE, Kippler M, Gardner R, Hawlader MD, Wagatsuma Y, et al. 2014. Arsenic exposure and cell-mediated immunity in pre-school children in rural bangladesh. Toxicological sciences : an official journal of the Society of Toxicology 141:166-175.

Akerstrom M, Barregard L, Lundh T, Sallsten G. 2013. The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. Toxicol Appl Pharmacol 268:286-293.

Akesson A, Berglund M, Schutz A, Bjellerup P, Bremme K, Vahter M. 2002. Cadmium exposure in pregnancy and lactation in relation to iron status. American journal of public health 92:284-287.

Akesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G, et al. 2006. Cadmium-induced effects on bone in a population-based study of women. Environmental health perspectives 114:830-834.

Akesson A, Julin B, Wolk A. 2008. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: A population-based prospective cohort study. Cancer research 68:6435-6441.

Akesson A, Barregard L, Bergdahl IA, Nordberg GF, Nordberg M, Skerfving S. 2014. Non-renal effects and the risk assessment of environmental cadmium exposure. Environmental health perspectives 122:431-438.

Akhtar E, Roy AK, Haq MA, von Ehrenstein OS, Ahmed S, Vahter M, et al. 2021. A longitudinal study of rural bangladeshi children with long-term arsenic and cadmium exposures and biomarkers of cardiometabolic diseases. Environ Pollut 271:116333.

Al-Rmalli SW, Jenkins RO, Haris PI. 2012. Dietary intake of cadmium from bangladeshi foods. Journal of food science 77:T26-33.

Alao ME, Perin J, Brooks WA, Hossain L, Goswami D, Zaman K, et al. 2021. Urinary arsenic is associated with wasting and underweight status in young children in rural bangladesh. Environmental research 195:110025.

Alfvén T, Elinder CG, Carlsson MD, Grubb A, Hellström L, Persson B, et al. 2000. Low-level cadmium exposure and osteoporosis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 15:1579-1586.

Ali I, Penttinen-Damdimopoulou PE, Mäkelä SI, Berglund M, Stenius U, Akesson A, et al. 2010. Estrogen-like effects of cadmium in vivo do not appear to be mediated via the classical estrogen receptor transcriptional pathway. Environmental health perspectives 118:1389-1394.

Almeida M, Iyer S, Martin-Millan M, Bartell SM, Han L, Ambrogini E, et al. 2013. Estrogen receptor-α signaling in osteoblast progenitors stimulates cortical bone accrual. The Journal of clinical investigation 123:394-404.

Amzal B, Julin B, Vahter M, Wolk A, Johanson G, Akesson A. 2009. Population toxicokinetic modeling of cadmium for health risk assessment. Environmental health perspectives 117:1293-1301.

Anderson PH, Lam NN, Turner AG, Davey RA, Kogawa M, Atkins GJ, et al. 2013. The pleiotropic effects of vitamin d in bone. The Journal of steroid biochemistry and molecular biology 136:190-194.

Ashrap P, Sánchez BN, Téllez-Rojo MM, Basu N, Tamayo-Ortiz M, Peterson KE, et al. 2019. In utero and peripubertal metals exposure in relation to reproductive hormones and sexual maturation and progression among girls in mexico city. Environmental research 177:108630.

ATSDR. 2012. Toxicological profile for cadmium. Atlanta: U.S. Department of Health and Human Services, Public Health Service. .

Barker DJ. 1997. Fetal nutrition and cardiovascular disease in later life. British medical bulletin 53:96-108.

Barregard L, Fabricius-Lagging E, Lundh T, Molne J, Wallin M, Olausson M, et al. 2010. Cadmium, mercury, and lead in kidney cortex of living kidney donors: Impact of different exposure sources. Environmental research 110:47-54.

Barregard L, Sallsten G, Fagerberg B, Borne Y, Persson M, Hedblad B, et al. 2016. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of swedish adults: The malmo diet and cancer study. Environmental health perspectives 124:594-600.

Barregard L, Sallsten G, Lundh T, Mölne J. 2022. Low-level exposure to lead, cadmium and mercury, and histopathological findings in kidney biopsies. Environmental research 211:113119.

Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. 2011. Bone mineral accrual from 8 to 30 years of age: An estimation of peak bone mass. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 26:1729-1739.

Bellinger DC. 2012. Comparing the population neurodevelopmental burdens associated with children's exposures to environmental chemicals and other risk factors. Neurotoxicology 33:641-643.

Benyi E, Sävendahl L. 2017. The physiology of childhood growth: Hormonal regulation. Hormone research in paediatrics 88:6-14.

Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, et al. 2010. Peak bone mass from longitudinal data: Implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 25:1948-1957.

Bergkvist C, Kippler M, Hamadani JD, Grandér M, Tofail F, Berglund M, et al. 2010. Assessment of early-life lead exposure in rural bangladesh. Environmental research 110:718-724.

Berglund M, Akesson A, Nermell B, Vahter M. 1994. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environmental health perspectives 102:1058-1066.

Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, et al. 2014. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: Results from a multicenter study using a new automated chemiluminescence igf-i immunoassay conforming to recent international recommendations. The Journal of Clinical Endocrinology & Metabolism 99:1712-1721.

Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. 2013. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet (London, England) 382:427-451.

Bonjour JP, Chevalley T. 2014. Pubertal timing, bone acquisition, and risk of fracture throughout life. Endocrine reviews 35:820-847.

Brzoska MM, Majewska K, Moniuszko-Jakoniuk J. 2005. Bone mineral density, chemical composition and biomechanical properties of the tibia of female rats exposed to cadmium since weaning up to skeletal maturity. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 43:1507-1519.

Brzoska MM, Moniuszko-Jakoniuk J. 2005. Bone metabolism of male rats chronically exposed to cadmium. Toxicol Appl Pharmacol 207:195-211.

Burns JS, Williams PL, Lee MM, Revich B, Sergeyev O, Hauser R, et al. 2017. Peripubertal blood lead levels and growth among russian boys. Environ Int 106:53-59.

Canelón SP, Boland MR. 2020. A systematic literature review of factors affecting the timing of menarche: The potential for climate change to impact women's health. International journal of environmental research and public health 17.

Carlson LA, Friberg L. 1957. The distribution of cadmium in blood after repeated exposure. Scandinavian journal of clinical and laboratory investigation 9:67-70.

Chatzi L, Ierodiakonou D, Margetaki K, Vafeiadi M, Chalkiadaki G, Roumeliotaki T, et al. 2018. Associations of prenatal exposure to cadmium with child growth, obesity, and cardiometabolic traits. American Journal of Epidemiology 188:141-150.

Chen Z, Myers R, Wei T, Bind E, Kassim P, Wang G, et al. 2014. Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. Journal of exposure science & environmental epidemiology 24:537-544.

Cheng L, Zhang B, Zheng T, Hu J, Zhou A, Bassig BA, et al. 2017. Critical windows of prenatal exposure to cadmium and size at birth. International journal of environmental research and public health 14.

Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO. 2012. Cadmium exposure and neurodevelopmental outcomes in u.S. Children. Environmental health perspectives 120:758-763.

Combs CE, Nicholls JJ, Duncan Bassett JH, Williams GR. 2011. Thyroid hormones and bone development. Minerva endocrinologica 36:71-85.

Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicological sciences : an official journal of the Society of Toxicology 44:185-190.

Cowley M, Skaar DA, Jima DD, Maguire RL, Hudson KM, Park SS, et al. 2018. Effects of cadmium exposure on DNA methylation at imprinting control regions and genome-wide in mothers and newborn children. Environmental health perspectives 126:037003.

Crews HM, Owen LM, Langford N, Fairweather-Tait SJ, Fox TE, Hubbard L, et al. 2000. Use of the stable isotope (106)cd for studying dietary cadmium absorption in humans. Toxicology letters 112-113:201-207.

Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, et al. 1993. Bone density at various sites for prediction of hip fractures. The study of osteoporotic fractures research group. Lancet (London, England) 341:72-75.

Day FR, Elks CE, Murray A, Ong KK, Perry JRB. 2015. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: The uk biobank study. Scientific reports 5:11208.

De Craemer S, Croes K, van Larebeke N, De Henauw S, Schoeters G, Govarts E, et al. 2017. Metals, hormones and sexual maturation in flemish adolescents in three cross-sectional studies (2002-2015). Environ Int 102:190-199.

De Loma J, Tirado N, Ascui F, Levi M, Vahter M, Broberg K, et al. 2019. Elevated arsenic exposure and efficient arsenic metabolism in indigenous women around lake poopó, bolivia. The Science of the total environment 657:179-186.

de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. 2007. Development of a who growth reference for school-aged children and adolescents. Bulletin of the World Health Organization 85:660-667.

De Sanctis V, Soliman A, Alaaraj N, Ahmed S, Alyafei F, Hamed N. 2021. Early and long-term consequences of nutritional stunting: From childhood to adulthood. Acta Biomed 92:e2021168-e2021168.

Delvaux I, Van Cauwenberghe J, Den Hond E, Schoeters G, Govarts E, Nelen V, et al. 2014. Prenatal exposure to environmental contaminants and body composition at age 7-9 years. Environmental research 132:24-32.

Dhooge W, den Hond E, Koppen G, Bruckers L, Nelen V, van de Mieroop E, et al. 2011. Internal exposure to pollutants and sex hormone levels in flemish male adolescents in a cross-sectional study: Associations and dose-response relationships. Journal of exposure science & environmental epidemiology 21:106-113.

Donangelo CM, Kerr BT, Queirolo EI, Vahter M, Peregalli F, Mañay N, et al. 2021. Lead exposure and indices of height and weight in uruguayan urban school children, considering co-exposure to cadmium and arsenic, sex, iron status and dairy intake. Environmental research 195:110799.

EFSA. 2009. Scientific opinion of the panel on contaminants in the food chain on a request from the european commission on cadmium in food.

EFSA. 2010. Scientific opinion on arsenic in food.

EFSA. 2013. Scientific opinion on lead in food.

Elhakeem A, Frysz M, Tilling K, Tobias JH, Lawlor DA. 2019. Association between age at puberty and bone accrual from 10 to 25 years of age. JAMA network open 2:e198918.

Esteban-Vasallo MD, Aragones N, Pollan M, Lopez-Abente G, Perez-Gomez B. 2012. Mercury, cadmium, and lead levels in human placenta: A systematic review. Environmental health perspectives 120:1369-1377.

Everson TM, Punshon T, Jackson BP, Hao K, Lambertini L, Chen J, et al. 2018. Cadmium-associated differential methylation throughout the placental genome: Epigenome-wide association study of two u.S. Birth cohorts. Environmental health perspectives 126:017010.

Fagerberg B, Barregard L. 2021. Review of cadmium exposure and smoking-independent effects on atherosclerotic cardiovascular disease in the general population. Journal of internal medicine 290:1153-1179.

Faroon O, Ashizawa A, Wright S, Tucker P, Jenkins K, Ingerman L, et al. 2012. In: Toxicological profile for cadmium. Atlanta (GA).

Flannery BM, Schaefer HR, Middleton KB. 2022. A scoping review of infant and children health effects associated with cadmium exposure. Regulatory toxicology and pharmacology : RTP 131:105155.

Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, et al. 1994. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. The Journal of biological chemistry 269:16896-16901.

Gardner RM, Hamadani J, Grandér M, Tofail F, Nermell B, Palm B, et al. 2011a. Persistent exposure to arsenic via drinking water in rural bangladesh despite major mitigation efforts. American journal of public health 101 Suppl 1:S333-338.

Gardner RM, Nermell B, Kippler M, Grandér M, Li L, Ekström EC, et al. 2011b. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Reproductive toxicology 31:210-218.

Gardner RM, Kippler M, Tofail F, Bottai M, Hamadani J, Grander M, et al. 2013. Environmental exposure to metals and children's growth to age 5 years: A prospective cohort study. Am J Epidemiol 177:1356-1367.

Gleason KM, Valeri L, Shankar AH, Hasan MO, Quamruzzaman Q, Rodrigues EG, et al. 2016. Stunting is associated with blood lead concentration among bangladeshi children aged 2-3 years. Environmental health : a global access science source 15:103.

Gliga AR, Malin Igra A, Hellberg A, Engström K, Raqib R, Rahman A, et al. 2022. Maternal exposure to cadmium during pregnancy is associated with changes in DNA methylation that are persistent at 9 years of age. Environ Int 163:107188.

Greenblatt MB, Tsai JN, Wein MN. 2017. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. Clinical Chemistry 63:464-474.

Gulson B, Mizon K, Smith H, Eisman J, Palmer J, Korsch M, et al. 2002. Skeletal lead release during bone resorption: Effect of bisphosphonate treatment in a pilot study. Environmental health perspectives 110:1017-1023.

Gulson B, Taylor A, Eisman J. 2016. Bone remodeling during pregnancy and post-partum assessed by metal lead levels and isotopic concentrations. Bone 89:40-51.

Gustin K, Tofail F, Vahter M, Kippler M. 2018. Cadmium exposure and cognitive abilities and behavior at 10years of age: A prospective cohort study. Environ Int 113:259-268.

Gustin K, Barman M, Stråvik M, Levi M, Englund-Ögge L, Murray F, et al. 2020. Low-level maternal exposure to cadmium, lead, and mercury and birth outcomes in a swedish prospective birth-cohort. Environmental Pollution 265:114986.

Gwatkin DRR, S,; Johnson, K.; Pande, R.P.; Wagstaff, A. 2000. Socio-economic differences in health, nutrition, and population in bangladesh. Washington , DC.

Hernandez CJ, Beaupré GS, Carter DR. 2003. A theoretical analysis of the relative influences of peak bmd, age-related bone loss and menopause on the development of osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 14:843-847.

Hightower L. 2000. Osteoporosis: Pediatric disease with geriatric consequences. Orthopedic nursing 19:59-62.

Hinton RJ, Jing Y, Jing J, Feng JQ. 2017. Roles of chondrocytes in endochondral bone formation and fracture repair. J Dent Res 96:23-30.

icddr,b. 2017. Registration of health and demographic events 2015.

Ignasiak Z, Slawinska T, Rozek K, Little BB, Malina RM. 2006. Lead and growth status of school children living in the copper basin of south-western poland: Differential effects on bone growth. Ann Hum Biol 33:401-414.

Interdonato M, Pizzino G, Bitto A, Galfo F, Irrera N, Mecchio A, et al. 2015. Cadmium delays puberty onset and testis growth in adolescents. Clin Endocrinol (Oxf) 83:357-362.

Jain RB. 2021. Contribution of diet and other factors for urinary concentrations of total arsenic and arsenic species: Data for us children, adolescents, and adults. Environmental science and pollution research international 28:50094-50116.

Jansen EC, Zhou L, Song PXK, Sánchez BN, Mercado A, Hu H, et al. 2018. Prenatal lead exposure in relation to age at menarche: Results from a longitudinal study in mexico city. Journal of developmental origins of health and disease 9:467-472.

Jin T, Nordberg G, Ye T, Bo M, Wang H, Zhu G, et al. 2004. Osteoporosis and renal dysfunction in a general population exposed to cadmium in china. Environmental research 96:353-359.

Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, et al. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nature medicine 9:1081-1084.

Jung SY, Kim S, Lee K, Kim JY, Bae WK, Lee K, et al. 2015. Association between secondhand smoke exposure and blood lead and cadmium concentration in community dwelling women: The fifth korea national health and nutrition examination survey (2010-2012). BMJ open 5:e008218.

Kanbur NO, Derman O, Kinik E. 2005. The relationships between pubertal development, igf-1 axis, and bone formation in healthy adolescents. Journal of bone and mineral metabolism 23:76-83.

Kim Y, Je Y. 2019. Early menarche and risk of metabolic syndrome: A systematic review and meta-analysis. Journal of women's health (2002) 28:77-86.

Kippler M, Goessler W, Nermell B, Ekstrom EC, Lonnerdal B, El Arifeen S, et al. 2009. Factors influencing intestinal cadmium uptake in pregnant bangladeshi women--a prospective cohort study. Environmental research 109:914-921.

Kippler M, Hoque AM, Raqib R, Ohrvik H, Ekstrom EC, Vahter M. 2010a. Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. Toxicology letters 192:162-168.

Kippler M, Nermell B, Hamadani J, Tofail F, Moore S, Vahter M. 2010b. Burden of cadmium in early childhood: Longitudinal assessment of urinary cadmium in rural bangladesh. Toxicology letters 198:20-25.

Kippler M, Tofail F, Gardner R, Rahman A, Hamadani JD, Bottai M, et al. 2012a. Maternal cadmium exposure during pregnancy and size at birth: A prospective cohort study. Environmental health perspectives 120:284-289.

Kippler M, Tofail F, Hamadani JD, Gardner RM, Grantham-McGregor SM, Bottai M, et al. 2012b. Early-life cadmium exposure and child development in 5-year-old girls and boys: A cohort study in rural bangladesh. Environmental health perspectives 120:1462-1468.

Kippler M, Wagatsuma Y, Rahman A, Nermell B, Persson LA, Raqib R, et al. 2012c. Environmental exposure to arsenic and cadmium during pregnancy and fetal size: A longitudinal study in rural bangladesh. Reproductive toxicology 34:504-511.

Kippler M, Engstrom K, Mlakar SJ, Bottai M, Ahmed S, Hossain MB, et al. 2013. Sex-specific effects of early life cadmium exposure on DNA methylation and implications for birth weight. Epigenetics 8:494-503.

Kippler M, Skröder H, Rahman SM, Tofail F, Vahter M. 2016. Elevated childhood exposure to arsenic despite reduced drinking water concentrations--a longitudinal cohort study in rural bangladesh. Environ Int 86:119-125.

Klaassen CD, Liu J, Choudhuri S. 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annual review of pharmacology and toxicology 39:267-294.

Kralick AE, Zemel BS. 2020. Evolutionary perspectives on the developing skeleton and implications for lifelong health. Frontiers in endocrinology 11:99.

Larsson SC, Orsini N, Wolk A. 2015. Urinary cadmium concentration and risk of breast cancer: A systematic review and dose-response meta-analysis. Am J Epidemiol 182:375-380.

Lee JJ, Cook-Wiens G, Johnson BD, Braunstein GD, Berga SL, Stanczyk FZ, et al. 2019. Age at menarche and risk of cardiovascular disease outcomes: Findings from the national heart lung and blood institute-sponsored women's ischemia syndrome evaluation. Journal of the American Heart Association 8:e012406.

Li Z, Li T, Leng Y, Chen S, Liu Q, Feng J, et al. 2018. Hormonal changes and folliculogenesis in female offspring of rats exposed to cadmium during gestation and lactation. Environ Pollut 238:336-347.

Lin CM, Doyle P, Wang D, Hwang YH, Chen PC. 2011. Does prenatal cadmium exposure affect fetal and child growth? Occupational and environmental medicine 68:641-646.

Liu Y, Téllez-Rojo MM, Sánchez BN, Zhang Z, Afeiche MC, Mercado-García A, et al. 2019. Early lead exposure and pubertal development in a mexico city population. Environ Int 125:445-451.

Lopez-Rodriguez D, Franssen D, Heger S, Parent AS. 2021. Endocrine-disrupting chemicals and their effects on puberty. Best practice & research Clinical endocrinology & metabolism 35:101579.

Lu Y, Kippler M, Harari F, Grandér M, Palm B, Nordqvist H, et al. 2015. Alkali dilution of blood samples for high throughput icp-ms analysis—comparison with acid digestion. Clinical Biochemistry 48:140-147.

Luo Y, McCullough LE, Tzeng JY, Darrah T, Vengosh A, Maguire RL, et al. 2017. Maternal blood cadmium, lead and arsenic levels, nutrient combinations, and offspring birthweight. BMC public health 17:354.

Lönnerdal B. 2017. Development of iron homeostasis in infants and young children. The American journal of clinical nutrition 106:1575s-1580s.

Maisonet M, Jaakkola JJ, Taylor CM, Marcus M, Golding J. 2014. Prenatal lead exposure and puberty timing in girls. Epidemiology (Cambridge, Mass) 25:153-155.

Marshall WA, Tanner JM. 1969. Variations in pattern of pubertal changes in girls. Archives of disease in childhood 44:291-303.

Morris HA, Turner AG, Anderson PH. 2012. Vitamin-d regulation of bone mineralization and remodelling during growth. Frontiers in bioscience 4:677-689.

Nageswara Rao R, Talluri MV. 2007. An overview of recent applications of inductively coupled plasma-mass spectrometry (icp-ms) in determination of inorganic impurities in drugs and pharmaceuticals. Journal of pharmaceutical and biomedical analysis 43:1-13.

Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, et al. 2007. Estrogen prevents bone loss via estrogen receptor alpha and induction of fas ligand in osteoclasts. Cell 130:811-823.

Nardone A, Ferreccio C, Acevedo J, Enanoria W, Blair A, Smith AH, et al. 2017. The impact of bmi on non-malignant respiratory symptoms and lung function in arsenic exposed adults of northern chile. Environmental research 158:710-719.

National Research Council Subcommittee to Update the Arsenic in Drinking Water R. 2001. In: Arsenic in drinking water: 2001 update. Washington (DC):National Academies Press (US). Copyright 2001 by the National Academy of Sciences. All rights reserved.

Navas-Acien A, Sharrett AR, Silbergeld EK, Schwartz BS, Nachman KE, Burke TA, et al. 2005. Arsenic exposure and cardiovascular disease: A systematic review of the epidemiologic evidence. Am J Epidemiol 162:1037-1049.

Navas-Acien A, Silbergeld EK, Streeter RA, Clark JM, Burke TA, Guallar E. 2006. Arsenic exposure and type 2 diabetes: A systematic review of the experimental and epidemiological evidence. Environmental health perspectives 114:641-648.

Nawrot T, Geusens P, Nulens TS, Nemery B. 2010. Occupational cadmium exposure and calcium excretion, bone density, and osteoporosis in men. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 25:1441-1445.

Nermell B, Lindberg AL, Rahman M, Berglund M, Persson LA, El Arifeen S, et al. 2008. Urinary arsenic concentration adjustment factors and malnutrition. Environmental research 106:212-218.

Nkomo P, Richter LM, Kagura J, Mathee A, Naicker N, Norris SA. 2018. Environmental lead exposure and pubertal trajectory classes in south african adolescent males and females. The Science of the total environment 628-629:1437-1445.

Nordberg GF. 2009. Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol 238:192-200.

Ohrvik H, Oskarsson A, Lundh T, Skerfving S, Tallkvist J. 2007. Impact of iron status on cadmium uptake in suckling piglets. Toxicology 240:15-24.

Olsson IM, Bensryd I, Lundh T, Ottosson H, Skerfving S, Oskarsson A. 2002. Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. Environmental health perspectives 110:1185-1190.

Osman K, Åkesson A, Berglund M, Bremme K, Schütz A, Ask K, et al. 2000. Toxic and essential elements in placentas of swedish women. Clinical Biochemistry 33:131-138.

Persson LA, Arifeen S, Ekstrom EC, Rasmussen KM, Frongillo EA, Yunus M, et al. 2012. Effects of prenatal micronutrient and early food supplementation on maternal hemoglobin, birth weight, and infant mortality among children in bangladesh: The minimat randomized trial. Jama 307:2050-2059.

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. 2012. Kisspeptins and reproduction: Physiological roles and regulatory mechanisms. Physiological reviews 92:1235-1316.

Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM. 2018. A comprehensive overview on osteoporosis and its risk factors. Ther Clin Risk Manag 14:2029-2049.

Quansah R, Armah FA, Essumang DK, Luginaah I, Clarke E, Marfoh K, et al. 2015. Association of arsenic with adverse pregnancy outcomes/infant mortality: A systematic review and meta-analysis. Environmental health perspectives 123:412-421.

Raggatt LJ, Partridge NC. 2010. Cellular and molecular mechanisms of bone remodeling. The Journal of biological chemistry 285:25103-25108.

Rahman A, Vahter M, Smith AH, Nermell B, Yunus M, El Arifeen S, et al. 2009. Arsenic exposure during pregnancy and size at birth: A prospective cohort study in bangladesh. American Journal of Epidemiology 169:304-312.

Rahman A, Granberg C, Persson LA. 2017. Early life arsenic exposure, infant and child growth, and morbidity: A systematic review. Arch Toxicol 91:3459-3467.

Rahman A, Kippler M, Pervin J, Tarafder C, Lucy IJ, Svefors P, et al. 2021. A cohort study of the association between prenatal arsenic exposure and age at menarche in a rural area, bangladesh. Environ Int 154:106562.

Raihan MJ, Briskin E, Mahfuz M, Islam MM, Mondal D, Hossain MI, et al. 2018. Examining the relationship between blood lead level and stunting, wasting and underweight- a cross-sectional study of children under 2 years-of-age in a bangladeshi slum. PloS one 13:e0197856.

Rasmussen AR, Wohlfahrt-Veje C, Tefre de Renzy-Martin K, Hagen CP, Tinggaard J, Mouritsen A, et al. 2015. Validity of self-assessment of pubertal maturation. Pediatrics 135:86-93.

Ravenscroft P, Brammer, H; Richards, K. 2009. Arsenic pollution: A global synthesis:Wiley-Blackwell.

Reynolds P, Canchola AJ, Duffy CN, Hurley S, Neuhausen SL, Horn-Ross PL, et al. 2020. Urinary cadmium and timing of menarche and pubertal development in girls. Environmental research 183:109224.

Rodriguez J, Mandalunis PM. 2016. Effect of cadmium on bone tissue in growing animals. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie 68:391-397.

Rollin HB, Kootbodien T, Channa K, Odland JO. 2015. Prenatal exposure to cadmium, placental permeability and birth outcomes in coastal populations of south africa. PloS one 10:e0142455.

Romano ME, Enquobahrie DA, Simpson C, Checkoway H, Williams MA. 2016. Maternal body burden of cadmium and offspring size at birth. Environmental research 147:461-468.

Saedi S, Shirazi MRJ, Zamiri MJ, Totonchi M, Dadpasand M, Sedaghati F. 2020. Impaired follicular development and endocrine disorders in female rats by prepubertal exposure to toxic doses of cadmium. Toxicology and industrial health 36:63-75.

Saha KK, Engstrom A, Hamadani JD, Tofail F, Rasmussen KM, Vahter M. 2012. Pre- and postnatal arsenic exposure and body size to 2 years of age: A cohort study in rural bangladesh. Environmental health perspectives 120:1208-1214.

Salari N, Darvishi N, Bartina Y, Larti M, Kiaei A, Hemmati M, et al. 2021. Global prevalence of osteoporosis among the world older adults: A comprehensive systematic review and meta-analysis. Journal of Orthopaedic Surgery and Research 16:669.

Salvatori F, Talassi CB, Salzgeber SA, Spinosa HS, Bernardi MM. 2004. Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. Neurotoxicology and teratology 26:673-680.

Samuel JB, Stanley JA, Princess RA, Shanthi P, Sebastian MS. 2011. Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative stress and impaired steroid hormone levels. Journal of medical toxicology : official journal of the American College of Medical Toxicology 7:195-204.

Satarug S. 2018. Dietary cadmium intake and its effects on kidneys. Toxics 6.

Schaffler MB, Kennedy OD. 2012. Osteocyte signaling in bone. Curr Osteoporos Rep 10:118-125.

Schoeters G, Den Hond E, Dhooge W, van Larebeke N, Leijs M. 2008. Endocrine disruptors and abnormalities of pubertal development. Basic & clinical pharmacology & toxicology 102:168-175.

Schutte R, Nawrot TS, Richart T, Thijs L, Vanderschueren D, Kuznetsova T, et al. 2008. Bone resorption and environmental exposure to cadmium in women: A population study. Environmental health perspectives 116:777-783.

Selevan SG, Rice DC, Hogan KA, Euling SY, Pfahles-Hutchens A, Bethel J. 2003. Blood lead concentration and delayed puberty in girls. The New England journal of medicine 348:1527-1536.

Signes-Pastor AJ, Doherty BT, Romano ME, Gleason KM, Gui J, Baker E, et al. 2019. Prenatal exposure to metal mixture and sex-specific birth outcomes in the new hampshire birth cohort study. Environmental epidemiology (Philadelphia, Pa) 3.

Skroder H, Hawkesworth S, Kippler M, El Arifeen S, Wagatsuma Y, Moore SE, et al. 2015. Kidney function and blood pressure in preschool-aged children exposed to cadmium and arsenic--potential alleviation by selenium. Environmental research 140:205-213.

Skroder H, Kippler M, Nermell B, Tofail F, Levi M, Rahman SM, et al. 2017. Major limitations in using element concentrations in hair as biomarkers of exposure to toxic and essential trace elements in children. Environmental health perspectives 125:067021.

Skröder H, Kippler M, De Loma J, Raqib R, Vahter M. 2018. Predictors of selenium biomarker kinetics in 4-9-year-old bangladeshi children. Environ Int 121:842-851.

Skröder Löveborn H, Kippler M, Lu Y, Ahmed S, Kuehnelt D, Raqib R, et al. 2016. Arsenic metabolism in children differs from that in adults. Toxicological sciences : an official journal of the Society of Toxicology 152:29-39.

Song Y, Wang Y, Mao W, Sui H, Yong L, Yang D, et al. 2017. Dietary cadmium exposure assessment among the chinese population. PloS one 12:e0177978.

Stoica A, Katzenellenbogen BS, Martin MB. 2000. Activation of estrogen receptor-alpha by the heavy metal cadmium. Molecular endocrinology (Baltimore, Md) 14:545-553.

Streicher C, Heyny A, Andrukhova O, Haigl B, Slavic S, Schüler C, et al. 2017. Estrogen regulates bone turnover by targeting rankl expression in bone lining cells. Scientific reports 7:6460.

Sughis M, Penders J, Haufroid V, Nemery B, Nawrot TS. 2011. Bone resorption and environmental exposure to cadmium in children: A cross--sectional study. Environmental health : a global access science source 10:104.

Svefors P, Rahman A, Ekstrom EC, Khan AI, Lindstrom E, Persson LA, et al. 2016. Stunted at 10 years. Linear growth trajectories and stunting from birth to pre-adolescence in a rural bangladeshi cohort. PloS one 11:e0149700.

Svefors P, Pervin J, Islam Khan A, Rahman A, Ekström EC, El Arifeen S, et al. 2020. Stunting, recovery from stunting and puberty development in the minimat cohort, bangladesh. Acta paediatrica (Oslo, Norway : 1992) 109:122-133.

Taylor CM, Golding J, Emond AM. 2016. Moderate prenatal cadmium exposure and adverse birth outcomes: A role for sex-specific differences? Paediatric and perinatal epidemiology 30:603-611.

Toro CA, Aylwin CF, Lomniczi A. 2018. Hypothalamic epigenetics driving female puberty. Journal of neuroendocrinology 30:e12589.

Towne B, Czerwinski SA, Demerath EW, Blangero J, Roche AF, Siervogel RM. 2005. Heritability of age at menarche in girls from the fels longitudinal study. American journal of physical anthropology 128:210-219.

Turgut S, Kaptanoglu B, Turgut G, Emmungil G, Genc O. 2005. Effects of cadmium and zinc on plasma levels of growth hormone, insulin-like growth factor i, and insulin-like growth factor-binding protein 3. Biological trace element research 108:197-204.

Vahter M, Berglund M, Slorach S, Jorhem L, Lind B. 1992. Integrated personal monitoring of cadmium exposure in sweden. IARC scientific publications:113-119.

Vahter M. 2002. Mechanisms of arsenic biotransformation. Toxicology 181-182:211-217.

Vahter ME, Li L, Nermell B, Rahman A, El Arifeen S, Rahman M, et al. 2006. Arsenic exposure in pregnancy: A population-based study in matlab, bangladesh. Journal of health, population, and nutrition 24:236-245.

Vandenput L, Kindblom JM, Bygdell M, Nethander M, Ohlsson C. 2019. Pubertal timing and adult fracture risk in men: A population-based cohort study. PLoS Med 16:e1002986-e1002986.

Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. 2008. Maternal and child undernutrition: Consequences for adult health and human capital. Lancet (London, England) 371:340-357.

Vidal AC, Semenova V, Darrah T, Vengosh A, Huang Z, King K, et al. 2015. Maternal cadmium, iron and zinc levels, DNA methylation and birth weight. BMC pharmacology & toxicology 16:20.

Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. 2016. The national osteoporosis foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. Osteoporosis International 27:1281-1386.

Werneck AO, Coelho ESMJ, Padilha CS, Ronque ERV, Cyrino ES, Szwarcwald CL, et al. 2018. Age at menarche and cancer risk at adulthood. Ann Hum Biol 45:369-372.

WHO. Ten chemicals of major public health concern. Available: https://www.who.int/ipcs/assessment/public_health/chemicals_phc/en [accessed 2021-02-09].

WHO. 2006. Who child growth standards.

WHO. 2017. Guidelines for drinking-water quality: Fourth edition incorporating first addendum, 4th ed + 1st add.

Willers S, Attewell R, Bensryd I, Schutz A, Skarping G, Vahter M. 1992. Exposure to environmental tobacco smoke in the household and urinary cotinine excretion, heavy metals retention, and lung function. Archives of environmental health 47:357-363.

Williams PL, Bellavia A, Korrick SA, Burns JS, Lee MM, Sergeyev O, et al. 2019. Blood lead levels and timing of male sexual maturity: A longitudinal study of russian boys. Environ Int 125:470-477.

Wilson AK, Cerny EA, Smith BD, Wagh A, Bhattacharyya MH. 1996. Effects of cadmium on osteoclast formation and activity in vitro. Toxicol Appl Pharmacol 140:451-460.

Wood CL, Lane LC, Cheetham T. 2019. Puberty: Normal physiology (brief overview). Best practice & research Clinical endocrinology & metabolism 33:101265.

Wu T, Buck GM, Mendola P. 2003. Blood lead levels and sexual maturation in u.S. Girls: The third national health and nutrition examination survey, 1988-1994. Environmental health perspectives 111:737-741.

Yang H, Huo X, Yekeen TA, Zheng Q, Zheng M, Xu X. 2013. Effects of lead and cadmium exposure from electronic waste on child physical growth. Environmental science and pollution research international 20:4441-4447.

Zamoiski RD, Guallar E, Garcia-Vargas GG, Rothenberg SJ, Resnick C, Andrade MR, et al. 2014. Association of arsenic and metals with concentrations of 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d among adolescents in torreon, mexico. Environmental health perspectives 122:1233-1238.

Zhang Q, Greenbaum J, Zhang WD, Sun CQ, Deng HW. 2018. Age at menarche and osteoporosis: A mendelian randomization study. Bone 117:91-97.

Zhang W, Pang F, Huang Y, Yan P, Lin W. 2008. Cadmium exerts toxic effects on ovarian steroid hormone release in rats. Toxicology letters 182:18-23.

Zhang Y, Xu X, Chen A, Davuljigari CB, Zheng X, Kim SS, et al. 2018. Maternal urinary cadmium levels during pregnancy associated with risk of sex-dependent birth outcomes from an e-waste pollution site in china. Reproductive toxicology 75:49-55.

Zhou CC, He YQ, Gao ZY, Wu MQ, Yan CH. 2020. Sex differences in the effects of lead exposure on growth and development in young children. Chemosphere 250:126294.