Renal Consequences of Cadmium Exposure Among Pregnant Women in Dhaka, Bangladesh Receiving Placebo or Vitamin D Supplementation: An Observational Cohort Study Nested Within a Randomized Controlled Trial

by

Anna Zuchniak

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Department of Nutritional Sciences
University of Toronto

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Abstract

Bangladeshi women are exposed to the toxic metal cadmium (Cd) through consumption of contaminated foods, and exposure to tobacco products. At high levels of vitamin D supplementation, increasing gastrointestinal uptake of Cd may occur. Cd exposure could lead to renal dysfunction, especially during a sensitive period such as pregnancy. In a cohort of pregnant Bangladeshi women enrolled in a randomized placebo-controlled trial of vitamin D supplementation in Dhaka, we examined the effect of vitamin D supplementation on maternal blood and cord blood Cd concentrations and the association of Cd with renal biomarkers. There was no effect of vitamin D supplementation on maternal blood Cd concentrations at delivery; however, vitamin D supplementation increased the probability of detecting Cd in cord blood. Maternal blood Cd concentrations were not associated with markers of renal functioning. Further work is required to clarify the potential effect of vitamin D on maternal-fetal transfer of Cd.

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List of Abbreviations

ACR: Albumin creatinine ratio

AFBM: Analytical Facility for Bioactive Molecules

As: Arsenic

BMD: Benchmark dose

BMDL: Benchmark dose (lower confidence limit)

BMI: Body mass index

B2MG: β_2-microglobulin

CACNA1C: Calcium channel alpha 1C

CaT1: Intestinal calcium transporter 1

Ca: Calcium

Cd: Cadmium

Cd-MT: Cadmium-metallothionein complex

CDC: Centres for Disease Control

CI: Confidence interval

CRP: C-reactive protein

CV: Coefficient of variation

DAG: Directed acyclic graph

DBP: Vitamin D binding protein

DEQAS: Vitamin D External Quality Assessment Scheme

DMT1: Divalent metal transporter 1

ECLIA: Electrochemiluminescence immunoassay

EFSA: European Food Safety Authority

eGFR: Estimated glomerular filtration rate

ELISA: Enzyme-linked immunosorbent assay

FFQ: Food frequency questionnaire

Fe: Iron

FPN1: Ferroportin

GFAAS: Graphite furnace atomic absorption spectrometry

Hg: Mercury

icddr,b: International Centre for Diarrhoeal Disease Research, Bangladesh

LAMP: Lead and Multielement Proficiency Program

LC/MS: Liquid chromatography-tandem mass spectrometry

LOD: Limit of detection

LOWESS: Locally weighted scatterplot smoothing

MAP: Mean arterial pressure

MDIG: Maternal Vitamin D for Infant Growth

MCHTI: Maternal and Child Health Training Institute

Mg: Magnesium

Mn: Manganese

MT: Metallothionein

NAG: *N*-acetyl-β-D-glucosaminidase

NBL: Nutritional Biochemistry Laboratory

NIBSC: National Institute for Biological Standards and Control

NIST: National Institute of Standards and Technology

NOAEL: No observed adverse effect level

Pb: Lead

PTH: Parathyroid hormone

PTMI: Provisional tolerable monthly intake

PTWI: Provisional tolerable weekly intake

ROS: Reactive oxygen species

Se: Selenium

SEPP1: Selenoprotein-1

TDI: Total daily intake

TWI: Tolerable weekly intake

WHO: World Health Organization

VITAL-EQA: Vitamin A Laboratory External Quality Assurance

Zn: Zinc

25(OH)D: 25-hydroxyvitamin D

1,25(OH)D: 1,25-hydroxyvitamin D

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Student Contributions

With the help of my supervisor, Dr. Daniel Roth, I conceptualized the study design. I completed and submitted the application for ethical approval through the Research Ethics Board at the Hospital for Sick Children in Toronto. I performed the laboratory techniques required for analysis of β_2-microglobulin in urine specimen and processed the raw data. In addition, I processed and analyzed the raw data for serum creatinine, urine albumin, as well as urine cotinine. I created a standard operating procedure for urine dipstick testing of specimen and performed the laboratory techniques required. I conducted the literature review, statistical analyses, and result interpretations of the thesis topic, as well as wrote up the thesis paper.

Chapter 1 Introduction

Toxic metal exposure is a pervasive problem in many low-middle income countries, due to pollution, industrial emissions, and lack of environmental regulations in these regions. Chronic exposure to toxic metals causes detrimental health effects, such as cardiovascular disease, renal disease, and neurocognitive developmental delays [1]. Heavy metal exposure during pregnancy can be particularly burdensome, as it can negatively affect the health of both the mother and the fetus [2, 3].

Cadmium (Cd) is a toxic heavy metal that has been found to be prevalent in the Bangladesh environment. The main route of Cd exposure in the Bangladeshi population is through food and tobacco usage [4, 5]. The populations that are the most vulnerable to Cd exposure are females of reproductive age, due to iron deficient women more readily absorbing Cd from the gut [6]. Low grade, chronic exposure to Cd has been associated with renal tubular dysfunction, as well as delayed cognitive development, growth, and increased oxidative stress in children of Cd-exposed women [7-10].

In recent years, micronutrient supplementation has become a common approach in attempting to alleviate the burden of disease in low-middle income countries. Supplementation trials commonly occur in pregnancy cohorts, due to the increased nutrient demand on the mother by the fetus [11]. Vitamin D supplementation trials have been conducted to address health concerns such as growth faltering and acute respiratory infections in young children [12, 13]. With the increased popularity of vitamin D supplementation trials, it is vital to comprehend any potential adverse effects that high vitamin D supplementation could have on toxic metal uptake, particularly in vulnerable populations such as pregnant, malnourished women [14].

The hypothesis that vitamin D supplementation may increase the absorption of toxic metals, such as Cd, has only been shown in an animal model [15]. The thought behind this hypothesis postulates that since Cd does not have specific endogenous transporters in the human body, it utilizes the transporters of structurally similar nutrients, such as calcium (Ca) and iron (Fe) [16]. As vitamin D increases the absorption of Ca via the gastrointestinal tract [17], it is plausible that Cd absorption may be upregulated as well.

In this study, we leveraged the randomized placebo-controlled design of the Maternal Vitamin D for Infant Growth (MDIG) trial in order to elucidate the effect of vitamin D supplementation on blood Cd concentrations in Bangladeshi women. We further aimed to better comprehend the potential renal consequences of Cd exposure in this population.

Chapter 2 Literature Review

2.1 Cadmium

2.1.1 Cadmium in the environment

Cadmium (Cd) is a naturally occurring chemical element and is classified as a divalent heavy metal. It is solid at room temperature and is bluish-white in colour [18]. Cd is released from the earth's crust as a side-product of zinc (Zn) and lead (Pb) smelting [19]. Certain areas have higher natural occurrence of Cd in the soil, such as in Poland, southern Sardinia, and the Goslar region of Germany [20]. Cd's commercial usage is plentiful, such as in the production of TV screens, lasers, paint pigments [21], as well as being used in electroplating and to control nuclear fission reactions. The metal was used in the production of rechargeable nickel Cd batteries [22], but this practice has been phased out, as nickel hydride batteries became more widely available [18].

Cd enters the environment from industrial emissions, such as from the mining industry, or phosphate fertilizer production. Another source of environmental exposure is urban pollution, such as the incineration of municipal solid waste [19]. High soil concentrations of Cd occur due to increasing industrialization, which account for 90% of Cd in the surface environment [21]. Cd enters the food supply through the usage of Cd contaminated pesticides and fertilizers on agricultural soils [23].

2.1.2 Quantification of Cd exposure

Cd exposure in humans is conventionally quantified based on the Cd concentration in blood or urine specimens. Urinary Cd is a biomarker of cumulative exposure, and does not fluctuate with recent changes in exposure [24]. Blood Cd reflects recent exposure (ie. within the last year) and is a suitable biomarker when Cd exposure is assumed to be consistent over time [25].

The half-life of blood Cd is 75-128 days, as shown in a study among workers of a copper-cadmium alloy factory. Cd moves from blood and accumulates in other tissues; it does not have any known biological functions in the human body but can readily cross various membranes and bind to proteins, such as metallothionein (MT) with high affinity [24].

2.1.3 Absorption of Cd

Human absorption of Cd occurs through respiratory, gastrointestinal, and in some cases, dermal contact [26]. Cd binds to the cysteine-rich protein MT post-absorption, which impacts the metal's long retention in tissues. The biological half-life of Cd in tissues is 10-30 years [27]. Clearance of Cd from tissues is extremely low [19]. Excretion of Cd occurs through urine, with only 0.007% of the total body burden of Cd being excreted on a daily basis [28].

MT isoforms are found in all organs, such as the kidney, liver, and intestines. MT binds to Zn and Cd, as well as other divalent metal ions such as iron (Fe^{2+}), lead (Pb^{2+}) and copper (Cu^{2+}) [29]. When Cd overload occurs, tissue expression of MT increases substantially, which leads to increased Cd binding to MT and retention in organs [27]. After absorption, Cd primarily accumulates in the liver and kidneys [21]. The sequestering of Cd by MT protects organs like the kidneys and liver from the toxic effects of Cd, but when the proportion of Cd exceeds MT availability, damage to organs can occur. In addition, once the Cd-MT complex enters the blood, it is filtered by the kidney and absorbed by proximal tubule cells which degrade the complex and release unbound Cd [27]. Studies point to additional forms of Cd transport and storage, as MT deficient mice were found to have similar Cd accumulation as wild-type mice [30]. Individual variation in MT expression could potentially mitigate the impact of Cd on tissues, with individuals with increased MT expression being less predisposed to Cd toxicity [31].

2.1.3.1 Gastrointestinal absorption

Divalent metal transporter 1 (DMT1) and metal transporter protein 1 (MTP1) are Fe transporters in mammals and have been shown to be involved in Cd uptake. An *in vitro* study found that DMT1 can transport Cd from the luminal membrane of the gastrointestinal tract, especially in an Fe-depleted state [32]. DMT1 transports Cd into the enterocyte, after which it is either stored in the cytoplasm bound to MT or transported to the basolateral membrane. Multiple transporters may be involved in Cd uptake and transport, such as Ca binding proteins (calbindin), Fe transporters (FPN1), and Zn transporters (ZIP8 and ZIP14) [33]. An animal study found that mice fed a low-Fe diet and administered Cd had higher body stores of Cd as compared to mice given an Fe-sufficient diet and administered an oral dose of Cd. Thereby, Fe depletion may lead to increased absorption of Cd from the gastrointestinal tract via the upregulated expression of DMT1 and MTP1 [34]. Approximately 6% of ingested Cd is absorbed, with Fe deficient individuals absorbing about 9% [28]. However, a study done on Japanese women (n=25) between the ages of 20-23 who were fed controlled Cd containing diets found absorption to be as high as 47%. The Cd in the diet was mostly originating from rice products, and may have been more effectively absorbed than sources of Cd measured in other studies, such as Cd artificially injected into food [35].

The type of diet consumed appears to have an influence on intestinal Cd uptake. Non-smoking Swedish women with vegetarian high fiber diets rich in root vegetables were shown to have higher Cd intakes than individuals on a non-vegetarian diet [36, 37]. Vegetarians appear to have a higher Cd intake, which may be due to the prevalence of Cd in leafy green vegetables and grain products [38]. The role of fiber in Cd absorption is contested. Fiber may decrease the bioavailability of Cd in foods. In one study, a high fiber intake was shown to

inhibit the amount of Cd absorbed in the intestinal tract [39]. However, high fiber may also inhibit the absorption of Fe, decrease stores, and thereby increase Cd uptake [36]. The intestinal absorption of Cd appears to be greatly modulated by the individual intake of nutrients such as Zn, Fe and Ca. This relationship is primarily due to Cd utilizing the same transport proteins and channels as these nutrients, especially in circumstances when deficiencies of these nutrients are present [33]. Rats fed rice diets low in Ca, Fe, and Zn were found to have Cd retention that was 8 times higher than rats who were fed nutrient sufficient diets [40].

2.1.3.2 Respiratory absorption

Cigarette smoking is a known source of Cd exposure, with one cigarette containing approximately 1-2 µg of Cd [41]. During cigarette smoking, Cd can pass through alveolar cells in the lungs and enter the bloodstream [42]. About 10-50% of inhaled Cd is taken up by the lungs. Smokers have been found to have blood Cd concentrations 4-5 times higher than those of non-smokers [7]. The method by which Cd enters the bloodstream is not completely understood. It appears that Cd uses the transporter channels dedicated to other ions and biomolecules in order to reach circulation pathways [22]. High Cd concentrations in air, such as in the vicinity of metal industries, have been linked to occurrence of emphysema, anosmia, and chronic rhinitis in exposed individuals. In occupationally exposed populations, Cd exposure has been linked to the incidence of lung cancer [43].

2.1.3.3 Transfer to the fetus and infant

Cd is partially transferred from the mother to the fetus through the placenta, or from the mother to the infant via breast milk. A study on a cohort of Polish women found that umbilical cord blood Cd concentrations were only 15% of maternal blood concentrations,

indicating that the placenta may act as a barrier for transfer [39]. On the other hand, Cd does seem to accumulate in the placenta, and could have detrimental impacts on placental functions such as the transport of Zn and Ca to the fetus, as shown in a study conducted in a rural Bangladeshi setting. However, these findings may be specific to malnourished females, as the women in this Bangladeshi cohort had high Cd exposure as well as prevalent Zn deficiency [44]. Placental accumulation of Cd may occur when the placenta is highly Fe depleted, however Fe uptake to the placenta is highly prioritized during pregnancy [6]. While the placenta may act as a barrier to transfer, placental accumulation of Cd may still have detrimental effects on the health of the offspring. In one study, the placentas of mothers who had low birth weight babies had higher Cd levels than the placenta's of mothers who had normal birth weight infants [38]. Pregnant Thai women who lived in either high or low Cd contaminated areas were found to have approximately 20-50% of maternal blood Cd transported across the placenta to umbilical cord blood. The study found an increase in Ca channel alpha 1C (CACNA1C, a type of voltage dependent Ca channel) mRNA in the placentas of high-Cd exposed women, which points to Cd utilizing CACNA1C as a placental transporter. The placental Ca concentrations of high-Cd exposed women were significantly lower than in women who resided in low-Cd exposed areas, in which case, Ca may be displaced in favour of Cd uptake. The placentas of women who were highly Cd exposed were found to have elevated levels of MT mRNA expression, which points to Cd being bound to this protein for transport and storage. The authors postulate that while MT might reduce the toxicity of Cd in the placenta, some Cd could still be transported into the cord blood [45]. A review on Cd breast milk concentrations showed that most studies (from low-middle income and high income countries) had participants with breast milk Cd

concentrations below 2 µg/L, a level that does not ignite significant health concern [46]. A systematic review conducted on Cd levels in breast milk from 1971-2014 found that breastmilk can be a pathway of excretion of maternal Cd. Half of the studies had breastmilk concentrations below the 1 µg/kg/day limit set by the WHO for Cd content in breast milk, while some countries were found to have elevated breastmilk Cd concentrations (such as countries in Asia) [47, 48]. The mammary gland may serve as a limited barrier to maternal-infant Cd transfer, with one study finding breast milk Cd concentrations to be 35% of those found in maternal blood [39]. Though, increasing blood Cd concentrations in mothers may be associated with higher breast milk Cd levels. The Cd that is being transferred from maternal circulation to breast milk may utilize the transporters of Fe and manganese (Mn) in order to do so, and at the same time, displace Ca transport to breast milk [49].

2.2 Cadmium and nutrient interactions

Cd and Zn are chemically similar metals, and both preferentially bind to MT for transport and storage purposes in the human body [50]. Cd displaces Zn from binding with cysteine binding site of MT, and interferes with Zn absorption, distribution, and metabolism [51, 52]. Zn deficiency appears to increase intestinal absorption of Cd, while Zn supplementation decreases Cd absorption [52]. In a study using NHANES data from 2003-2012, individuals with greater Zn consumption were found to have lower Cd concentrations [53]. In addition, individuals with low serum Zn concentrations were found to have an elevated risk of Cd nephrotoxicity [54]. Cd has also been associated with the trace mineral manganese (Mn). Cd may be entering cells through a Mn transporter, as shown in a study where Cd-resistant cells had reduced Mn uptake. This relationship remained after adjusting for Fe status [55].

2.2.1 Vitamin D

Vitamin D is a pro-hormone, which is actively synthesized in the skin from 7-dehydrocholesterol into cholecalciferol in the presence of ultraviolet light. Vitamin D can also be absorbed from food items, such as fatty fish, beef liver, and dairy products [56]. Deficiencies in vitamin D are associated with a variety of harmful consequences, such as rickets in children and osteomalacia in adult populations [57]. Excessive vitamin D supplementation can also cause negative health effects, such as hypercalcemia [58]. A review on vitamin D and toxic elements called into question the lack of cohesion on the topic of optimal vitamin D dosage and avoidance of detrimental health consequences due to toxic metals [14]. It is widely recognized that vitamin D increases the uptake of Ca. Yet, high levels of vitamin D may also theoretically increase the absorption of toxic elements such as aluminum, Cd, and Pb. Non-essential heavy metals do not have naturally occurring transporters in the human body, but can be taken up by various ions, amino acids and small molecule transporters by mimicking other essential nutrients [59]. Thus, vitamin D may increase the co-absorption of metals, such as Cd. The most significant study on vitamin D supplementation and Cd was conducted on baby chicks. After being fed vitamin D deficient diets and displaying symptoms of rickets, chicks were randomized to receive either 2000 IU vitamin D_3 orally and a diet containing 1000 IU vitamin D_3 per 100 g of feed, or no supplementation. Chicks in the supplemented and non-supplemented groups then were divided to receive either oral or subcutaneous doses of Cd^{115}, as well as Zn^{65}, and mercury (Hg^{203}). Researchers found that chicks that were supplemented with vitamin D had increased uptake of both Zn and Cd in the bones, specifically those that were dosed orally with Cd or Zn. As the oral dose of Cd led to increased uptake, this would indicate that vitamin D

increased gastrointestinal absorption of Cd [15]. Cd may also have an effect on vitamin D metabolism, as postmenopausal women with itai-itai disease (Cd poisoning disease which causes joint pain and kidney failure) living near the Cd contaminated Jinzu river in Japan were found to have increased urinary excretion of vitamin D binding protein (DBP) associated with long-term Cd exposure. The authors hypothesized that increased excretion occurred due to Cd exposure leading to renal tubular dysfunction and disrupted vitamin D metabolism in the kidneys, leading to bone lesions [60]. Of note, the women who were most severely affected by abnormalities such as proteinuria and glucosuria were post-menopausal, malnourished, and multiparous [61].

Contrary to the aforementioned findings in the chick study, a study conducted on pregnant Canadians from the MIREC cohort found that higher vitamin D intake was associated with lower Cd concentrations. In this cohort, blood Cd levels had a median of 0.20 μg/L. No differences in Cd concentrations existed over the course of the pregnancy. It is important to note that vitamin D intake was determined using food frequency questionnaires and a dietary supplement questionnaire, and no biochemical measures were obtained, thus vitamin D status was not directly ascertained [62]. Since vitamin D intake was measured through an imprecise proxy measure, and vitamin D status was not quantified using for example, serum 25-hydroxyvitamin D (25(OH)D) concentrations, this observed association might not be indicative of the true relationship of vitamin D on Cd absorption.

A study with similar findings was conducted among Korean women with Fe deficiency or Fe deficiency and anemia. Data were obtained from a cross-sectional, nationally representative survey and interpreted using path analysis. It was found that low 25(OH)D levels were related to Fe deficiency anemia, while Fe deficiency anemia increased blood Cd

concentrations [63]. Some animal research has shown that Cd exposure may reduce serum 25(OH)D levels, and potentially lead to impairments in bone metabolism [52], but evidence is conflicting with additional studies showing no effect [64]. Clearly, current research is lacking regarding the topic of vitamin D and Cd. It is unknown at what level vitamin D increases absorption of Cd. Potentially, the effect of vitamin D on Cd absorption may have a U-shaped effect, with nutritional deficiency, as well as excessively high levels increasing the absorption of Cd. While vitamin D may theoretically increase the absorption of Cd by upregulating intestinal absorption of the toxic metal, Cd may also may adversely affect the renal production of $1,25(OH)_2D_3$, which could lead to metabolic bone disease, as found in a rat study [65, 66]. However, this suppressive relationship of Cd on $1,25(OH)_2D_3$ was not replicated in a study in monkeys and should be verified with further research [64].

2.2.2 Calcium

Ca binding proteins can bind Cd, as well as other divalent cations [67]. In a study conducted in rats, those fed a low-Ca diet had 60% more Cd accumulating in kidneys and the liver than those fed a Ca-sufficient diet [68]. A mouse study found that oral $CdCl_2$ injections of 1 mg/kg a day for 5 days, and a Ca deficient diet caused hepatic and renal Cd accumulation. It was also shown that the intestinal mRNA expression of intestinal Ca transporter 1 (CaT1), was higher in mice fed the Ca deficient diet, independent of DMT1 expression, pointing to CaT1 being a potential stimulator of Cd absorption [69]. The relevance of these findings to human populations is not yet fully understood. In a rural female Bangladeshi population, Ca and blood Cd were found to be inversely related [70], which may occur due to Cd inhibiting Ca intestinal uptake, or vice versa. Thus, low Ca intake might upregulate the absorption of Cd via CaT1 in this population.

Supplementation with vitamin D increases Ca absorption, which theoretically could then decrease the amount of Cd absorbed. However, in a Ca deficient population, vitamin D supplementation could possibly increase Cd absorption, and Cd could substitute for Ca at the site of Ca transportation, such as CaT1.

2.2.3 Iron

Cd may alter cellular Fe availability, by inducing ferroportin expression, which is an Fe transporter in mammals found at the basolateral membrane in enterocytes and macrophages [71, 72]. A study conducted on macrophage cells found that low-dose Cd treatment increased ferroportin expression at the translational level, and thereby was associated with decreased cellular Fe levels [72]. In mouse bone marrow macrophages, it was shown that Zn and Cd activate FPN1 transcription through the action of Metal Transcription Factor-1 (MTF-1), which is a unique Zn finger transcription factor [73]. A study conducted on mouse cells showed that Cd upregulates FPN1 expression in a transcription-dependent manner through the generation of oxidative radicals. Consequently, Cd may interfere with Fe homeostasis [74]. As previously mentioned, individuals who are Fe deficient may have increased Cd absorption. A study of pregnant Bangladeshi women found low Fe stores were associated with increased urinary Cd levels in individuals with adequate Zn stores, which appears to be occurring due to increased DMT1 expression in iron-deficient states [75].

Fe deficient individuals who were supplemented with vitamin D may possibly have increased Cd levels, due to increased gastrointestinal Cd absorption, and Cd binding substituting for Fe at the site of Fe transporters, such as DMT1.

2.2.4 Selenium

While selenium (Se) is an essential element, it can gain entry into cells in a similar way to Cd, by mimicking nutrients at the site of their endogenous transporters. Se transport into cells may be influenced by biomolecules such as glutathione and cysteine [16]. Se has been found to antagonize oxidative stress caused by Cd exposure, by isolating Cd into biologically inert conjugates, and through its role as an antioxidant [76]. Se may also bind to Cd and form a Se-Cd complex, thus decreasing the concentration of free form Cd and preventing toxicity [77]. Mice dosed orally with both Cd and Se were found to have lower Cd concentrations in the kidney as compared to mice given only oral Cd supplementation [77]. Suckling rats given oral Se supplementation as a pre-treatment had lower Cd concentrations in their blood, brain, liver, and kidneys, as well as decreased Se in their plasma, kidney and brain [78]. In theory, if vitamin D supplementation increased Cd concentrations, high Se concentrations could have antagonized the detrimental effects of Cd, and even decreased Cd absorption.

2.2.5 The association of vitamin D with Ca, Fe, and Se

Vitamin D is associated with the absorption and physiological processes of various essential nutrients, such as Ca, Fe and Se. Vitamin D is known to up-regulate the absorption of Ca from the intestine. In situations where there is an increased demand on Ca stores (such as during pregnancy), or when Ca intake is insufficient, the active form of vitamin D, 1-25-dihydroxyvitamin D_3 (1-25$(OH)_2D_3$) is amplified, which causes an increase in Ca absorption from the gut. In the event that intestinal Ca uptake is not enough to maintain serum Ca levels, 1-25$(OH)_2D_3$ and parathyroid hormone (PTH) work together to increase Ca mobilization from the bone and kidneys [17].

Studies have shown a relationship between low vitamin D and Fe deficiency [79]. While the exact mechanisms behind this association is not fully understood, it may be due to vitamin D having a suppressive effect on hepcidin, causing iron dysregulation and erythropoiesis [71]. Vitamin D may also increase the absorption of Se in a similar process as Cd, but further research needs to confirm this relationship [14].

2.2.6 Associations of Cd with other heavy metals

Environmental exposure to Cd often co-occurs with exposure to other toxic metals, such as lead (Pb), arsenic (As), or mercury (Hg) especially in low-income industrialized areas. The interactive effects of these toxic metals may lead to amplified negative health consequences [80]. Mixed metal studies have mostly focused on the relationship of Pb and other toxic metals, such as Cd. A Korean prospective study on prenatal metals exposure found a synergistic interaction between maternal Pb and high Cd blood concentrations on neuro-development measures in 6-month old children [81]. Other research has found interactive effects of Pb and Cd as reproductive toxicants in peripubertal girls [82]. Mixed metals are an emerging field of research that is fraught with complications. Metals are metabolized in various ways and may interact at particular thresholds of concentration or time frames, which is difficult to accurately measure and quantify [80]. If vitamin D was found to increase blood Pb concentrations in a similar way to blood Cd, then high concentrations of both toxic metals could exacerbate adverse health outcomes, such as neurocognitive developmental delays in children.

2.3 Cadmium and human health

2.3.1 Overall health impact

Exposure to Cd is considered dangerous for human health and has been listed as a category 1 known human carcinogen by the International Agency for Research on Cancer [83]. The Environmental Protection Agency has Cd classified as a Group B1 probable human carcinogen [28]. Cd causes oxidative stress, leading to tissue injury [84]. It also has been shown to inhibit heme synthesis [85]. High Cd exposure has been linked to kidney and bone effects, itai-itai disease, and the possible occurrence of cancers, such as lung and prostate, in humans [26]. It may also be an *in vivo/in vitro* endocrine disruptor through estrogen and androgen-like activities [86]. Acute effects of Cd toxicity, which predominantly occur through occupational respiratory exposure, lead to pulmonary edema, chemical pneumonitis, and can cause severe respiratory effects years after exposure. Acute, oral ingestion of Cd causes severe symptoms of gastroenteritis. If the amount of ingested Cd is sufficient enough, liver or kidney necrosis and metabolic acidosis can occur [28].

2.3.2 Renal health impact

Cd's effect on renal function is one of the most well-established health outcomes of chronic exposure. Tubular dysfunction is the primary renal effect of chronic Cd exposure. The low molecular weight of the Cd-MT complex readily allows the passage from the glomerular kidney membrane into the tubular fluid. Damage to the tubules may occur if free Cd exceeds the amount of bound Cd-MT, and begins to cause degradation to the proximal tubules [87]. Renal effects appear to occur above a urinary Cd threshold of 2-10 μg/g creatinine, with the lower levels of urinary Cd causing increased excretion of renal biomarkers, and higher levels of Cd detrimentally impacting proximal tubule functioning [88]. One of the earliest observed

nephrotoxic effects of Cd exposure in individuals is the elevated excretion of *N*-acetyl-β-D-glucosaminidase (NAG, an indicator of renal proximal tubular damage) in urine, as well as low molecular weight proteins, such as β2-microglobulin (B2MG). These proteins are almost completely reabsorbed under normal conditions [89]. At urinary levels of 1000 µg urine B2MG/g creatinine, renal tubular dysfunction is considered irreversible [90]. A 1% decrease in proximal tubule function could lead to a 30 fold increase in the excretion of urinary proteins such as B2MG [91]. In one cross-sectional population study, urine Cd levels were abnormal (above the 95th percentile) in 10% of participants when excretion of B2MG was 305 µg per day [92].

The European Food Safety Authority (EFSA) suggests a reference point of 1 µg Cd/ g creatinine as the dose-response relationship between urinary Cd and elevated B2MG excretion [93]. In a population of adult Japanese women, a urinary Cd concentration of over 2 µg/L or 2 µg/g creatinine caused 5% of the study population to have excretion of B2MG, and NAG above the 95th percentile. This Cd concentration was linked to a tubular response of 323 µg B2MG/g of creatinine [94]. This level of B2MG is within the 300 and 1,000 µg/g creatinine proposed to be the bounds of tubular dysfunction caused by Cd exposure [95]. Various studies have been conducted on the dose dependent relationship of Cd and renal functioning. A benchmark dose (BMD) method is used to assess the risks of being exposed to dangerous compounds and is defined as the exposure limit that corresponds to an increase in the probability of an unfavourable response, as compared to zero background exposure. When the lower 95% confidence limit of BMD is used, it is referred to as the benchmark dose lower confidence limit (BMDL) and serves as a replacement of the no observed adverse effect level (NOAEL) [96]. It appears that urinary Cd concentrations that lead to elevated

levels of renal indicators vary, but usually are above the 1 µg/g creatinine reference set out by the EFSA. However, the presence of high levels of urine B2MG in women in itself may not indicate Cd-induced tubular dysfunction, as B2MG should be evaluated in combination with other factors, such as Cd concentrations [95].

The Joint FAO/WHO Expert Committee on Food Additives established the provisional tolerable monthly intake (PTMI) of 25 µg/kg body weight to account for long and short-term risks of disease due to Cd exposure. This amount corresponds to a weekly intake of 5.8 µg/kg body weight [97]. A previous report had an established provisional tolerable weekly intake (PTWI) of 7 µg/kg body weight. The report ascertained the risk of tubule dysfunction to increase when urinary excretion of Cd began to exceed 2.5 µg/g of creatinine. The EFSA endorsed a tolerable weekly intake (TWI) of 2.5 µg/kg body weight, a decision that was then confirmed in a subsequent report [19, 98]. In environmentally exposed populations, dietary intakes of Cd that exceed 0.5 µg/kg of body weight a day has been shown to be associated with an increased prevalence of renal tubule dysfunction [99]. There are very few established recommendations for safe Cd concentrations in pregnant women. The Federal Environmental Agency of Germany has the established limit of 1 µg/L in whole blood for non-smoking adults between the ages of 18-69 [100]. However, Cd has no physiologic functions in the human body and ideally should not be present in any concentration.

2.3.2.1 Vitamin D and renal health

The relationship of vitamin D and renal health in the context of Cd exposure has limited research. One recent study in a Chinese cohort found that high levels of serum 25(OH)D mitigated Cd associated renal tubular dysfunction [101]. It is pertinent to consider the confounding effects that vitamin D intake or status could have on the association of Cd and

renal outcomes. Research on vitamin D and renal health has shown that low vitamin D status has been associated with end stage renal disease [102], as well as chronic kidney disease, due to impaired uptake of 25(OH)D by the kidneys [103].

2.3.3 Impact on maternal health

2.3.3.1 Health effects of Cd in pregnancy

A small body of research exists on the health outcomes of Cd-exposed pregnant women. Most studies have been focused on the association of Cd levels, preeclampsia and pregnancy. Preeclampsia affects approximately 2-8% of pregnancies and is diagnosed when maternal blood pressure is >140/90 mmHg on two separate occasions (measured 4 hours apart), along with the occurrence of proteinuria (urinary protein excretion of >300 mg/day, protein/creatinine ratio >0.3, or protein > 30 mg/dL or 1+ on a urine dipstick) [104-107]. A significant reduction in estimated glomerular filtration rate (eGFR) occurs in preeclamptic women, and is more common in those with pre-existing kidney dysfunction [104]. Unfortunately, the only known cure for preeclampsia is delivery [108]. In a study in rats, administering a prenatal low dose of Cd led to the development of preeclampsia-like symptoms, such as hypertension, proteinuria, and fetal growth restriction [109]. A study in a cohort of American women found that Cd exposure was associated with preeclampsia risk. This association significantly increased when placental Se levels were below the median, and with decreasing placental Zn levels, pointing to a protective effect of Se and Zn on the relationship between placental Cd levels and preeclampsia [110]. Pregnant women in a Turkish cohort study who had preeclampsia were found to have higher serum levels of Cd as compared to normotensive pregnant women, and healthy non-pregnant women. The study also found decreased Zn levels in preeclamptic and normotensive pregnant women compared

to non-pregnant controls [111]. A systematic review of studies concerning Cd exposure and reproductive health issues in women found the greatest association between Cd levels and preeclampsia, as well as hypertension in pregnant females [112]. In a more recent Chinese case-control study, women with preeclampsia were found to have higher placental and blood Cd concentrations in comparison to normotensive women [113].

The mechanism of Cd influenced preeclampsia risk is unknown, but it may occur through Cd altering the expression of miRNA which target pathways related to preeclampsia [114], or through Cd inducing oxidative stress and reactive oxygen species (ROS) [115]. Cd is one of a few heavy metals that has been found to be elevated in women with high-risk pregnancies. Preeclamptic women in their third trimester (n=29) were found to have significantly increased Pb, Cd, Cu and magnesium (Mg), and decreased Zn in their amniotic fluid when compared to normotensive women (n=101). In this study population, Cd levels were elevated 18% in the preeclamptic women [116]. In contrast, some studies have shown no relationship between Cd levels and preeclampsia. A cohort study in Iran found that preeclamptic women had higher levels of Pb, Mn and antimony in umbilical cord blood, but not Cd [117]. However, in a study on pregnant smoking and non-smoking women in former Yugoslavia, normotensive and hypertensive smokers had the highest mean (± standard deviation) maternal blood Cd concentrations (2.2± 1.1 µg/L and 1.9 ± 0.6 µg/L, respectively), and had decreased Se levels [118].

During pregnancy, Cd uptake may increase due to increasing demands for Fe and micronutrient absorption [6], which increases DMTI density at the enterocyte apical surface of the intestine, as observed in a study conducted on pregnant female rats [119]. A study on pregnant rural Bangladeshi women given multiple micronutrient supplementation or usual

care (micronutrient containing Fe and folic acid) found Cd levels to be 1.1 µg/kg erythrocyte (or 0.5 µg/L in whole blood) in early pregnancy, with a range of 0.30-5.4 µg/kg, and an increase of 0.2 µg/kg from early pregnancy to 6 months postpartum [70]. These levels are similar to those shown in Swedish middle-aged women after chronic exposure and could be associated with detrimental effects on kidney and bone health [120, 121]. In addition, multiparous Bangladeshi women were found to have higher Cd levels compared to primiparous women, which has also been shown in previous literature [6, 70]. In participants of the Bangladeshi study, Cd levels increased postpartum, but there was no difference in postpartum Cd levels between women given multiple micronutrient supplements versus control. Women with adequate Zn status but low Fe stores showed increased blood Cd, which is evidence of Cd being absorbed more readily when Fe stores are insufficient, as Zn may play a role in up-regulating DMT1 expression [70].

A systematic review/meta-analysis found a positive relationship between blood Cd concentrations and blood pressure in women [122]. However, in a study on Cd exposed pregnant women and blood pressure, women with urine Cd concentrations in the highest tertile and in their third trimester of pregnancy who had a normal or underweight BMI had decreased mean arterial pressure (MAP). In normal pregnancy, MAP usually increases in the third trimester (37-40 weeks) compared to earlier pregnancy. Interestingly, the women with increased urine Cd were also found to have increased dietary consumption of micronutrients (Ca, Zn, Mg, and Se) and urine Cd concentrations increased with higher intake of Zn and non-heme Fe containing foods. No significant difference was found in urine Cd concentrations between preeclamptic and non-preeclamptic patients [123]. In the above mentioned systematic review, urine Cd and hypertension were found to have an inverse

relationship [122]. Evidently, the relationship between Cd levels in pregnancy and blood pressure is not completely clear. The biological mechanism behind Cd lowering blood pressure in later pregnancy maybe due to its estrogenic effects on certain target organs (shown in animal studies), or through Cd binding to Ca binding sites, activating calmodulin dependent enzymes, and thus increasing dopamine synthesis in the brain which leads to decreased sympathetic nerve activity and blood pressure [124, 125].

2.3.3.2 Renal biomarkers in pregnancy

Few studies have measured urine B2MG concentrations during pregnancy. It appears that a significant increase in concentration occurs during pregnancy, with one study showing normotensive pregnant women at 30 weeks of gestation having mean (± standard deviation) B2MG levels of 642 ± 634 μg B2MG/g creatinine, while non-pregnant women had a mean concentration of 80 ± 37 μg B2MG /g creatinine. This is an approximate 8-fold increase in concentration between pregnant and nonpregnant women. In the study, urine B2MG did not differ significantly between gestational weeks 37, 30 and 20. The study also found increased urine albumin/creatinine (ACR) concentrations in later pregnancy (30 and 37 weeks) compared to early pregnancy (20 weeks) and non-pregnant controls [126]. This is comparable to previous research which has found increased albumin concentrations in second and third trimester pregnancy compared to nonpregnant controls [127]. The authors of the study postulated that increased urine B2MG concentrations may be occurring due to decreasing renal tubular reabsorption during pregnancy, while albumin increased due to increased glomerular permeability [126].

The connection between Cd exposure and renal function may be of particular importance during pregnancy, when women have an increase in blood volume, heart rate, cardiac output,

as well as changes in GFR and renal plasma flow [128]. Glomerular hyper-filtration increases 40-60% in the second half of pregnancy, and renal plasma flow increases up to 80% by the 12th week of gestation [129]. For this reason, pregnant women with high Cd exposure may have an increased risk of renal dysfunction and health complications, especially in a population with a high prevalence of Cd exposure.

2.3.4 Impact on fetal/infant health

In a study of rural pregnant Bangladeshi women, increased maternal Cd levels were found to be positively associated with Cd levels in breastmilk [49]. This could affect the levels of Cd accumulation in infants, as the gastrointestinal tracts of infants uptake more Cd than adults, as shown when adults and infants were fed a porridge made with the stable isotope ^{106}Cd, at a concentration found in normal diets [130]. In addition, the breast milk of Bangladeshi women has been shown to be elevated in Cd as compared to women in other countries (with the exception of Japan and India) [44]. As previously mentioned, Cd can be transferred from maternal stores to the fetus by crossing the placenta, albeit with the placenta acting as a partial barrier [131]. Maternal Cd concentrations have been associated with decreased birthweight of infants [132], and increased odds of infants being born small-for-gestational age in observational epidemiological studies [133]. A cross-sectional study on early life Cd exposure showed a correlation with bone resorption in 10-year old children, pointing to a potential demineralization effect [134]. Prenatal Cd exposure, and low-grade exposure in children in early life have also been linked to lower intelligent scores in 5-year-olds in rural Bangladesh [9].

2.4 Cadmium in Bangladesh

2.4.1 Food sources of Cd in Bangladesh

Unlike other toxic elements, such as As, Cd has not been found to be elevated in Bangladeshi drinking water [135]. Cd is abundant in a variety of foods commonly consumed by humans such as crustaceans, cereals, rice, and leafy vegetables [136]. Several studies have tested food products in Bangladesh to characterize specific dietary sources of Cd, and have found significant levels of Cd in the regional food chain [136, 137]. A thorough analysis of commonly consumed Bangladeshi food products purchased in the United Kingdom estimated that the total daily intake (TDI) of Cd through food products was 34.55 μg/day, which is elevated compared to other non-occupationally exposed populations studied thus far [4]. A more recent analysis of frequently consumed Bangladeshi foods such as rice, vegetables and fish concluded that Cd exposure from consumption of all three surpassed allowable daily intakes. The study additionally found that Cd in rice may also be above allowable carcinogenic levels for children and adults [138]. Rice and leafy green vegetables, two staple food products in the Bangladeshi diet, retain high amount of Cd due to the heavy metal leaching from agricultural soils into food products. Leafy green vegetables contain Cd due to the metal accumulating more in the leaves of plants than the vegetable seeds or roots [139]. Both urban and rural populations are exposed to Cd-contaminated rice, as rice is grown in paddies in rural areas and is later sold at urban markets [138]. Discarding the water in which rice is cooked does not decrease the amount of Cd in rice, which was found to have a mean concentration of 30 μg/kg in one study [136]. The Cd concentration of rice products is alarming, seeing as rice is a staple of the Bangladeshi diet and is consumed at least twice a day. Rice is consumed in large

quantities, with adults consuming approximately 1,500 grams of rice per day, and accounts for about 76% of a regular daily caloric intake [138, 140, 141].

2.4.2 Tobacco sources of Cd in Bangladesh

Cigarette smoking is the most significant source of Cd exposure in humans [142]. Cigarette smoking among Bangladeshi females is considered a social taboo, and few admit to the behaviour. Thus, reported smoking rates (4% among Bangladeshi women) could be underestimated [143]. In a Dhaka based study on smoking rates, about 5% of women and 36% of men were found to be smokers [144]. Individuals who are cigarette smokers have been found to have blood and urine Cd and As levels twice that of non-smokers [145]. Second-hand smoking is also a source of Cd exposure. A Korean based cohort study found that second-hand tobacco exposure in a workplace setting was related to elevated blood Cd levels in women [146]. The source of heavy metal exposure in tobacco plants in Bangladesh appears to be due to excessive usage of commercial fertilizers, contaminated animal manures, as well as metal-based pesticides in agricultural soils. This study also presented evidence on the enhanced mobility of Cd from soil to the tobacco plant as compared to other toxic metals [147]. Betel quid, commonly known as *paan* (a combination of areca nut, betel leaf, lime and tobacco leaves), is popular stimulant used by the Bangladeshi population [5]. A 2009 survey showed that in individuals over the age of 15, 25% of women and 24% of men were consumers of betel quid. Among users, 80.4% chewed betel quid along with tobacco [148]. Rural pregnant Bangladeshi women had reported rates of 11% chewing betel quid with tobacco [149]. It has been found that women are more likely than men to chew betel quid multiple times a day [150]. Betel quid is a significant source of Cd, with one betel quid (mean weight of 7g) containing about 0.028 mg kg^{-1} dry weight of Cd. An intake of 6 betel quid a

day (a modest consumption, as some individuals reported chewing up to 30 a day) can contribute to 1.9% of the provisional tolerable monthly intake (PTMI) of Cd. Tobacco, flavoured tobacco, and lime had the highest Cd concentrations of the various components of the betel quid [5]. Betel quid consumption has been linked to detrimental health outcomes, such as oral cancer occurrence [151, 152].

2.4.3 Additional sources of Cd in Bangladesh

The prevalence of geophagy in pregnant women in Bangladesh, specifically the consumption of baked clay (sikor), could also be a source of Cd exposure. This product is promoted as a source of micronutrients during pregnancy and is even sold in certain Bangladeshi stores. However, sikor has been found to contain high amounts of toxic heavy metals, such as Pb, As, as well as Cd [153]. An additional under-studied exposure in Bangladesh is Cd that can leach out during usage of low-quality aluminum cookware. Out of the 42 cookware items tested from Bangladesh, 23 had detectable levels of Cd, with one pot having a maximum estimated exposure of 7.5 µg per usage [154].

2.4.4 Cadmium concentrations in Bangladeshi women

The absorption of Cd is higher in Bangladeshi women than in men [155]. Bangladeshi women with low Fe and Zn stores were shown to be prone to higher Cd concentrations in urine [75], which may be caused by increased gastrointestinal absorption by Fe-deficient women of child-bearing age [6]. In a study in rural Bangladesh, pregnant women (n=1616) were found to have a median urinary Cd level of 0.63 µg/L [156]. An additional study on pregnant Bangladeshi women (n=408) found erythrocyte concentrations of Cd to have a median of 1.1 µg/kg at 14 weeks gestation, which is equivalent to approximately 0.5 µg/L Cd in whole

blood. Blood Cd concentrations were found to increase 15% from early pregnancy to 6 months postpartum, possibly due to enhanced Fe absorption in later pregnancy [70].

2.4.5 Cadmium exposure in Bangladesh compared to Canadian exposure

Comparing Cd exposure in Bangladeshi and Canadian populations shows clear differences. The mean intake of Cd from diet in Canada was estimated to be 0.014 mg/day, (or 14 μg/day) [157]. In females between the ages of 20-39, the dietary Cd intake was 0.21 μg/kg of body weight a day [158]. A report analyzing the results from the Canadian Health Measures survey found the greatest contributors to blood and urine Cd levels in Canadians were age and smoking status, with diet being a modest contributor to exposure overall. In this report, Canadian women (aged 20-39, smokers and non-smokers) had a geometric mean blood Cd level of 0.31 μg/L. [159]. Newcomer Canadian women from East and South Asian countries (aged 19-45) have been shown to have slightly elevated blood Cd levels, as compared to Canadian-born individuals of a similar age group, with a geometric mean of 0.39 μg/L. Newcomer Bangladeshi women had some of the most elevated blood levels in the study with a geometric mean of 0.49 μg/L [160]. Thus, it does appear that immigrant Canadian women have elevated blood Cd concentrations, potentially as a result of living in Cd contaminated environments for a substantial portion of their lives, and due to Cd's long retention in organs.

Chapter 3 Rationale, Aims

3.1 Rationale

There have been no published randomized controlled trials that test the effect of vitamin D supplementation on toxic metal burden, specifically Cd. Our proposed study aimed to assess the causal effect of vitamin D supplementation on blood Cd concentrations during pregnancy. The *a priori* hypothesized role of Ca, Se and Fe on the effect of vitamin D supplementation and blood Cd were also examined. In addition, our study estimated the impact of blood Cd concentrations on the renal function of pregnant Bangladeshi women, while considering the main environmental sources of Cd, particularly food and tobacco products. Certain micronutrients, such as Se, Fe, and vitamin D may modulate the relationship between Cd and renal function, as shown in animal models and observational studies on the topic of Cd and health (renal) outcomes in pregnancy. We aimed through exploratory assessment, to gain a more thorough understanding of the role of these micronutrients as modifiers of the relationship of blood Cd and renal function.

3.2 Aims and hypotheses

In a cohort of pregnant Bangladeshi women, we aimed:

1: To investigate the dose-dependent effect of prenatal vitamin D supplementation on maternal blood and cord blood Cd concentrations among pregnant women at delivery.

1.1: To examine habitual calcium intake during pregnancy as a potential modifier of the effect of vitamin D supplementation on maternal blood and cord blood cadmium levels.

1.2: To explore whether selected minerals (including selenium, and iron) modified the effect of vitamin D supplementation on maternal blood and cord blood cadmium level

2: To investigate the association of maternal blood cadmium levels with urine β_2-microglobulin/creatinine ratio and other biomarkers of renal function (serum creatinine and urine albumin/creatinine ratio).

2.1: To examine whether selected minerals including selenium, and iron (based on circulating serum ferritin concentrations) acted as modifiers of the association between maternal blood cadmium levels and renal function.

2.2: To examine whether prenatal vitamin D supplementation modified the association between maternal blood cadmium levels and renal function.

We hypothesized that high-dose vitamin D supplementation (28,000 IU/week) would increase maternal blood Cd concentrations at delivery in this cohort of pregnant Bangladeshi women. This dose-dependent relationship may be modulated by Ca status, with participants who consume low Ca diets, in conjunction with receiving higher dose vitamin D supplementation, showing increased circulating Cd concentrations. We did not expect to see a substantial effect on cord blood Cd concentrations, as transfer of Cd is mitigated by the placenta acting as a partial barrier. It was expected that participants with increased blood Cd levels would show evidence of renal dysfunction, as indicated by increased levels of excretion of urine B2MG. In addition, we expected blood Cd to be positively correlated with urine ACR, and serum creatinine concentrations. The effect of Cd on renal function may be attenuated by an individual's status with respect to other minerals/micronutrients; for example, a high whole blood Se concentration may mitigate the negative impact of Cd on renal dysfunction. Owing to competitive intestinal absorption, we expected that participants

with increased Cd exposures would have lower Fe and Ca absorption, and conversely that higher intake and/or absorption of these minerals would protect against the effects of Cd.

Chapter 4 Methodology

4.1 Cohort description

4.1.2 Background on MDIG study

Our proposed study was nested within the Maternal Vitamin D for Infant Growth (MDIG) trial, a randomized, dose-ranging, placebo-controlled design of the MDIG trial that enables causal inferences with respect to the effect of vitamin D supplementation on Cd exposure [161]. The main objectives of the trial were to establish the effect of maternal prenatal vitamin D_3 supplementation (4200 IU/week, 16,800 IU/week, 28,000 IU/week) versus placebo on infant length at 1 year of age in Dhaka, Bangladesh, as well as the effect of maternal postpartum vitamin D_3 supplementation (28,000 IU/week) versus placebo on infant length at 1 year of age in infants born to women in the trial who received 28,000 IU/week prenatally (see Figure 1 for treatment group allocation of MDIG participants). With respect to supplementation, all women were given 500 mg/day of Ca as Ca carbonate, as well as Fe (66 mg/day) and folic acid (350 μg/day) for the duration of the intervention. Secondary objectives that were addressed during the trial included anthropometric outcomes, and clinical, biochemical, and microbiological surveillance of infant-mother pairs.

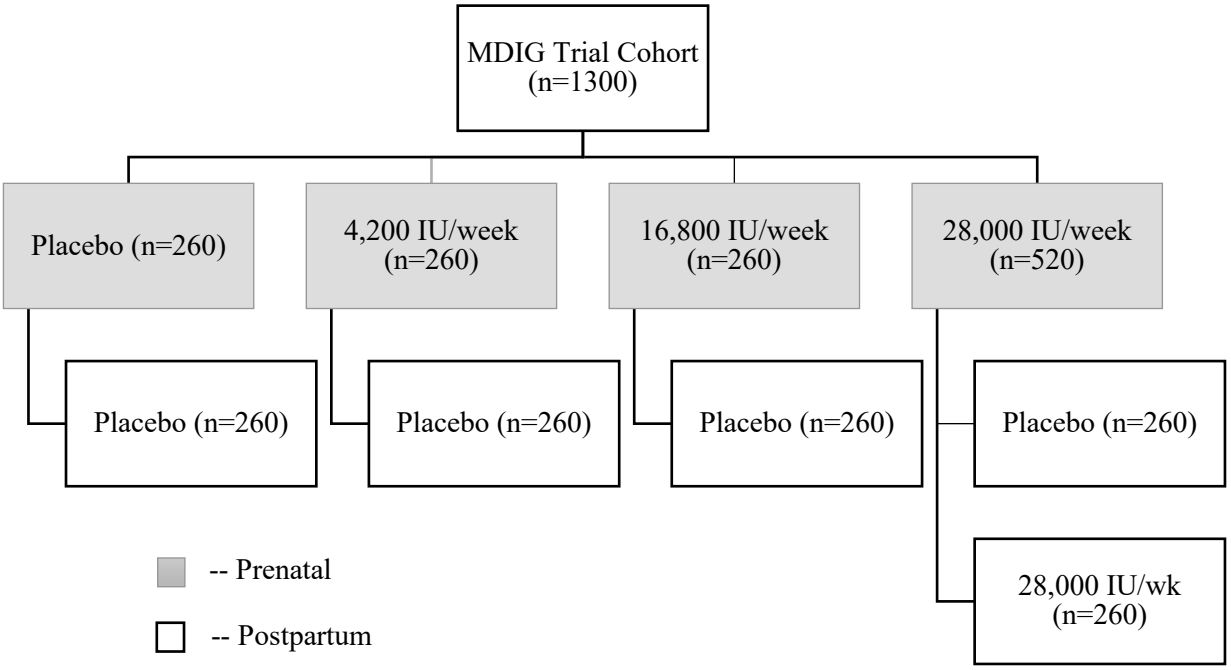

Figure 1: Maternal Vitamin D for Infant Growth (MDIG) trial treatment group allocation.

The design of the MDIG trial, the vitamin D deficient participants, as well as the observed effects of supplementation on vitamin D status made for a particularly useful setting to test the question of the effect of vitamin D supplementation on blood Cd concentrations. As well, high quality micronutrient data was available for most study participants. Adherence to supplementation was strong along with good follow-up for participants, with 90% of doses taken by over 90% of participants during the prenatal period [162].

4.1.3 MDIG study setting

The MDIG trial was conducted in the capital city of Dhaka, Bangladesh. Participants of the trial resided in the following catchment areas: Kamrangir char, Azimpur, Lalbag, and Hazaribag. A high number of participants in the trial resided in the Kamrangir char, a large urban slum. Participant enrolment and all clinical activities and specimen collection occurred in the Maternal

and Child Health Training Institute (MCHTI), also referred to as the Azimpur Maternity Center. The MCHTI provides low cost health care to pregnant women and their children (MDIG protocol).

4.1.4 MDIG study participants and inclusion/exclusion criteria

Participants in the current study were a sub-cohort of Bangladeshi women enrolled in the MDIG trial (ClinicalTrials.gov identifier NCT01924013). Inclusion criteria for the MDIG trial were as follows: being aged 18 years or older, having the intention to reside in the trial area for at least 18 months, and having 17-24 weeks of completed gestation. Exclusion criteria were as follows: history of any medical condition or medications that may predispose to vitamin D sensitivity, altered vitamin D metabolism, and/or hypercalcemia; high-risk pregnancy (severe anemia, moderate-severe proteinuria, hypertension, multiple gestation, major congenital anomaly, severe oligohydramnios); unwillingness to stop taking non-study vitamin D or Ca supplements or a multivitamin containing Ca and/or vitamin D; or, currently prescribed vitamin D supplements as part of a physician's treatment plan for vitamin D deficiency. In total, n=1300 women were enrolled in the primary trial, with approximately 260 women in each trial arm [161]. From the primary trial population of 1300 women, the sample for this sub-study consisted of all women for whom a blood Cd concentration was measured (n=619) in a specimen collected at or around the time of delivery (n=798). For some analyses, the sample size was lower based on the availability of other biomarker data (such as decreased cord blood availability). This sub-sample was expected to be representative of the general MDIG trial population.

As not all samples sent to the CDC for blood Cd analysis yielded measured results (due to clotting), the sample size for the primary analysis for Aims 1 and 2 decreased from 798, to 619 (Table 1). Comparison of baseline characteristics of participants with clotted samples versus

those with un-clotted samples showed no significant differences between the two groups (Appendix Table 6). No additional exclusion criteria were applied.

Table 1: Participants eligible for the Cd study among all participants enrolled in the MDIG trial.

MDIG trial	n=1300	
Aim 1: Cd study	**Maternal blood**	**Cord blood**
Samples sent for CDC Cd testing	n=798	n=552
Samples with CDC results (no clotting)	n=619	n=516
Maternal-cord Cd comparison	n=375	
Aim 2: Cd study	**Participants with serum creatinine results**	**Participants with urine albumin results**
Samples with obtained results	n=311	n=444
Serum creatinine-albumin with obtained results	n=266	

4.2 Data sources

4.2.1 Study Forms

Study participants were interviewed by trained personnel at varying time points during the trial. For the purposes of this study, we used questionnaire data from baseline and delivery timepoints. Questionnaires included items about participant's personal and household characteristics, behaviours, and clinical symptoms at delivery.

4.2.2 Food Frequency Questionnaire

A targeted 41-item food frequency questionnaire (FFQ) was administered to study participants at enrolment and 6 months postpartum by study personnel. Participants were asked about frequency of intake of food items, ranging from never, to multiple times per day. Responses to the FFQ were collapsed into the never category if participants stated never consuming the food item in the past month. Responses to the FFQ were collapsed into the ever category if participants stated any of the following responses: 1 time per month, 2-3 times per month, 1 time per week, 2-4 times per week, 5-6 times per week, 1 time per day, or more than 1 time per day. Food item consumption was dichotomized into the never/ever categories due to low response rates within many of the frequency categories of the FFQ. The FFQ had not been previously validated but was designed to capture Ca, phosphorus, and phytate intake in the past month before the administration of the FFQ. A relative Ca intake was derived for each study participant using food frequency questionnaire data for an additional MDIG sub-study. As portion size was not included in the FFQ, an estimate was chosen based on typical portion sizes of food items and was guided by additional resources (Dietary Guidelines for Bangladesh, the Dietary Guidelines for Indians, as well as the Canadian Nutrient File). The portion size and Ca content per portion size of food item were assumed to be constant across all participants. To develop the relative Ca intake

measure for each woman, a nominal portion size was assigned to each food item in the FFQ, which provided a weight to each item. For food items that encompassed multiple food items (eg. the other vegetables category encompasses 7 types of vegetables), the average Ca content per portion for all foods within the food item was computed, and the value was multiplied by the reported frequency of intake. Subsequently, each item was converted into Ca consumed per day. All items were then summed together for each study participant to generate a final estimate of relative Ca intake. Ca intake was expressed as a daily intake (mg).

4.2.3 Biological specimens

4.2.3.1 Maternal venous blood collection at delivery

Two mL of venous blood was collected by a trained phlebotomist using a serum tube in addition to 6 mL being collected into an EDTA tube and centrifuged at a low speed for 15 minutes. Plasma or serum were transferred into 0.25 mL aliquots. Aliquots were stored at ≤ -70 °C for future analysis. Specimens were considered to be collected at 'delivery' if collection occurred within 24 hours of delivery. Blood specimens were used for analysis of Cd, Pb, Se, ferritin (marker of body Fe stores), creatinine, and 25(OH)D, while plasma was used for analysis of CRP.

4.2.3.2 Maternal urine collection at delivery

Study participants urinated in sterile dry collection containers, and the specimens were stored in 1.5 mL aliquots. One aliquot was stored at 2-8°C for same or next-day analysis of urine creatinine. All additional aliquots for future analyses were stored in a -80°C freezer and transferred to the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) or SickKids Hospital for long term storage, also at -80°C. Urine samples were used for the analysis of creatinine, cotinine, albumin, and B2MG.

4.2.3.3 Cord blood collection

The attending physician or birthing attendant were tasked with deciding the technique and timing of umbilical cord clamping and cutting, as this was not part of the study protocol. Dry cotton gauze was used to clean the umbilical cord at a site attached to the placenta within 10 minutes of delivery, after which the umbilical vein was cannulated, and blood was collected (MDIG protocol). Cord blood was used for the analysis of Cd and Se.

4.2.3.4 Specimen processing

Immediately following blood collection, steps to process blood specimen were taken. Within 30 minutes of blood being drawn, maternal and cord blood samples were centrifuged and placed in a portable freezer at <-60°C (Shuttle ULT-25N; Stirling Ultracold, Athens, OH, USA), and transported to SickKids Hospital for long-term storage. Maternal and cord blood samples in the serum tubes were kept at room temperature for a time period between 30 minutes and an hour before being centrifuged. Samples were then placed in the portable freezer for transport and storage. Whole blood was removed from the vacutainer right after blood was drawn and before centrifugation.

4.2.4 Laboratory techniques

The metals panel analysis of blood (maternal and cord) specimen for Cd, Pb, and Se occurred at the Centres for Disease Control in Atlanta, Georgia. The metals panel also included Hg and Mn. Blood was analyzed for Cd and serum ferritin at the Nutritional Biochemistry Laboratory (NBL) at the icddr,b in in Dhaka, Bangladesh. Urine creatinine was measured at the Clinical Biochemistry Laboratory (CBL) at the icddr,b. Urine cotinine and serum 25(OH)D were quantified at the Analytical Facility for Bioactive Molecules (AFBM) laboratory at the Hospital

for Sick Children (SickKids) in Toronto, Canada. Serum creatinine, urine B2MG and urine albumin were analyzed at the at The Hospital for Sick Children Research Institute (SickKids).

4.2.4.1 Measurement of maternal whole blood and cord blood Cd at delivery

Measurements of blood Cd levels were used, as blood provides the most accurate assessment of recent exposure (i.e. in the last year) from all environmental sources, such as air, food, and water [163]. Blood Cd is a good indicator of Cd body burden in individuals with consistent, low-grade exposure, due to the half-life of Cd in blood having a fast component (3-4 months), and slow component (10 years) [24, 164]. Cd concentrations in blood samples were assumed to be reflective of consistent Cd exposure in the study population, due to regular exposure via food intake and second-hand tobacco exposure, as well as Cd having a half-life of approximately 10-30 years in human organs [26].

Sample analysis for Cd in maternal whole blood samples at time of delivery (n=798) and venous cord blood samples (n=552) was conducted at the U.S. Centers for Disease Control (Atlanta, Georgia) using inductively coupled dynamic reaction cell plasma mass spectrometry (ICP/MS, DLS3016.8) in the Division of Laboratory Sciences, National Center for Environmental Health, CDC. National Institute of Standards and Technology (NIST) standards (SRM 955c Toxic elements in Caprine Blood) were used during analysis. The CDC laboratory also tested NIST SRM 955c quarterly as an additional form of quality control. During the time frame that blood Cd was being measured in our study population (January-June 2018), the laboratory completed three quarterly tests of SRM 955c levels 2-4. For each test, results obtained were within the method's experimental precision (all differences from NIST target value were less than 8%). NIST SRM 955c level 2 (target value= 2.14 μg/L) was closest to Cd concentrations found in our study population and were within 0.1μg/L of the NIST target value. During the study time frame,

the CDC laboratory also took part in two rounds of proficiency testing for blood Cd and received 100% scores for each test. During proficiency testing, samples tested had assigned blood Cd target values from undetectable to 62.0 μg/L. For those that were most similar to our blood Cd concentrations (<5 μg/L), all had results within 0.1 μg/L of the target value. The CDC utilizes the laboratory standardization Lead and Multielement Proficiency Program (LAMP), in order to improve the precision and accuracy of blood Pb, Cd, and Hg measurements. Over 100 laboratories participate in the program, by analyzing CDC-provided blood samples and sharing their obtained results with the CDC. The CDC then provides feedback to the labs outlining how well their analyses were performed. LAMP results are not meant to provide accreditation or certification.

The CDC laboratory found clotting in a high portion of our blood samples (22% of the total). In accordance with CDC quality control criteria, they were excluded from analysis due to inhomogeneity issues from inconsistent distribution of cellular material in the blood sample [163]. Therefore, the sample size decreased to n=619. One sample for which the Cd result was below the limit of detection (0.1 μg/L) was imputed as 0.1 μg/L and included in analyses. Timing of analyses at the CDC for whole blood and cord blood was from January-June 2018.

4.2.4.1.1 Discrepancies between Cd datasets from different laboratories

The Cd dataset described above, referred to as the CDC dataset, was meant to be used in a related MDIG sub-study on toxic metals, but was adopted for use in the present study when it became available. The originally proposed Cd dataset for our study (referred to as the NBL dataset) was part of a MDIG trial biochemical sub-study dataset (n=550) for which maternal delivery whole blood Cd analysis was conducted at the Nutritional Biochemistry Laboratory (NBL) at the International Centre for Diarrheal Disease Research, Bangladesh (iccdr,b) using

graphite furnace atomic absorption spectrometry (GFAAS) in June 2014-February 2016. The NBL dataset was considered as a secondary/alternative source of Cd data from an overlapping group of MDIG participants (matched results with the CDC dataset were n=299). During analysis, the NBL used a four-point calibration curve in each lot from the commercial Cd standard solution (Cica-Reagent, Kanto Chemical Co. INC, Japan). The concentrations used were 0.2, 0.4, 0.8, and 1.2 μg/L. Diluted blood samples for testing were then aspirated into the GFAAS, and results were obtained by calculating from the lot specific standard curve. In-house prepared pooled blood samples were used as a form of internal quality control, with values being assigned against standards (NIST SRM 955c) in order to check for accuracy and precision in each lot. Accuracy of Cd results were confirmed by cross-checking with left over blood samples from the LAMP program. A comparison of the CDC and NBL datasets (Figure 2) with matched participant ID's led to the finding that very minimal correlation existed between the two (n=299), with a correlation coefficient of r=0.16 (p=0.01). The geometric mean (95% CI) of the NBL dataset (n=550) was 1.91μg/L (1.86 μg/L, 1.96 μg/L), (minimum: 0.34 μg/L; maximum: 3.91 μg/L), while the CDC dataset was significantly lower, at 0.66 μg/L (0.64 μg/L, 0.69 μg/L) (minimum: 0.1 μg/L; maximum: 2.6 μg/L). The geometric mean (95% CI) of NBL results (n=299) that had obtained CDC results was 1.87 μg/L (1.80 μg/L, 1.95 μg/L). The causes of the major difference between the two datasets are uncertain. It was previously reported that Cd levels were found to be stable in human blood for 5 years when samples were kept frozen at -20°C [165]. In a similar quality control study using bovine blood, Cd remained stable over a period of 2 years, when held at -20 or 4°C [166]. Therefore, it is unlikely that a lag in timing of specimen analysis (i.e., CDC analyses were conducted at a later date than the original analyses) would lead

to such variations in Cd concentrations. Methodological and inter-lab variations may have occurred due to Cd being tested using two distinct methods; however, agreement between the two methods should have been greater than observed, with one study finding a correlation of r=0.96 between blood Cd measured using ICP-MS and blood Cd measured using GFAAS. In the study, ICP-MS was found to give slightly lower results than GFAAS, and the agreement between the two results were found to be better when blood Cd concentrations were above 2 µg/L [167].

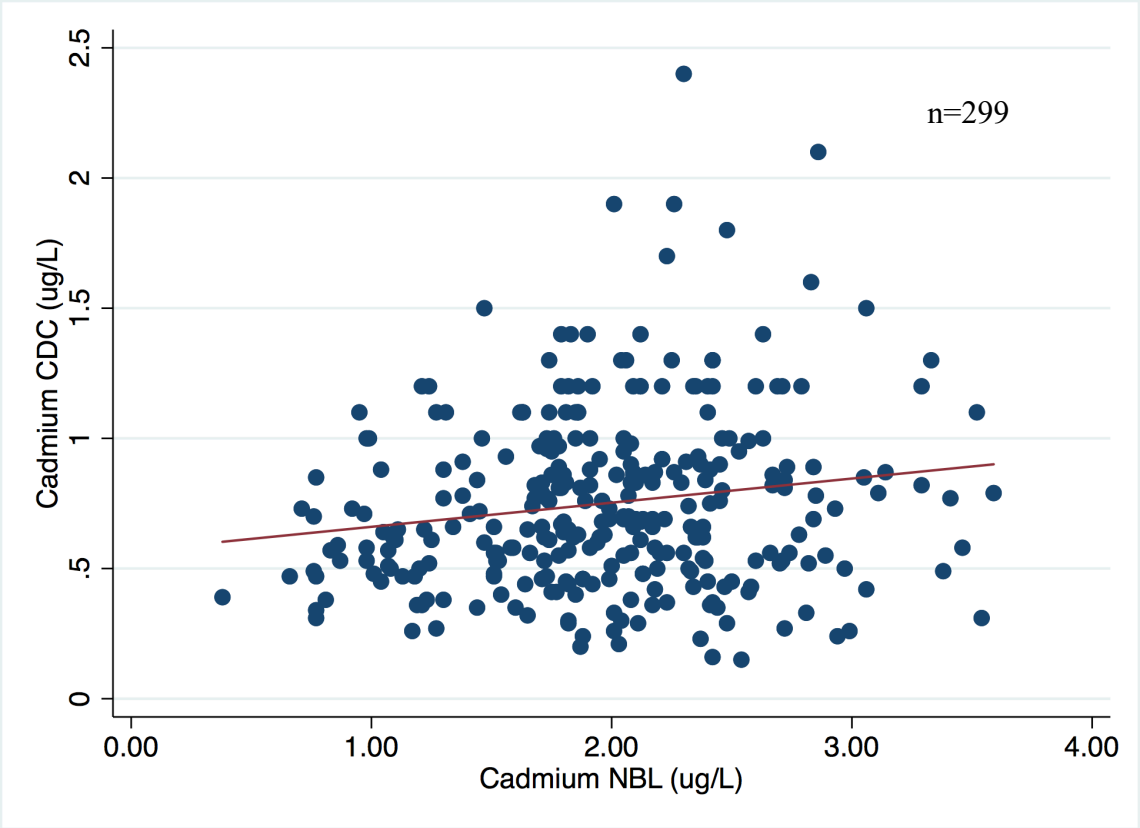

Figure 2: Scatterplot of NBL Cd results and CDC Cd results (n=299). The red line signifies a line of best fit. Variation between the two datasets appears to increase with increasing measured Cd concentrations in the datasets.

The low correlation between the two datasets may have been due to one laboratory having stricter quality control over the other, but that is difficult to ascertain. As a form of quality

control, we sent 50 randomly selected maternal blood samples to be re-tested for Cd concentrations at the NBL in Dhaka in February 2019. By having samples re-tested, we hoped to have a better understanding of the discrepancy between CDC and NBL results. These selected samples had previous Cd results from both CDC and the original NBL analyses. The geometric mean (95% CI) of the NBL re-tests (n=50) was 0.22 µg/L (0.15 µg/L, 0.32 µg/L), compared to the geometric mean (95% CI) of the original NBL results (n=50) of 1.87 µg/L (1.70 µg/L, 2.06 µg/L), and a geometric mean (95% CI) for CDC results (n=50) of 0.64 µg/L (0.55 µg/L, 0.73 µg/L). The correlation coefficient between NBL re-tests and CDC results was r=0.93, while the correlation between NBL re-tests and original NBL results was r=0.15. The mean inter-assay coefficient of variation (CV) of CDC results and NBL re-tests is 43.8%, while the mean inter-assay CV for NBL re-tests and NBL results was 90.7%. Bland-Altman plots were created to compare CDC results and re-tests, as well as NBL results and NBL re-tests (Figure 3, Figure 4, Table 2).

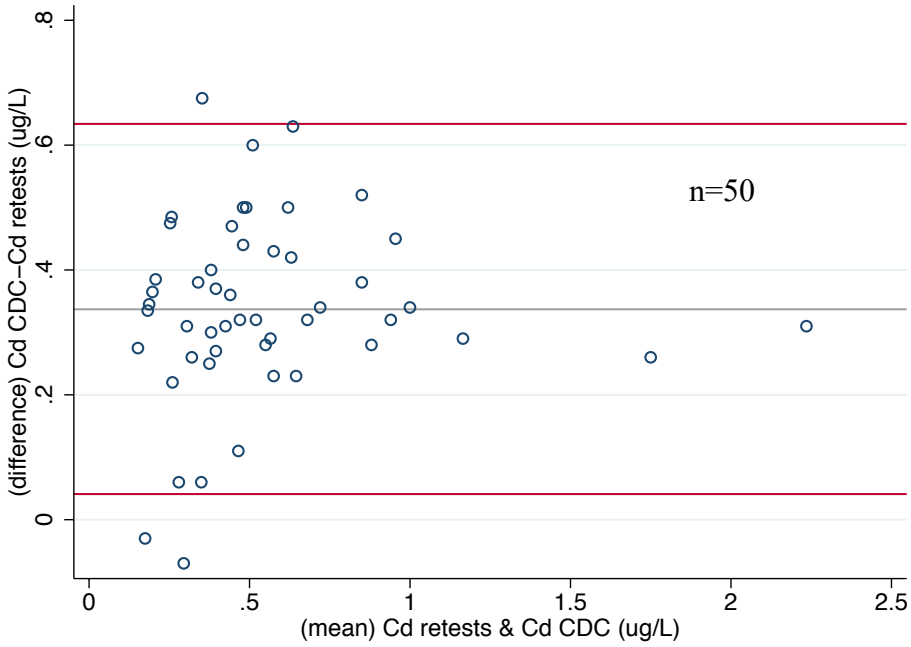

Figure 3: Bland-Altman plot showing the agreement between CDC Cd results and NBL re-test results (n=50). The red horizontal lines represent the limits of agreement (0.04 & 0.63 µg/L), while the grey horizontal line represents the mean (0.34 µg/L).

Table 2: Summary of Bland-Altman plot for NBL results and NBL re-tests and CDC results and NBL re-tests (n=50).

	NBL and NBL retests	CDC and NBL retests
Limits of agreement	0.30, 2.88	0.04, 0.63
Mean difference and CI	1.59 (1.40-1.77)	0.34 (0.30-0.38)
Range (in difference of paired measurements)	0.39-2.19	0.15-2.24
Pitman's test of difference in variance (null hypothesis= variances are equal)	r=0.39, p=0.005	r=0.07, p=0.61

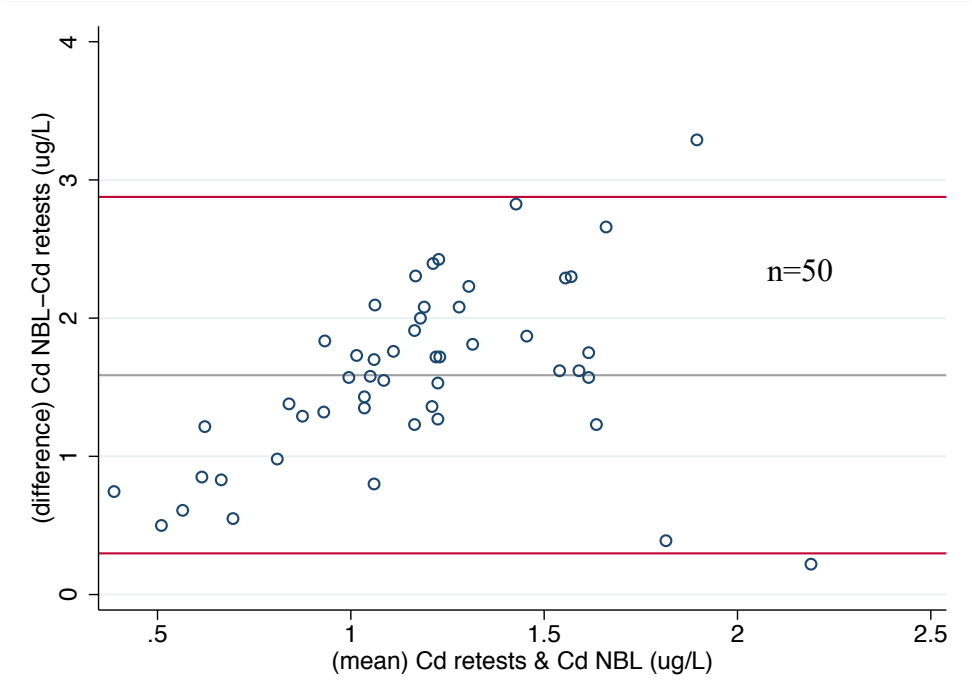

Figure 4: Bland-Altman plot showing the agreement between NBL Cd results and NBL re-test results (n=50). The red horizontal lines represent the limits of agreement (0.30 & 2.88 µg/L), while the grey horizontal line represents the mean (1.59 µg/L).

The limits of agreement between CDC Cd results and NBL re-tests were much narrower than between NBL Cd results and NBL re-tests. From this we can interpret that CDC results and NBL re-tests were more similar than NBL results and NBL re-tests. NBL original results and re-tests also show a clear trend of differences getting larger as the average increases. The re-tested samples were run with serially diluted NIST Cd standards (Table 3).

Table 3: Summary of serially diluted NIST Cd standards as compared to target NIST values.

	Target value		**Aliquot 1**	**Aliquot 2**	**Aliquot 3**	**Mean concentration (µg/L)**
Concentration 1	21.4	NBL Observed	18.26	17.78	15.76	17.3
		% difference	-15%	-17%	-26%	
Concentration 2	52.0	NBL Observed	51.49	52.12	52.87	52.2
		% difference	-0.9%	0.2%	1.7%	
Concentration 3	98.5	NBL Observed	100.42	100.53	106.59	102.6
		% difference	1.9%	2.1%	8.2%	

In addition to 50 blood samples, we also tested 2 pooled blood samples, all run in triplicate. The CV for aliquots of pooled sample 1 was 41.9%, while for pooled sample 2 it was 3.4%.

The strong correlation between the CDC results and NBL re-tests supports the reliability of the CDC results with respect to between-individual differences in our study population. It is important to note that a decline in concentrations initially noted between the original NBL results and CDC results appeared to be continued when comparing CDC results to NBL re-tests. Given the reported stability of Cd in blood (as noted above), it is possible that the decline in concentrations may be due to an unexplained factor, such as the presence of an inhibitor in the blood specimen which could decrease Cd concentrations over time. There is also the potential that this inhibitor did not lead to a uniform Cd concentration decline across all samples. However, the high correlation of CDC results and NBL re-tests suggested that the relative ranking of participants and between-person differences regarding Cd concentrations were robust, even though we cannot be certain absolute concentrations reflect real values. Due to the Center for Disease Control's reputation as a reliable reference laboratory, we also assume adequate internal validity of the CDC dataset. For these reasons, we have chosen to primarily use the CDC

dataset for our data analysis, as we believe it is the most robust dataset for estimating Cd's associations with other variables within the cohort.

4.2.4.1.2 Potential contamination of blood Cd samples

While in the process of testing serum specimens for Zn, the CDC discovered that Zn leaching from the O-rings inside the specimen vials was contaminating the specimen and leading to elevated concentrations. The CDC laboratory tested the contaminated O-rings used in the serum vials for the blood metals panel (Cd, Pb, Mn, Hg, and Se). The O-rings were soaked in ultrapure water for 5 days and afterwards the water was tested for the metals. It did not appear that the O-rings were contaminated with metal concentrations that could have compromised our blood samples. However, an additional test was conducted where vials used for blood storage were tested using 0.5% nitric acid as a leaching agent. It was found that Cd and Pb could be leaching from the vials into the specimens inside, potentially contaminating results. Vials that were inverted were found to have the highest degree of contamination, pointing to the caps of the vials being the main source of contamination.

However, the testing using nitric acid may have not been the ideal procedure for testing for contamination, as human blood has a close to neutral pH and may not have leached Cd out of the vials in the same manner that nitric acid would have. In addition, the water test was done on serum vials while the nitric acid test was done on blood vials, which could have been manufactured in a different batch. For this reason, we decided to re-test blood vials for Cd, and shipped 50 vials to the CDC in order for them to repeat the procedure using water samples with low native concentrations of Cd. The CDC did not find that leaching of metals occurred under these pH neutral conditions. Thus, we are reassured that our blood Cd and Pb concentrations were not likely to be contaminated.

4.2.4.1.3 Measurement of additional metals at delivery

The CDC whole blood metals panel run to obtain blood Cd results also included blood Pb, Se, Mn, and Hg. The procedure for laboratory analyses were the same as for blood Cd, outlined above. No Pb, Hg, Mn or Se results were found to be below the limit of detection. As blood metals were tested in the same panel as Cd, 22% of Pb, Hg, Mn and Se concentrations have no obtainable results due to clotting of blood, with a final n=619 for all.

4.2.4.2 Measurement of urine biomarkers at delivery

4.2.4.2.1 Urine B2MG

B2MG, a biomarker of renal tubular function, is often measured in Cd-induced nephrotoxicity studies, as occupational or environmental Cd exposure leads to increased excretion of this low-molecular weight proteins due to proximal tubule impairment [168]. B2MG is usually present in very low amounts in urine, but can increase substantially in cases of tubular impairment [169]. B2MG excretion may also increase due to malignancies, lymphoproliferative or autoimmune diseases. B2MG analysis using enzyme-linked immunoassay (ELISA) technique has high sensitivity, with one drawback being that high variations in results may be due to partial breakdown of B2MG in samples with pH below 5.5, which can occur in highly acidic urine [170, 171]. Urine creatinine measured at delivery (available from the MDIG trial) was used to express the B2MG ratio (µg/g creatinine). Urine B2MG was measured using a commercial ELISA kit (Abcam, ab181423) at the SickKids Research Institute in Toronto in April 2018-September 2018. The assay utilized an affinity tag labelled capture antibody, and a receptor conjugated detector antibody which immunocapture the sample analyte in solution. Initial testing and optimization of the assay involved running test samples in order to determine the necessity of diluting samples, as well as to confirm the performance of the standard curve. Maternal urine samples from five

random women were tested, at four varying dilutions (undiluted, 1:100, 1:1000, and 1:3000). The first plate assayed had samples diluted 1000-fold, but dilutions were adjusted for further plates due to unexpectedly high concentrations of B2MG. The range of dilutions for all plates was between 150-fold to 1900-fold. The lowest dilution used (150-fold) occurred due to a calculation error while preparing the assay. Levey-Jennings plots were generated to monitor internal controls and standards over time. One standard value was higher than ± 2 standards deviations from the mean of the standard value (plate 6, standard 7), as the standards for this plate were not loaded properly and had to be adjusted by backwards calculating the expected standard concentrations. All additional plates had consistent standards in each run.

We experienced significant difficulty working with the urine B2MG dataset. We decided to test urine for this biomarker due it being measured prevalently in studies on Cd exposure and renal dysfunction. The results we obtained from our assays were difficult to interpret. Some results were exceptionally high, with concentrations an order of magnitude higher than those that are normally seen in urine (eg. 11665 μg B2MG/g creatinine). This originally led us to test urine samples for presence of blood using urine dipsticks. However, we did not find an association between presence of blood and B2MG (Section 5.3.2.2). The urine samples were also tested for pH using urine dipsticks, as low pH could have a negative effect on B2MG concentrations. No samples were found to have a pH<5, at which point the breakdown of B2MG in urine could occur due to acidic conditions. We decided to re-test a sub-set of urine samples (which originally had either high or low B2MG results) for B2MG, in order to verify whether the high results we were observing were repeatable. We found that on average, concentrations declined substantially for participants who originally had urine samples with high B2MG levels. The re-tests had a mean of 254 μg/L, while the original results (that were re-tested) had a mean of 561 μg/L (n=53).

The correlation coefficient of the two datasets was r=0.67. We created a Bland-Altman plot of the original results and re-tests to compare the two datasets (Table 4, Figure 5). Concordance in the upper range of concentrations was particularly low, while at lower B2MG concentrations, the correlation was high.

Table 4: Summary of Bland-Altman plot for β_2-microglobulin results and re-tests (n=53).

	Urine β_2-microglobulin (µg/L)
Limits of agreement	-713 to 1346
Mean difference and CI	317 (175, 459)
Range (in differences of paired measurements)	14, 1620
Pitman's test of difference in variance (null hypothesis= variances are equal)	R=0.89, p<0.00 (n=53)

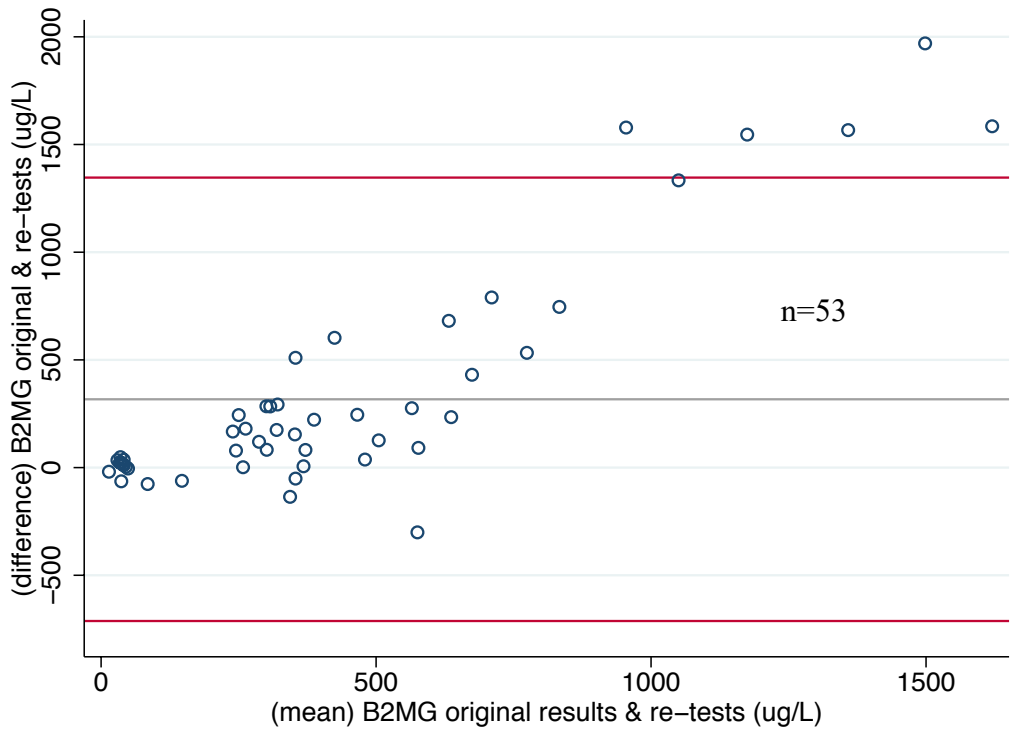

Figure 5: Bland-Altman plot of original and re-tested B2MG results (n=53). The limits of detection are represented by the red horizontal lines (at -712 & 1346 µg/L) and the mean difference (317 µg/L).

This cast doubt on the validity of our B2MG results, particularly samples with high concentrations. This decline in re-test concentration could have been due to urine samples being subject to multiple freeze-thaw cycles (3), even though studies have shown that B2MG is freeze-thaw stable [172]. The noted instability of B2MG in urine due to acidity, in combination with prevalent hematuria in urine (64% of tested urine samples) collected at delivery in our study population may have led to us observing such discordant B2MG results. We decided not to proceed with modelling using B2MG as a biomarker of tubular dysfunction. We were not reassured that the high concentrations we were originally seeing in our dataset were true values, due to significant decreases in concentration upon re-testing the samples. We considered modelling our dataset in quintiles for descriptive purposes, but because of the aforementioned issue, we could not be certain that the highest values we were observing would actually belong in the highest quintile. A high proportion of our dataset was below the limit of detection of the assay (11% of the dataset) or above the highest standard (14%). While we have used imputations in urine albumin and urine cotinine datasets in order to deal with elevated concentrations or values below the LOD (eg. the LOD multiplied by the dilution factor for values below the limit of detection), these previous datasets were not missing such substantial portions of data. Imputing such a high number of results would lead to a truncated dataset with multiple samples having the same imputed concentration. We contemplated modelling the dataset using Tobit regression, in which upper and lower levels of censoring could be used for these ‘clusters’ of data. However, as multiple dilutions were used for different plates, there were no clear values at which to censor the data. Thus, we concluded that we had a flawed B2MG dataset, as well as no appropriate method for analyzing it, and thus could not continue analysis with the data.

4.2.4.2.2 Urine albumin

The albumin/creatinine ratio (ACR) is a sensitive method of detecting proteinuria in pregnant women and is an important general marker of chronic renal dysfunction. It is the preferred method of detecting elevated proteins in pregnant women's urine [173]. Increased urine Cd excretion was shown to be positively associated with urine albumin in individuals with low-grade environmental exposure to Cd. However, this association could be mediated by additional factors, such as urinary flow, and physiological variability, and thus the risk of renal toxicity from Cd exposure could be overestimated [174]. Urine creatinine measured at delivery (available from the MDIG trial, explained in further detail in 4.2.4.2.4) was used for the ratio (mg/g). Urine albumin was measured using a commercial ELISA kit (Abcam, ab108788) at the SickKids Research Institute in April 2018-September 2018. After standards or specimen samples were pipetted into wells, an albumin-specific biotinylated detection antibody was added, washed out with wash buffer, and then followed by Streptavidin-Peroxidase Complex. TMB was added to visualize the Streptavidin-Peroxidase reaction and was then catalyzed by Streptavidin-Peroxidase to form a blue colour product, which changed to a yellow colour. The density of yellow coloration that occurred after a stop solution was added to wells was proportional to the concentration of albumin in each specimen sample. As with B2MG, test samples were initially run for optimization of the assay. Five maternal urine samples were used, all diluted 200-fold. The first assay conducted was diluted 100-fold, and subsequent plates were diluted 200-fold, 250-fold, and 300-fold, due to detection of high concentrations of albumin in some specimens. Samples with unobtainable results due to high concentrations of albumin, or that had high results extrapolated from the standard curve were re-tested, (n=103), with a portion of samples being run in duplicate. For samples that had duplicate results, the mean of both results was used if the

duplicate results had a CV that was within 15%. If the CV was greater than 15%, the result that was closer to the median of the dataset (excluding duplicates) was used. For samples run in duplicate, the mean intra-assay CV was 10.9%. Levey-Jennings plots were also generated for urine albumin and showed consistent standards in each assay run.

4.2.4.2.3 Urine cotinine

Cotinine is the ideal biomarker for measuring tobacco exposure as it is found in various bodily fluids, such as urine, blood, and saliva, in addition to having a longer half-life (16-20 hours) as compared to nicotine. However, urine cotinine is indicative of recent (48 hour) tobacco exposure, so it is the most accurate when recent exposure is representative of regular exposure [175]. Maternal urine at delivery was tested for cotinine using a solid phase sandwich ELISA kit (Origene, EA100901) at the Analytical Facility for Bioactive Molecules (AFBM) at the SickKids Research Institute in January 2019. The wells of the assay kit were coated with an anti-cotinine antibody, to which urine specimen and cotinine enzyme conjugate were added. Cotinine enzyme conjugate subsequently competed with cotinine in urine specimen for binding sites. A wash buffer was used to wash off any unbound cotinine and cotinine enzyme conjugate. The color development which occurred after addition of the substrate was inversely proportional to the amount of cotinine in urine specimen. Quantification of concentrations occurred relating colour intensity to a prepared standard curve. An optimization test was conducted on three samples with varied dilutions (undiluted, 10-fold, 100-fold). It was determined that samples would be run undiluted, as these gave a more consistent reading than diluted samples, and urine cotinine concentrations were not expected to be high in our study population. Obtained results that were below the LOD or with high concentrations (n=29) were re-tested. Dilutions were used when re-testing specimen above the highest standard (20-fold). All re-tested samples were run in

duplicate, with the mean intra-assay CV being 13.2%. Levey-Jennings plots were generated for urine cotinine and showed consistent standards in each assay run. For samples that had duplicate results (n=4), the mean of both results was used if the duplicate results had a coefficient of variation that was within 15%. If the CV was greater than 15%, the result that was closer to the median of the dataset (excluding duplicates) was used. No duplicate results had CVs within 15% of each other, thus all duplicate results were assigned the result that was closer to the median of the dataset, (excluding duplicates). A few (n=6) participants who had unobtainable results due to high concentrations on the original plates were found to have particularly high urine cotinine concentrations on the re-tested plate (>1000 ng/mL). Results were corroborated using baseline maternal and paternal tobacco exposure questionnaire data. All participants, except for one, were found to have tobacco exposure, be it second hand exposure from husbands who smoked cigarettes, or from maternal usage of chewing tobacco. Thus, these high cotinine results were considered to be biologically plausible. One particularly elevated result had discordant intra-run duplicate results, in which case the result that was closest to the standard range was used as the final concentration.

4.2.4.2.4 Urine creatinine

Urine creatinine was measured at the Clinical Biochemistry Laboratory (CBL) at icddr,b in Dhaka using Beckman Coulter AU analyzers in routine clinical lab testing from April 2014-December 2016. The procedure used a kinetic modification of the Jaffe procedure, wherein creatinine in the sample reacted with picric acid at an alkaline pH to form a coloured complex. The absorbance was then measured at 520-800 nm and the rate of change was proportional to the amount of creatinine in the specimen.

4.2.4.2.5 Urine analysis for presence of blood

Preliminary results showed unexpectedly elevated delivery urine B2MG concentrations, which remained even after standardizing for urine creatinine. After discussion with assay manufacturers and biochemists, we were urged to test urine samples for the presence of red blood cells in urine, as B2MG in blood can be an order of magnitude higher than in urine. As urine samples were collected within 24 hours of delivery, there is an elevated risk that blood could be present in our urine samples. This information prompted us to test urine for blood using urine dipsticks (Siemens Multistix 8 SG Labstix Uristix Reagent Strips®). Urine samples were thawed, and 0.5 mL of urine was pipetted onto the dipstick square designated for blood testing. Following the instructions of the tests strips, after an elapsed time of 60 seconds, we compared the colour of the test square to those on the colour chart (yellow=negative; yellow with minimal green flecks= non-hemolyzed trace; yellow with green flecks= non-hemolyzed moderate; light yellow-green= hemolyzed trace; light green= small; medium green= moderate; dark green= large) and results were recorded. Results were interpreted in the following way: non-hemolyzed trace and hemolyzed trace results were grouped into the same category (trace), and non-hemolyzed moderate and hemolyzed moderate were grouped together (moderate). All other results were kept as their own separate level of blood in urine. Results of each urine biomarker were compared to level of blood group using pairwise linear regression models. Results of the urine dipstick analysis are discussed in detail in section 5.3.2.

4.2.4.3 Measurement of serum creatinine at delivery

Serum creatinine is a widely used marker of renal function, as it measures the waste product that forms when muscle creatine is broken down. Serum creatinine is often used for the calculation of glomerular filtration rate (eGFR). However, it should be used in combination with other

measurements of renal functioning for increased accuracy [176]. Consideration was given to deriving an eGFR based on serum creatinine, if a formula validated for pregnancy was identified. Serum creatinine was measured using a colorimetric assay (Cayman, 700460) at the SickKids Research Institute. The assay was based on a Jaffe reaction, in which the metabolite was treated with an alkaline picrate and colour development was observed. The colour intensity was directly proportional to the amount of creatinine in the specimen and was quantified at an absorbance between 490-500nm. No dilutions were used when testing specimen, as the assay instructions did not specify the necessity of dilutions. All samples were run in duplicate. The average inter-assay CV of 16 plates was 1.7%. Levey-Jennings plots were generated for serum creatinine and showed that two standards had values that were higher than ± 2 standard deviations from the mean standard value (plate 4a, standard 3; plate 1a, standard 8). All other standards showed consistency in each assay run.

4.2.4.4 Measurement of serum ferritin at delivery

Serum ferritin is used as an indicator of Fe stores in the body [177]. Ferritin was measured in serum in a sub-sample of women (n=610) at the NBL in Dhaka using an electrochemiluminescence immunoassay (ECLIA) with a Roche automated immunoassay analyzer, Bobas e601 (03737551, Roche Diagnostics, IN, USA) from June 2014-February 2016. The ferritin assay was standardized against NIST standards 80/602. The assay used was standardized against the WHO International Standard NIBSC (National Institute for Biological Standards and Control, code 03/178). Two lyophilized quality control serums (PreciControl Varia, Level 1& 2, Roche Diagnostics) were used as internal quality controls and were run each day to confirm both accuracy and precision of the ferritin assays. In order to ensure quality of results, the laboratory participated in the Vitamin A Laboratory External Quality Assurance

(VITAL-EQA) program, as well as the College of American Pathologists. We accounted for elevated serum ferritin concentrations caused by inflammation by adjusting models with serum ferritin for C-reactive protein (CRP), a non-specific marker of inflammation [178].

4.2.4.4.1 C-reactive protein

C-reactive protein (CRP) was measured in maternal plasma at delivery using Quantikine® ELISA kits (DCRP00, R&D Systems, MN, USA) at the SickKids Research Institute in September 2018.

4.2.4.5 Measurement of serum 25(OH)D at baseline and delivery

Serum 25(OH)D concentrations were measured using liquid chromatography-tandem mass spectrometry at the Analytical Facility for Bioactive Molecules (AFBM), a core laboratory facility at the SickKids Research Institute from March 2014-September 2016. AFBM participated in the Vitamin D Quality Assessment Scheme (DEQAS) certification. The laboratory method used allowed for specific quantification of 25(OH)D_3, 25(OH)D_2, and 25(OH)D_3 C-3 epimer concentrations. For our analyses, only results of 25(OH)D_3 were used. For quality control purposes, NIST SRM 972a standards and DEQAS standards (451, 452, 453, 454, 455) were run with each lot of tested specimen. DEQAS standards were monitored on a regular basis to ensure accuracy (calculated concentrations were compared to expected concentrations). As well, a pooled plasma sample was run in duplicate with each lot of assayed specimen. The mean inter-assay CV was 10%, and the mean intra-assay CV was 5%.

4.3 Data analysis

4.3.1 Aim 1: The effect of vitamin D supplementation on maternal blood Cd concentrations

4.3.1.1 Study variables

Table 5: Composition and derivation of variables used as part of Aim 1 analyses.

Variable	Unit	Type of Variable	Classification	Derivation of Variable
Maternal 25(OH)D at delivery	nmol/L	Continuous	-	-
Maternal 25(OH)D at baseline	nmol/L	Continuous	-	-
Maternal blood Cd and Se at delivery	μg/L	Continuous	-	-
Venous cord blood Cd	-	Categorical	Detectable/undetectable	-
Venous cord blood Se	μg/L	Continuous	-	-
Maternal serum ferritin at delivery	μg/L	Continuous	-	-
Maternal plasma C-reactive protein at delivery	mg/L	Continuous	-	-
Maternal Ca intake at baseline	mg/day	Continuous	-	Relative Ca intake derived from FFQ data
Vitamin D treatment group	IU/week	Categorical	0 IU/week; 4200 IU/week; 16800 IU/week; 28000 IU/week	-

4.3.1.2 Descriptive analysis

Continuous variables were visualized as histograms and box plots, and categorical variables were tabulated. Outliers were interrogated through manual review of hardcopy study forms or lab reports. The extent of missing data was assessed. As substantial attrition occurred due to clotting of blood samples, baseline characteristics of participants whose blood samples clotted, versus those that had un-clotted specimen were compared to assess the extent of any selection bias in

the analytical sample. No significant differences were found between the two groups, on all baseline characteristics (Appendix Table 4).

4.3.1.3 General analytical approach

Maternal blood Cd concentration (μg/L) was a continuous variable with a non-normal distribution (due to a right skew of the data). It was expressed as a median with interquartile range, stratified by treatment arm. In order to detect a possible dose-response relationship, median and interquartile range were visualized using dot plots as well as box plots. Maternal blood Cd concentration at delivery was compared across treatment/placebo arms using pairwise comparisons in a linear regression model. We intended to use the Holm test to adjust for multiple comparisons if results were found to be statistically significant. Because we were interested in the effect of prenatal vitamin D supplementation at delivery, the prenatal and postnatal 28000 IU/week supplementation groups were combined. Relative daily Ca intake was a continuous variable and was included as a main effect, as well as in interaction terms with vitamin D supplementation group. Whole blood Se and serum ferritin were continuous variables, and were included as main effects, as well as in separate interaction terms with vitamin D supplementation group. Interaction terms with categorical variables were tested for significance using the Wald test. As mentioned, serum ferritin models were adjusted for plasma CRP to account for inflammation that could lead to elevated ferritin concentrations. As a form of secondary analysis, we assessed the effect of vitamin D supplementation on venous cord blood Cd concentrations. As a substantial amount of cord blood concentrations were found to be below the limit of detection (76% of samples), cord blood Cd was analyzed as a categorical variable (detectable/undetectable) in a log-binomial model.

4.3.2 Power calculation

For aim 1, a power calculation was determined after post-hoc analysis of preliminary results using a two-sample means test. It was based on an estimated minimum sample size of 100 in three of the supplementation groups (4200 IU/week, 16800 IU/week, and 28000 IU/week) and the placebo group, a two-tailed type I error rate of 0.05. Cd results (n=619) give a mean of 0.74 μg/L, and a standard deviation of 0.35 μg/L. We had 80% power to detect a difference in means of 0.14 μg/L.

4.3.3 Sensitivity and additional analyses

4.3.3.1 25(OH)D

We assessed the relationship of vitamin D and Cd by plotting serum 25(OH)D concentrations at delivery and maternal blood Cd delivery concentrations with a fitted a locally weighted scatterplot smoothing (LOWESS) line in order to visualize a relationship between the two parameters. We also visualized baseline 25(OH)D concentrations and maternal blood Cd concentrations at delivery using the same methodology.

4.3.3.2 Serum ferritin

We assessed the relationship of vitamin D and Cd with serum ferritin as a covariate, excluding outliers (>1000 ng/mL) and adjusting for plasma CRP. As well, we included serum ferritin as a covariate in the model without adjusting for plasma CRP in separate models with outliers excluded and included.

4.3.3.3 Month of enrolment and month of delivery

Due to uneven distribution of participants by month of enrolment (fewer women were enrolled in the months of December-February), as a sensitivity analysis, we adjusted for month of enrolment in Aim 1 models to assess whether it impacted model inferences. By chance, no infants were

born to mothers in the sub-study in the months of March-May. Month of delivery was also adjusted for in Aim 1 models to assess whether it impacted model inferences.

4.3.4 Aim 2: The association of maternal blood Cd concentrations and renal biomarkers

4.3.4.1 Study variables

Table 6: Composition and derivation of variables used as part of Aim 2 analyses.

Variable	Unit	Type of Variable	Classification	Derivation of Variable
Maternal age	years	Continuous	-	-
Maternal 6 months post-partum BMI	kg/m^2	Continuous	-	Derived from maternal weight (kg) at 6 months postpartum, and maternal height (cm) at baseline
Maternal education	Level of education attained	Categorical	No schooling; primary incomplete; primary complete; secondary incomplete; secondary complete or higher	Based on school curriculum in Bangladesh
Household asset index	-	Categorical	Quintiles	The asset index was derived from principal component analysis of variables that accounted for household wealth. The ownership of various items (private toilet, electricity, radio, TV, mobile phone, non-mobile phone, fridge, almirah/wardrobe, table, chair, electric fan, DVD/CD player, auto bike, rickshaw/van, bicycle, motorcycle/motor scooter/tempo/CNG, livestock/herds/farm animals/poultry, homestead, and land) was used to generate a wealth score, with higher scores

				indicating higher relative wealth.
Gravidity	-	Continuous	-	Self-reported number of pregnancies, including current pregnancy
Gestational age at birth	Weeks	Continuous	<37 weeks is considered a pre-term birth; ≥ 37 weeks is a term birth	Last recalled menstrual period, as well as ultrasound performed at the MHCTI
Maternal blood metals/ minerals (Cd, Pb, Se) at delivery	µg/L (µg/dL for Pb)	Continuous	Detectable/undetectable for cord blood Cd	-
Maternal serum creatinine at delivery	µmol/L	Continuous	-	-
Maternal urine cotinine at delivery	ng/mL	Continuous	-	-
Maternal urine ACR at delivery	µg/mg	Continuous	-	-
Maternal urine B2MG/creatinine at delivery	µg/g	Continuous	-	-
Maternal serum ferritin at delivery	µg/L	Continuous	-	-
Maternal plasma C-reactive protein at delivery	mg/L	Continuous	-	-
Maternal coconut meat intake at baseline	Ever, never	Categorical	Never consumed, ever consumed	-
Maternal poultry meat intake at baseline	Ever, never	Categorical	Never consumed, ever consumed	-
Maternal beef, mutton and pork intake at baseline	Ever, never	Categorical	Never consumed, ever consumed	-
Maternal large fish intake at baseline	Ever, never	Categorical	Never consumed, ever consumed	-
Maternal organ meat intake at baseline	Ever, never	Categorical	Never consumed, ever consumed	-
Vitamin D treatment group	IU/week	Categorical	0 IU/week; 4200 IU/week; 16,800 IU/week; 28,000 IU/week	-

4.3.4.2 Covariates

Directed acyclic graphs (DAG) were generated to identify covariates to include in multivariable models. DAGs allow for the conceptualization of relationships between variables in a visual

form. Chosen variables were based off of known relationships described in literature as well as available information from the MDIG dataset. Variables that were identified as confounders for inclusion in adjusted models were as follows: maternal age, maternal BMI (6 months postpartum), maternal education, household asset index, gravidity, gestational age at birth, poultry meat consumption, coconut meat consumption, beef/mutton/pork consumption, organ meat consumption, large fish consumption, blood Pb and Se concentrations at delivery, vitamin D supplementation group, and urine cotinine at delivery (Figure 6). The food items included in the model were found to be significantly associated with Cd concentrations in initial analyses ($p<0.1$), not theoretical considerations.

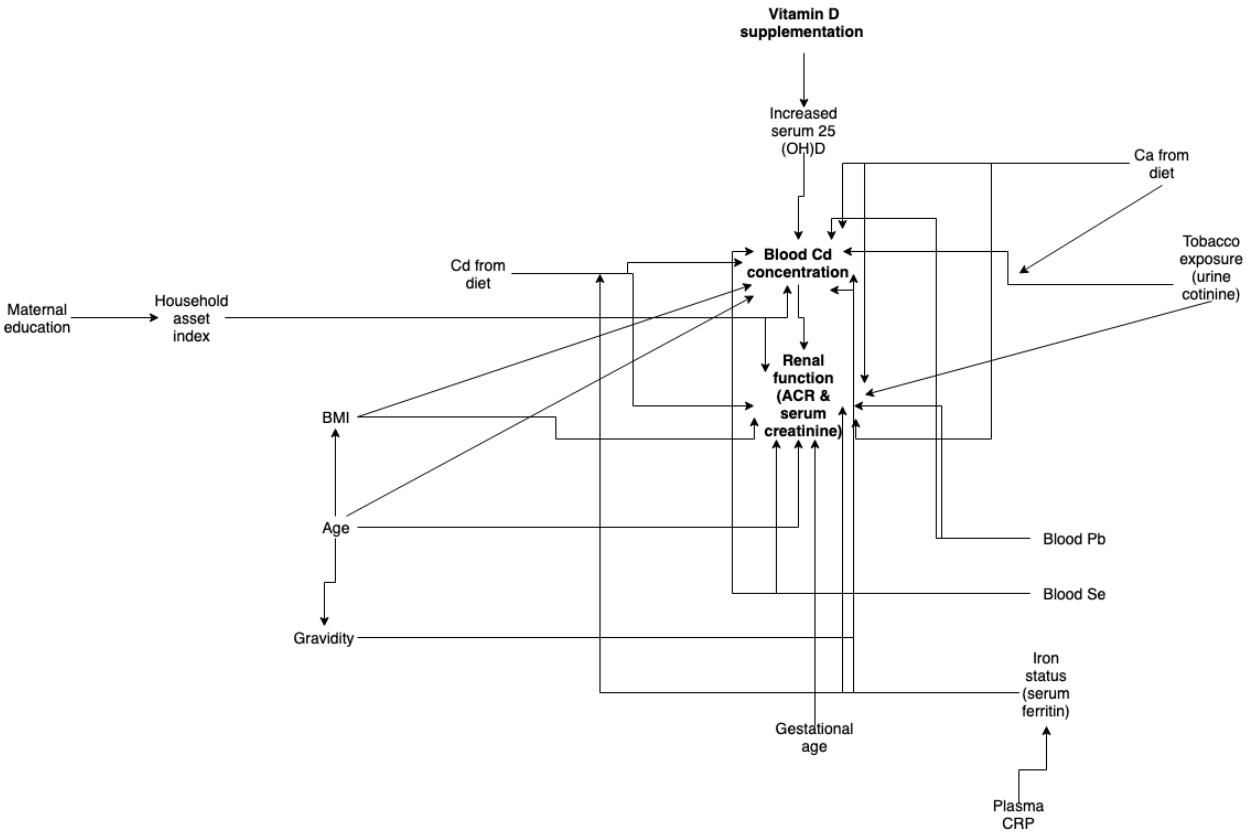

Figure 6: Conceptual model of the vitamin D, blood Cd, and renal function relationship.

4.3.4.3 Descriptive analysis

As with Aim 1, continuous variables were visualized as histograms and box plots, and categorical variables were tabulated. Summary statistics were calculated for all continuous variables, and included: means, and standard deviations for normally distributed variables, and medians and interquartile ranges for non-normally distributed variables.

The following covariates were expressed as continuous variables: maternal BMI, maternal age, gestational age at birth, gravidity, blood Pb and Se concentrations, and urine cotinine. Asset index, maternal education, food item intake, and vitamin D supplementation group were assessed as categorical variables. For any analyzed biochemical data that was below the LOD, the result was imputed as the LOD (multiplied by the dilution factor, if appropriate).

4.3.4.4 Analytical approach

In multivariable linear regression modeling to address Aim 2, the primary exposure variable was maternal blood Cd concentrations at delivery (μg/L), and the outcome variable was one of the following markers of renal function: serum creatinine concentration (μmol/L), and urine ACR (mg/g). Renal biomarkers were log-transformed in analyses, if distributions were found to be non-normal. All markers of renal function were expressed as continuous variables. Separate models were run for each Cd-renal function outcome variable relationship. Univariate models were run as a form of preliminary analysis for each Cd-renal marker variable, as well as each exposure/covariate in the model.

Multivariate linear regression models were used to assess the relationship between Cd concentrations at delivery and measures of renal function. All renal markers were assessed independently, as each marker measures a slightly different physiological process, with serum creatinine being an indicator of longer term renal dysfunction, while urine albumin is a sensitive

marker of proteinuria or damage to the kidneys [173, 176]. Directed acyclic graphs (DAGs) were used to guide the inclusion of specific hypothesized confounders in multivariable models. We controlled for confounding by dietary and environmental (tobacco) sources of Cd, by including these variables as confounders in our models. These variables have been shown to be well-established contributors to both Cd burden and renal outcomes. Linear regression models were used to estimate the association between tobacco exposure and food items (the main exposure variables of interest) with maternal blood Cd concentrations (μg/L) at delivery. This ensured the establishment of an appropriate model of the urine cotinine-renal function relationship in the context of primary multivariable models. Urine cotinine, a continuous variable, was analyzed as the primary biomarker of overall tobacco exposure at delivery. We also secondarily considered self-reported personal tobacco and related product usage (e.g., chewing tobacco) by the pregnant women; however, overall usage in this population was low and not expected to vary greatly within the sample.

To address associations between usual intake of particular foods or food groups and Cd burden, we used a 41-question food frequency questionnaire (FFQ) administered to study participants at enrolment [161]. Consumption of each food or food group were dichotomized into never/ever consumed categories and were compared to blood Cd concentrations in separate univariate linear regression models. Food items that are found to be significantly associated with blood Cd concentrations ($p<0.1$) were included in multivariable models of blood Cd and renal biomarkers. Particular nutrients and metals/minerals may be effect modifiers in the relationship of blood Cd levels and renal outcomes. Literature pointed to Se being potentially mitigatory in the relationship of Cd exposure and renal function, including the risk of preeclampsia [110]. Other nutrients, such as Fe, and vitamin D, are not as well established in literature as modifiers of the

Cd exposure and adverse renal outcome relationship. As a form of exploratory analysis, they were considered *a priori* using interaction variables in our model. Interactions with categorical variables were tested for significance using the Wald test. Serum ferritin (adjusted for inflammation using CRP) was measured in a sub-sample of our study population (n=610), while whole blood Se data was available for all of our study participants. As previously mentioned, the data set has missing Cd results, due to clotting of blood samples. All analysis was completed using Stata version 15 (College Station, Texas).

4.3.5 Sensitivity and additional analyses

4.3.5.1 Maternal blood Cd and eGFR

As a form of sensitivity analysis, we assessed the association of maternal blood Cd concentrations and eGFR from the CKD-EPI equation (calculated using maternal serum creatinine concentrations at delivery).

4.3.5.2 Including month of enrolment and month of delivery as covariates in Aim 2 models

All Aim 2 models were adjusted for month of enrolment and month of delivery to confirm whether these variables were confounding the association of maternal blood Cd and renal biomarkers. These variables were not initially considered to be confounders of the association but empirical evidence (uneven distribution of participants into variable categories) led us to consider these variables as potential confounders.

4.3.6 Power calculation

As one of the supplementation groups has an (approximately) doubled sample size, an additional power calculation was obtained to increase specificity. It was based on an estimated sample size of 200, and a two-tailed type I error rate of 0.05. Cd results (n=619) had a mean of 0.74 μg/L,

and a standard deviation of 0.35 μg/L. We had 80% power to detect a difference in means of 0.12 μg/L between treatment group and placebo.

The maximum sample size for all aims was fixed, based on the number of maternal delivery whole blood samples for which a Cd concentration value can be generated at the CDC. Inferences were drawn primarily from multivariable models involving Cd as the primary exposure variable and a continuous health measure as the outcome (Aim 2); we adjusted for numerous other covariates that represented potential confounders. Estimation of the power or minimum detectable effect size in the context of multivariable regression modeling is complex and reliant on numerous unverifiable assumptions. However, the extent to which we were able to detect associations of Cd concentration with other continuous variables can be demonstrated by considering the minimum detectable Pearson correlation coefficient for bivariate (unadjusted) associations: given a projected sample size of n=619, for any two continuous variables, we had 80% power to detect a minimum statistically significant correlation coefficient of 0.019, assuming a stringent significance level of p=0.01. Therefore, we expected to have a sufficient sample size to detect even relatively weak associations between Cd and continuous outcome measures.

Chapter 5 Results

5.1 Participant characteristics

The median age (years) of mothers in the sub-study was 23 (range: 18-40). All participants were married, and 94% reported homemaking as their primary occupation. The median number of times participants were ever pregnant in the sub-study was 2 (range: 1-9). Regarding educational attainment, 61% of participants had at least a primary education completed. The study sample had a relatively even split between the 5 quintiles of the household asset index. Participants did not significantly differ between the MDIG study and the sub-study in baseline characteristics, except for negligibly small differences in maternal weight and BMI. Differences in season of enrolment between the two studies were due to a decreased proportion of participants being enrolled in the December-February months, as compared to the MDIG trial (Table 7). Month of enrolment was not found to differ significantly between the vitamin D supplementation groups (Appendix Table 7). Maternal blood Cd concentrations were significantly higher for participants who enrolled in the months of December, January, or February as compared to participants who were enrolled in the months of March, April, or May (Appendix Table 8a). Maternal blood Cd concentrations did not differ by month of delivery (Appendix Table 8b). The risk of detectable cord blood Cd was higher in participants who enrolled between the months of September, October, or November and December, January, or February as compared to those who enrolled in March, April, or May (Appendix Table 9a). As well, the risk of detectable cord blood was lower in participants who delivered in September, October, or November and higher for participants who delivered in the months of December, January, or February as compared to those that delivered in the months of June, July, or August (Appendix Table 9b).

Table 7: Maternal characteristics at enrolment, comparison of the MDIG and Cd study.

Enrolled participants, N	**MDIG Trial 1298**	**Cd study 619**	***P*[f]**
Age (years), median (min, max)	22 (18, 40)	23 (18,40)	0.06
Marital status, n (%)[a]			0.17
Married	1281 (99.8)	617 (100)	
Not Married	2 (0.2)	0 (0)	
Level of education, n (%)			0.62
No schooling	58 (4.5)	25 (4.04)	
Primary incomplete	277 (21.3)	127 (20.5)	
Primary complete	179 (13.8)	90 (14.5)	
Secondary incomplete	498 (38.4)	232 (37.5)	
Secondary complete or higher	286 (22.0)	145 (23.4)	
Primary occupation, n (%)[b]			0.65
Homemaker	1202 (93.7)	580 (94.0)	
Other	81 (6.3)	37 (6.00)	
Asset index quintiles, n (%)[c]			0.94
1 (lowest)	261 (20.4)	122 (19.8)	
2	251 (19.6)	123 (20.0)	
3	256 (20.0)	128 (20.8)	
4	257 (20.1)	123 (20.0)	
5 (highest)	255 (19.9)	120 (19.5)	
Gravidity[d], median (min, min)	2 (1, 9)	2 (1, 9)	0.27
Weight (kg), mean ± SD	54.0 ±10.0	54.7 ± 10.0	0.02*
Height (cm), mean ± SD	151 ± 5.4	151 ± 5.47	0.86
BMI (kg/m^2)	23.7 ± 4.05	24.0 ± 4.00	0.02*
Month of enrolment, n (%)			0.00*
March-May	465 (35.8)	269 (43.5)	
June-August	412 (31.7)	237 (38.3)	
September-November	225 (17.3)	77 (12.4)	
December-February	196 (15.1)	36 (5.82)	
Hemoglobin (g/L), mean ± SD	106.2 ± 11.4	106.0 ± 11.2	0.71
Serum 25(OH)D[e], mean ± SD	27.5 ± 14.0	27.0 ± 13.7	0.27

[a] n_{MDIG}= 1285, $n_{Cd\text{-}study}$=617
[b] n_{MDIG}= 1283, $n_{Cd\text{-}study}$=617
[c] n_{MDIG}=1280, $n_{Cd\text{-}study}$=616
[d] Number of pregnancies, including the current pregnancy
[e] n_{MDIG}=1283, $n_{Cd\text{-}study}$=615
[f] Parametric and non-parametric tests were used to assess any differences between groups (t-test, Mann-Whitney test, chi-squared test); Cd study participants were compared to MDIG participants who were not eligible for inclusion in the Cd study (n=679); $p<0.05$ is considered significant, significant results are marked with a *

5.2 Aim 1: The effect of vitamin D supplementation on maternal blood Cd concentrations

5.2.1 Metal and mineral concentrations

Maternal blood Cd concentrations (n=619) in the study had a non-normal distribution with a median of 0.68 μg/L (IQR: 0.42 μg/L). The minimum concentration was 0.15 μg/L (with one result below the limit of detection imputed as 0.1 μg/L) and the highest was 2.6 μg/L. These concentrations were higher than blood concentrations measured previously in pregnant rural Bangladeshi women, who had a median blood concentration of 0.5 μg/L [70]. Of the n=124 detectable cord blood Cd results, the geometric mean (95% CI) was 0.14 μg/L (0.13 μg/L, 0.14 μg/L). Maternal blood Se at delivery (n=619) had a normal distribution, with a mean of 135 μg/L (SD: 18.5 μg/L). It appears that women in the study on average had sufficient blood Se concentrations [179]. Baseline relative daily Ca intake from food (n=619) had non-normal distribution with a median of 441 mg/day (IQR: 336 mg/day). On average, most women in our study were not consuming high amounts of Ca from foods, as recommended daily intakes of Ca for individuals over 19 years of age vary between 700 to 1000 mg [180]. Maternal serum ferritin at delivery (n=610) had a non-normal distribution, with a median of 45.8 ng/mL (IQR: 46.1 ng/mL). Serum ferritin concentrations of less than 15 ng/mL, which is the World Health Organization's cut-off for iron deficiency in adults, were measured in 10% of the study population [181]. Plasma CRP at delivery (n=553) had a non-normal distribution with a median of 10.1 mg/L (IQR: 20.2 mg/L). Descriptive statistics of all variables (including delivery 25(OH)D concentrations) for all study participants and stratified by vitamin D treatment arm, are presented in Appendix Table 6. There were no differences in baseline characteristics across treatment groups (Appendix Table 7). As

already reported in the MDIG trial, vitamin D supplementation also had a dose-dependent effect on maternal 25(OH)D concentrations at delivery in our study cohort, with increasing supplementation leading to higher 25(OH)D concentrations (Appendix Table 6) [162].

5.2.2 The effect of vitamin D on maternal blood Cd concentrations

There was no significant effect of vitamin D supplementation group on maternal blood Cd concentrations, as compared to placebo (Table 8).

Table 8: Pairwise regression of vitamin D_3 supplementation group and maternal blood Cd concentrations at delivery.

Treatment group	n	% difference	(% difference) 95% CI	P[a]
Placebo	118	ref	ref	ref
4200 IU/ week	141	6.58	-5.09, 19.7	0.28
16800 IU/week	121	4.86	-7.01, 18.3	0.44
28000 IU/week	239	1.61	-8.48, 12.8	0.77

[a] $p<0.05$ is considered significant in this model

5.2.3 Considerations of iron, Ca, and Se as modifiers of the effect of vitamin D on maternal blood Cd

5.2.3.1 Univariate associations of iron, Se, Ca with maternal blood Cd

Serum ferritin at delivery (adjusted for plasma CRP), whole blood Se, and daily Ca intake (at baseline) were not significantly associated with blood Cd (μg/L) at delivery (Table 9).

Table 9: Univariate regression of serum ferritin (adjusted for plasma CRP), Ca, and Se with whole blood Cd at delivery.

Variable[a]	n	% difference	(% difference) 95% CI	P[b]
Serum ferritin (ng/10 mL)[c]	550	-0.29	-0.63, 0.04	0.09
Plasma CRP (mg/L)	550	-0.02	-0.12, 0.09	0.73
Daily Ca intake (100 mg)[d]	619	-0.65	-2.04, 0.76	0.37
Maternal whole blood Se (μg/L)	619	0.11	-0.09, 0.31	0.28

[a] Separate univariate linear regression models were run for each covariate
[b] $p<0.05$ is considered significant in this model
[c] Variable scaled to ng/10 mL
[d] Variable scaled to 100 mg/day

5.2.3.2 The effect of vitamin D on maternal blood Cd concentrations with iron, Se, and Ca as covariates

A multivariable model to test the effect of vitamin D on blood Cd, including serum ferritin and plasma CRP as covariates, was not statistically significant (Table 10). Similarly, the multivariable model on the effect of vitamin D on maternal blood Cd that included maternal blood Se as a covariate with increasing vitamin D supplementation as compared to placebo was not statistically significant (Table 10).

The multivariable model on the effect of vitamin D on maternal blood Cd with relative Ca intake as a covariate also showed a non-significant relationship (Table 10).

5.2.3.3 The effect of vitamin D on maternal blood Cd concentrations with iron, Se, and Ca as interaction terms

Including serum ferritin, daily Ca intake, and maternal Se as interaction terms with vitamin D supplementation group in separate models did not show any significant modification of the effect of vitamin D supplementation group on maternal blood Cd concentrations (Table 10).

Table 10: Effect of vitamin D supplementation on maternal blood Cd concentrations, adjusting for daily Ca intake, serum ferritin (adjusted for plasma CRP), and whole blood Se with and without interactions of vitamin D group with daily Ca intake, serum ferritin (adjusted for plasma CRP) and whole blood Se included as effect modifiers.

	Adjusted model[a]				*Interaction model*[b]			
	N	**% difference**	**95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Daily Ca intake (100 mg/day)[g]	619	-0.59	-1.99, 0.83	0.41	619	-0.93	-4.02, 2.26	0.56
Vitamin D treatment group								
Placebo	118	ref	ref	ref	118	ref	ref	ref
4200 IU/week	141	6.27	-5.38, 19.4	0.30	141	9.25	-15.0, 40.4	0.49
16800 IU/week	121	4.68	-7.19, 18.1	0.46	121	4.85	-17.6, 33.5	0.70
28000 IU/week	239	1.53	-8.55, 12.7	0.78	239	-4.36	-23.3, 19.3	0.69
Interaction								
Placebo	-	-	-	-	118	ref	ref	ref
4200 IU/week*Ca intake	-	-	-	-	141	-0.65	-5.18, 4.10	0.79[d]
16800 IU/week*Ca intake	-	-	-	-	121	-0.06	-4.25, 4.32	0.98[d]
28000 IU/week*Ca intake	-	-	-	-	239	1.22	-2.65, 5.25	0.54[d]
Model C					**Model D**			
Serum ferritin (ng/10 mL)[h]	550	-0.29	-0.63, 0.04	0.09	550	-0.25	-0.63, 0.13	0.20
Plasma CRP (mg/L)	550	-0.02	-0.12, 0.08	0.70	550	-0.02	-0.12, 0.09	0.74
Vitamin D treatment group								
Placebo	115	ref	ref	ref	115	ref	ref	ref
4200 IU/week	122	5.77	-6.15, 19.2	0.36	122	6.85	-6.76, 22.4	0.34
16800 IU/week	105	1.56	-10.3, 15.0	0.81	105	4.84	-13.3, 26.8	0.63
28000 IU/week	208	0.15	-10.0, 11.5	0.98	208	1.33	-12.0, 16.6	0.85
Interaction								
Placebo	-	-	-	-	115	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	122	-0.13	-0.99, 0.73	0.76[e]
16800 IU/week*ferritin	-	-	-	-	105	-0.50	-2.77, 1.82	0.67[e]
28000 IU/week*ferritin	-	-	-	-	208	-0.02	-0.12, 0.09	0.81[e]
Model E					**Model F**			
Whole blood Se (µg/L)	619	0.11	-0.09, 0.31	0.28	619	0.30	-0.18, 0.78	0.22
Vitamin D treatment group								
Placebo	118	ref	ref	ref	118	ref	ref	ref
4200 IU/week	141	6.56	-5.10, 19.7	0.28	141	-11.2	62.8, 112	0.78
16800 IU/week	121	4.74	-7.13, 18.1	0.45	121	92.0	-19.3, 359	0.14
28000 IU/week	239	1.60	-8.48, 12.8	0.77	239	54.7	-30.2, 243	0.28
Interaction								
Placebo	-	-	-	-	118	ref	ref	ref
4200 IU/week*Se	-	-	-	-	141	0.13	-0.50, 0.78	0.68[f]
16800 IU/week*Se	-	-	-	-	121	-0.45	-1.08, 0.19	0.17[f]
28000 IU/week*Se	-	-	-	-	239	-0.31	-0.89, 0.27	0.30[f]

[a] Separate adjusted models were run with daily Ca intake (100 mg/day, Model A), serum ferritin (ng/10 mL) and plasma CRP (mg/L, Model C), and cord blood Se (µg/L, Model E) as covariates
[b] Separate interaction models were run with daily Ca intake (100 mg/day, Model B), serum ferritin (ng/10 mL) and plasma CRP (mg/L, Model D), and cord blood Se (µg/L, Model E) as interaction terms
[c] $p<0.05$ is considered significant
[d] Interaction tested using Wald test (p=0.80)
[e] Interaction tested using Wald test (p= 0.96)
[f] Interaction tested using Wald test (p=0.18)
[g] Variable scaled to 100 mg/day
[h] Variable scaled to ng/10 mL

5.2.4 The effect of vitamin D on venous cord blood Cd concentrations

Due to a significant proportion of cord blood Cd concentrations (76%) being below the limit of detection (0.1μg/L), cord blood Cd was dichotomized to a detectable/undetectable variable. Log binomial regression was used to establish the relative risk of vitamin D supplementation on the detectability of cord blood Cd in each vitamin D supplementation group as compared to the placebo group. Vitamin D supplementation increased the detectability of cord blood Cd, as compared to placebo. The risk of having detectable cord Cd was 2.18-fold higher in the 4200 IU/week group (CI: 1.27, 3.75; p=0.01), 1.38-fold higher in the 16800 IU/week group (CI: 0.76, 2.50; p=0.29), and 1.72-fold higher in the 28000 IU/week group (CI: 1.02, 2.91; p=0.04) (Table 10). The effect remained significant when all vitamin D supplementation groups were combined together and compared to placebo, with the risk of having detectable cord Cd being 1.75-fold higher in the supplemented group as compared to the placebo group (CI: 1.07, 2.86; p=0.03).

Table 11: Pairwise regression of vitamin D supplementation group and detectable cord blood Cd.

Treatment group	n	Risk Ratio	95% CI	P[a]
Placebo	100	ref	ref	ref
4200 IU/week	104	2.18	1.27, 3.75	0.01*
16800 IU/week	111	1.38	0.76, 2.50	0.29
28000 IU/week	201	1.72	1.02, 2.91	0.04*

[a] $p<0.05$ is considered significant, significant results are marked with a *

5.2.5 Considerations of iron, Ca, and Se as modifiers of the effect of vitamin D on venous cord blood Cd

5.2.5.1 Univariate associations of Fe, Se, and Ca with venous cord blood Cd

There was no significant association of serum ferritin, relative daily Ca intake, or cord blood Se with the risk of having detectable cord blood Cd (Table 12). Visualizations of the

relationships of blood Cd with serum ferritin, Ca intake, and blood Se are shown in Appendix Figures 1, 2 and 3.

Table 12: Pairwise regression of detectable cord blood Cd with serum ferritin, plasma CRP, cord blood Se, and daily Ca intake.

Variable	n	Risk Ratio	95% CI	P[a]
Serum ferritin (ng/ 10 mL)[b]	465	1.00	0.98, 1.01	0.62
Plasma CRP (mg/L)	465	1.00	1.00, 1.01	0.09
Daily Ca intake (100 mg)[c]	516	1.02	0.97, 1.07	0.43
Cord blood Se (μg/L)	516	1.00	0.99, 1.01	0.87

[a] $p<0.05$ is considered significant
[b] Variable scaled to ng/10 mL
[c] Variable scaled to 100 mg/day

5.2.5.2 The effect of vitamin D on cord blood Cd concentrations with iron, Se, and Ca as covariates

A multivariable model on the effect of vitamin D on detectability of cord blood Cd with relative Ca intake as a covariate showed little influence of Ca intake on the model (Table 13, Model A). Similarly, a multivariable model on the effect of vitamin D on cord blood Cd with serum ferritin and plasma CRP as covariates appeared to have very little impact on the model (Table 13, Model B). Including cord blood Se as a covariate showed almost no impact of the variable on the effect of vitamin D supplementation on detectability of cord blood Cd (Table 13, Model C).

5.2.5.3 The effect of vitamin D on maternal blood Cd concentrations with iron, Se, and Ca as interaction terms

As evident in the multivariable models, the variables ferritin, Ca intake, and Se appeared to have little impact on the effect of vitamin D supplementation on detectability of cord blood

Cd. Including the variables as interaction terms with supplementation group showed no significant effect modification (Table 13).

Table 13: Effect of vitamin D supplementation on maternal blood Cd concentrations, adjusting for daily Ca intake, serum ferritin (adjusted for plasma CRP), and whole blood Se with and without interactions of vitamin D group with daily Ca intake, serum ferritin (adjusted for plasma CRP) and whole blood Se included as effect modifiers.

	Adjusted model[a]				*Interaction model*[b]			
	N	**Risk Ratio**	**95% CI**	***P*[c]**	**N**	**Risk Ratio**	**95% CI**	***P*[c]**
Model A					**Model B**			
Daily Ca intake (100 mg)[g]	516	1.03	0.97, 1.08	0.35	516	1.00	0.86, 1.17	0.98
Vitamin D treatment group								
Placebo	100	ref	ref	ref	100	ref	ref	ref
4200 IU/week	104	2.21	1.28, 3.80	0.004*	104	2.36	0.81, 6.83	0.11
16800 IU/week	111	1.38	0.77, 2.50	0.28	111	1.07	0.35, 3.23	0.91
28000 IU/week	201	1.72	1.02, 2.89	0.04*	201	1.46	0.53, 4.02	0.47
Interaction								
Placebo	-	-	-	-	100	ref	ref	ref
4200 IU/week*Ca	-	-	-	-	104	0.98	0.81, 1.19	0.86
16800 IU/week*Ca	-	-	-	-	111	1.05	0.88, 1.26	0.60
28000 IU/week*Ca	-	-	-	-	201	1.03	0.87, 1.23	0.72
Model C					**Model D**			
Serum ferritin (ng/10 mL)[h]	465	1.00	0.98, 1.01	0.79	465	0.95	0.84, 1.09	0.49
Plasma CRP (mg/L)	465	1.00	1.00, 1.01	0.16	465	1.00	1.00, 1.01	0.13
Vitamin D treatment group								
Placebo	95	ref	ref	ref	95	ref	ref	ref
4200 IU/week	90	2.14	1.24, 3.70	0.01*	90	1.93	0.75, 4.96	0.17
16800 IU/week	100	1.13	0.60, 2.12	0.70	100	0.70	0.22, 2.19	0.54
28000 IU/week	180	1.62	0.95, 2.75	0.08	180	0.91	0.36, 2.28	0.84
Interaction								
Placebo	-	-	-	-	95	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	90	1.02	0.88, 1.18	0.84[e]
16800 IU/week *ferritin	-	-	-	-	100	1.09	0.92, 1.29	0.34[e]
28000 IU/week*ferritin	-	-	-	-	180	1.10	0.96, 1.26	0.17[e]
Model E					**Model F**			
Cord blood Se (µg/L)	516	1.00	1.00, 1.01	0.84	516	1.00	0.97, 1.01	0.47
Vitamin D supplementation group								
Placebo	100	ref	ref	ref	100	ref	ref	ref
4200 IU/week	104	2.18	1.27, 3.75	0.05*	104	0.25	0.01, 9.95	0.46
16800 IU/week	111	1.39	0.77, 2.51	0.28	111	1.92	0.02, 18.3	0.78
28000 IU/week	201	1.73	1.03, 2.93	0.04*	201	0.85	0.02, 36.1	0.93
Interaction								
Placebo	-	-	-	-	100	ref	ref	ref
4200 IU/week*Se	-	-	-	-	104	1.01	0.99, 1.04	0.25
16800 IU/week*Se	-	-	-	-	111	1.00	0.97, 1.03	0.91
28000 IU/week*Se	-	-	-	-	201	1.00	0.98, 1.03	0.70

[a] Separate adjusted models were run with daily Ca intake (100 mg/day, Model A), serum ferritin (ng/10 mL) and plasma CRP (mg/L, Model C), and cord blood Se (µg/L, Model E) as covariates
[b] Separate interaction models were run with daily Ca intake (100 mg/day, Model B), serum ferritin (ng/10 mL) and plasma CRP (mg/L, Model D), and cord blood Se (µg/L, Model F) as interaction terms
[c] $p<0.05$ is considered significant, significant results are marked with a *
[d] Interaction tested using the Wald test (p=0.84)
[e] Interaction tested using the Wald test (p=0.25)
[f] Interaction tested using the Wald test (p=0.37)
[g] Variable scaled to 100 mg/day
[h] Variable scaled to ng/10 mL

5.2.6 Additional analyses

5.2.6.1 25(OH)D and maternal Cd concentrations

To further explore the observed null effect of vitamin D supplementation on maternal blood Cd concentrations at delivery, we assessed the relationship of serum 25(OH)D concentrations at delivery and baseline with maternal blood Cd concentrations. The relationship with delivery 25(OH)D was not found to be significant, with each one unit increase in serum 25(OH)D concentrations leading to a -0.06% decrease in blood Cd concentrations (CI: -0.18%, 0.07%; p=0.37). The relationship with baseline 25(OH)D was also not significant, with each unit increase in serum 25(OH)D concentrations leading to a -0.12% decrease in blood Cd concentrations (CI: -0.40, 0.15; p=0.37). The weak associations between baseline and delivery 25(OH)D concentrations and maternal blood Cd are visualized in Figures 7 and 8 as scatterplots with horizontal LOWESS lines.

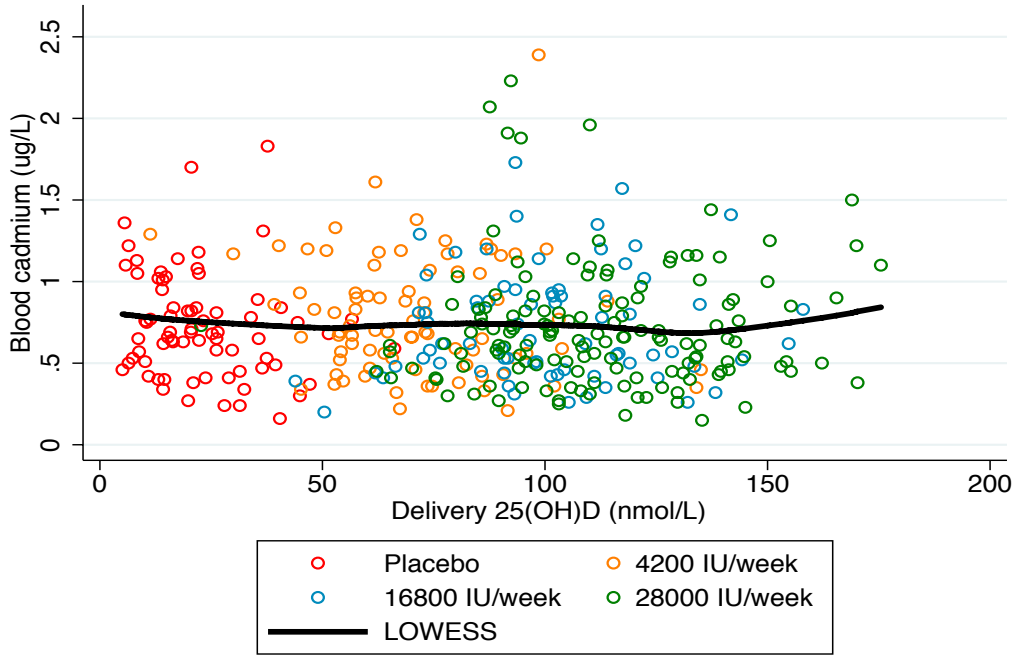

Figure 7: Scatterplot of the association of delivery 25(OH)D concentrations and maternal blood Cd at delivery.

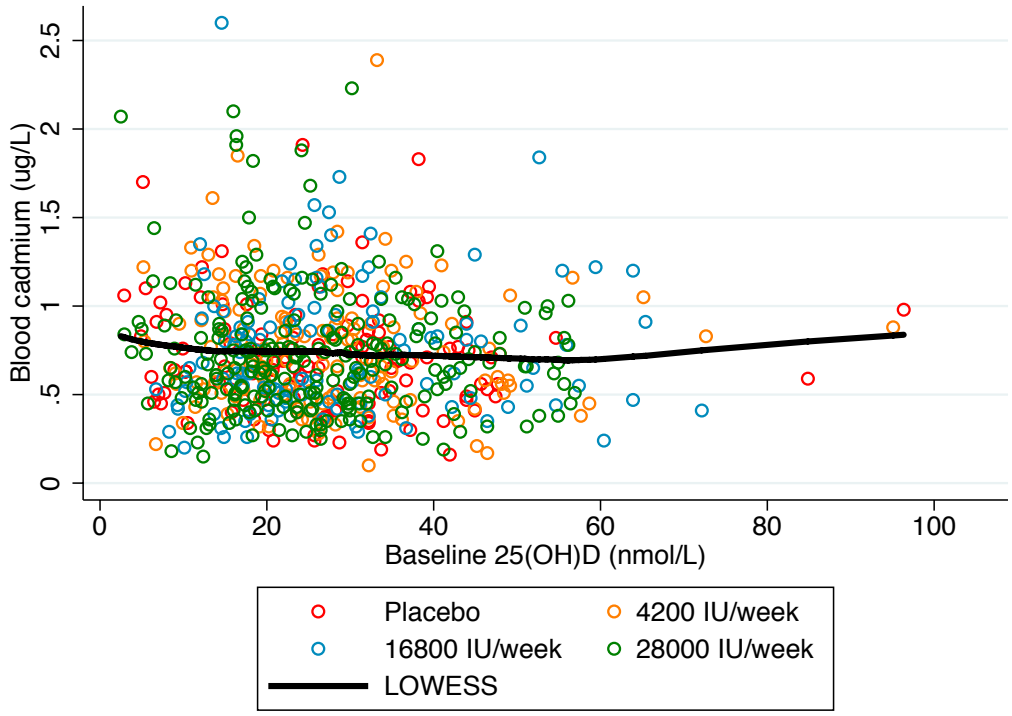

Figure 8: Scatterplot of the association of baseline 25(OH)D concentrations and maternal blood Cd at delivery.

5.2.7 Sensitivity analyses

5.2.7.1 Excluding influential outliers in models utilizing serum ferritin

Two influential outliers in the serum ferritin dataset were found to significantly impact the association of serum ferritin and blood Cd concentrations. These serum ferritin concentrations were very high, at >1000 ng/mL, values which are only seen in cases of acute inflammation [182]. Reviewing patient information at the time of labour, it was found that both these participants experienced clinical symptoms which may have caused inflammation, with one woman being diagnosed with placenta previa, and the other patient experiencing an induced operative vaginal delivery. In additional, both women had high delivery plasma CRP concentrations of >100 mg/L, which signal acute inflammation. For these reasons, we decided to include these outliers in our main model and include serum ferritin with these

outliers removed as part of a sensitivity analysis. We did not find significant differences between the two models regarding the interaction effect of serum ferritin on the vitamin D supplementation/maternal blood Cd model, as well as the cord blood Cd model (Appendix Table 2 and 3). Models using serum ferritin without adjusting for plasma CRP also did not significantly change the inference (Appendix Table 2 and 3).

5.2.7.2 Including month of enrolment and month of delivery as covariates in Aim 1 models

All separate models in Aim 1 were adjusted for month of enrolment (March-May, June-August, September-November, December-February) as well as month of delivery (June-August, September-November, December-February). Due to chance, no mothers in our study delivered infants in the months of March, April, or May. Adjusting for month of enrolment and month of delivery did not lead to a significant impact on the effect of vitamin D supplementation on maternal blood Cd concentrations (Appendix Table 10a and Appendix Table 11a). Adjusting for month of enrolment and month of delivery did lead to some attenuation of the effect of vitamin D supplementation on the detectability of cord blood Cd (Appendix Table 10b and Appendix Table 11b).

5.3 Aim 2: The association of maternal blood Cd and renal biomarkers

5.3.1 Renal biomarker concentrations

Serum creatinine at delivery (n=311) had a non-normal distribution and a median of 61.2 μmol/L (IQR 27.0 μmol/L). As previously mentioned, issues with blood in urine and missing data/ uncertain laboratory results for urine β_2-microglobulin (B2MG) led to incomplete and potentially inaccurate datasets. Raw urine B2MG (n=449) data (with results below the LOD imputed as the LOD multiplied by the dilution factor) had a non-normal distribution with a

median of 162.0 μg/L (IQR: 322.0). The raw data (n=449) of urine B2MG/creatinine was non-normally distributed and had a median of 357.9 μg/g (IQR: 578.6 μg/g). Urine albumin (with results below the limit of detection imputed as the LOD multiplied by the dilution factor) had a non-normal distribution with a median of 11.2 μg/mL (IQR: 22.8 μg/mL). Urine ACR had a non-normal distribution (n=444) with a median of 20.2 μg/mg (IQR: 40.9 μg/mg). Urine cotinine (n=429) had a non-normal distribution with a median of 3.08 ng/mL (IQR: 9.70 ng/mL).

5.3.2 Presence of blood in urine samples in our cohort

Considerably elevated urine β_2-microglobulin results led us to test urine samples (n=419) for presence of blood using urine dipsticks. Table 14 shows the levels of blood in our urine samples.

Table 14: Urine samples in the Cd study with blood present.

Level of blood in urine	n (%)
Negative	150 (35.8)
Trace	44 (10.5)
Small	45 (10.7)
Moderate	76 (18.1)
Large	104 (24.8)

We assessed the associations of blood in urine with each of the urine biomarkers (albumin, B2MG, creatinine, cotinine) in pairwise linear regression models comparing each level of blood present to the negative group.

5.3.2.1 Urine albumin

Presence of blood in urine appeared to steadily and significantly increase urine albumin concentrations with each increasing level (Table 15, Model A). Due to this known

relationship between presence of blood and urine albumin results, all regression models in which urine albumin was used as a variable were adjusted for presence of blood in urine.

5.3.2.2 Urine β_2-microglobulin

We found no significant association between urine B2MG and presence of blood (Table 15, Model B). Due to issues with the interpretability of assay results, missing data due to the vast range of concentrations, urine B2MG will not be used as a variable in modelling the association of maternal blood Cd concentrations on renal function.

5.3.2.3 Urine cotinine

We found no significant association between urine cotinine and presence of blood, except for the trace group (Table 15, Model C). Urine cotinine concentrations were -47.5% lower in samples with trace amounts of blood, as compared to samples with negative blood (CI: -71.2%, -4.16; p=0.04). However, a regression model with presence of blood dichotomized as a detectable/undetectable variable was not found to significantly differ in urine cotinine concentration (results not shown).

5.3.2.4 Urine creatinine

We found no significant association between urine creatinine and presence of blood, except for the trace group (Table 15, Model D). Urine creatinine concentrations were -25.6% lower in the samples with trace amounts of blood, as compared to samples with negative blood (CI: -42.1%, -4.55%; p=0.02). However, a pairwise regression model with presence of blood dichotomized as an undetectable/detectable variable was not found to differ significantly in urine creatinine concentrations (results not shown).

Table 15: Pairwise linear regression analysis of the association of blood in urine and urine biomarkers.

Urine biomarker[a]	Presence of blood in urine	N	% difference	(% difference) 95% CI	*P*[b]
Model A					
Albumin (μg/L)					
	Negative	142	ref	ref	ref
	Trace	41	87.5	11.8, 214	0.02*
	Small	40	112	25.9, 258	0.01*
	Moderate	67	151	63.2, 287	0.00*
	Large	94	381	226, 608	0.00*
Model B					
B2MG (μg/L)					
	Negative	143	ref	ref	ref
	Trace	42	-35.2	-58.8, 1.97	0.06
	Small	40	-13.6	-45.6, 37.0	0.53
	Moderate	69	-24.1	-48.0, 10.7	0.15
	Large	95	-12.1	-37.5, 23.7	0.46
Model C					
Cotinine (ng/mL)					
	Negative	150	ref	ref	ref
	Trace	44	-47.5	-71.2, -4.16	0.04*
	Small	44	-30.4	-61.9, 26.9	0.24
	Moderate	76	-7.95	-43.8, 50.8	0.74
	Large	103	-13.1	-44.5, 36.1	0.54
Model D					
Creatinine (mg/L)					
	Negative	142	ref	ref	ref
	Trace	41	-25.6	-42.1, -4.55	0.02*
	Small	40	-12.5	-32.0, 12.6	0.30
	Moderate	67	-14.5	-30.6, 5.35	0.14
	Large	94	-5.07	-21.3, 14.5	0.59

[a] Separate pairwise regression linear models were run for each urine biomarker
[b] $p<0.05$ is considered significant, significant results are marked with a *

5.3.3 Food sources and blood Cd concentrations

Maternal blood Cd concentrations and ever (in the past month before enrolment) intake of each food item in the FFQ were assessed in separate pairwise linear regression models, compared to the never consumed group. Six food items were found to be associated with blood Cd concentrations as specified by the predetermined criteria: coconut meat consumption (Table 16, Model A), poultry meat consumption (Table 16, Model B), beef/mutton/pork meat consumption

(Table 16, Model C), organ meat consumption (Table 16, Model D) and large fish consumption (Table 16, Model E). Wood apple and other nut consumption were not included in models due to low variation in consumption. Rice consumption was not found to be significantly associated with blood Cd concentrations, potentially due to very low variability of intake in our study participants. Separate associations of maternal blood Cd with all food items are shown in Appendix Table 1. With increasing asset index quintile, a greater proportion of participants stated ever consuming coconut, large fish, and beef/mutton/pork (results not shown).

Table 16: Pairwise linear regression of maternal blood Cd concentrations and food consumption showed a significant association between Cd concentrations and poultry, coconut beef/mutton/pork, organ and large fish meat consumption.

Food item[a]	**Consumption**	**n**	**% difference**	**(% difference) 95% CI**	**P**[b]
Model A					
Coconut meat	Never	489	ref	ref	ref
	Ever	130	-10.2	-18.0, -1.61	0.02*
Model B					
Poultry meat	Never	92	ref	ref	ref
	Ever	527	-11.3	-20.1, -1.54	0.02*
Model C					
Beef/mutton/pork meat	Never	140	ref	ref	ref
	Ever	479	-8.42	-16.2, 0.10	0.05*
Model D					
Organ meat	Never	375	ref	ref	ref
	Ever	244	-6.25	-13.1, 1.18	0.10*
Model E					
Large fish	Never	124	ref	ref	ref
	Ever	495	-8.54	-16.7, 0.37	0.06*

[a] Separate pairwise linear regression models were run for each food item
[b] $p<0.1$ is considered significant, significant results are marked with a *

5.3.4 Tobacco exposure and maternal blood Cd concentrations

In a univariate model, urine cotinine was not found to be associated with maternal blood Cd concentrations (Table 17). The breakdown of reported maternal tobacco exposure at baseline showed almost no women smoked cigarettes, with 2.1% reporting usage of chewing tobacco (*zarda*), and approximately 10% using betel leaf and related products. Smoking appears to be much more prevalent in husbands of participants, with 52% reporting smoking cigarettes at baseline (Table 18). Urine cotinine concentrations stratified by reported tobacco usage at enrolment are shown in Table 19. Urine cotinine concentrations were found to be significantly higher in mother who ever reported usage of *zarda* (chewing tobacco), *paan* (betel leaf), *chuna* (lime paste), or *supari* (areca nut). Paternal cigarette smoking was not found to be associated with maternal urine cotinine concentrations.

Table 17: Univariate linear regression of urine cotinine and maternal blood Cd concentrations at delivery.

	n	**% difference**	**(% difference) 95% CI**	**P**[a]
Urine cotinine (ng/10 mL)[b]	419	0.00	-0.00, 0.00	0.88

[a] $p<0.05$ is considered significant
[b] Variable scaled to ng/10 mL

Table 18: Reported tobacco product usage of participants in Cd study and their husbands/partners at enrolment.

	Yes (n, %)	
Substance type	**Women[1]**	**Husbands/partners[2]**
Cigarettes	1 (0.2)	260 (52)
Zarda (sweetened tobacco)	13 (2.1)	39 (7.8)
Paan (betel leaf)	59 (9.5)	96 (19)
Supari (areca nut)	17 (12)	103 (21)
Chuna (lime paste)	55 (9)	86 (17)

[1]n=619
[2]n=502

Table 19: Urine cotinine concentrations stratified by tobacco exposure at enrolment.

		Urine cotinine (ng/mL)		
Tobacco exposure		**N**	**Median (IQR)**	***P*[a]**
Maternal chewing tobacco intake				0.00*
	Never	412	3.08 (9.37)	
	Ever	6	613 (1527)	
Maternal *paan* intake				0.00*
	Never	384	2.97 (9.42)	
	Ever	35	8.23 (29.0)	
Maternal *chuna* intake				0.00*
	Never	385	2.97 (9.41)	
	Ever	34	8.52 (28.6)	
Maternal *supari* intake				0.01*
	Never	372	2.95 (9.40)	
	Ever	47	6.98 (15.2)	
Paternal smoking				0.16
	Never	163	2.60 (7.95)	
	Ever	180	4.99 (9.98)	

[a] $p<0.05$ is considered significant; separate non-parametric tests were performed to assess between group differences (Kruskal-Wallis test); significant results are marked with a *

5.3.5 The association of maternal blood Cd and serum creatinine concentrations at delivery

5.3.5.1 Unadjusted model

In unadjusted models, maternal blood Cd concentrations at delivery (n=311) were not found to be associated with serum creatinine concentrations (Table 20).

5.3.5.2 Multivariable model

A multivariable model of the association of maternal blood Cd concentrations at delivery on serum creatinine concentrations at delivery was run (n=242), and adjusted for the following covariates: gravidity, maternal BMI, blood Pb and Se concentrations at delivery, gestational age at birth, maternal age, urine cotinine concentrations at delivery, maternal education, asset index, coconut meat consumption, poultry consumption, beef/mutton/pork consumption, organ meat consumption, large fish consumption, and vitamin D supplementation group (Table 20). The categorical variable maternal education had the groups ‘no schooling’ and ‘primary incomplete’ collapsed into one group, due to a low number of participants in the ‘no schooling’ group, which greatly biased the results. There was no evidence of an association of maternal blood Cd with serum creatinine.

Table 20: Separate univariate models of covariates and serum creatinine at delivery, and the association of maternal blood Cd and serum creatinine, adjusted for covariates.

	Univariate model[a]				*Multivariate model*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Blood Cd (μg/L)	311	0.92	-10.8, 14.2	0.88	242	-2.67	-16.8, 13.9	0.73
Gravidity	311	1.44	-2.41, 5.45	0.47	242	1.15	-5.28, 8.02	0.73
Maternal BMI (kg/m^2)	306	0.59	-0.47, 1.67	0.27	242	0.49	-0.90, 1.88	0.49
Blood Pb (μg/dL)	311	1.12	0.03, 2.21	0.04*	242	1.15	-0.18, 2.50	0.09
Blood Se (μg/L)	311	0.08	-0.16, 0.33	0.50	242	-0.03	-0.33, 0.27	0.83
Gestational age at birth (weeks)	311	1.01	-2.11, 4.24	0.53	242	2.90	-1.02, 6.98	0.15
Maternal age (years)	311	0.82	-0.25, 1.89	0.13	242	0.68	-0.93, 2.33	0.40
Urine cotinine (ng/10 mL)[d]	247	0.003	-0.00, 0.01	0.42	242	0.04	-0.04, 0.12	0.33
Maternal education								
No schooling or primary incomplete	81	ref	ref	ref	58	ref	ref	ref
Primary complete	41	-2.95	-16.2, 12.4	0.69	32	11.3	-6.49, 32.4	0.23
Secondary incomplete	106	4.91	-6.33, 17.5	0.41	88	17.5	2.70, 34.5	0.02*
Secondary complete or higher	83	4.88	-6.98, 18.3	0.43	64	15.1	-0.74, 33.4	0.06
Asset index (quintiles)								
1 (lowest)	64	ref	ref	ref	48	ref	ref	ref
2	60	-2.69	-15.2, 11.7	0.70	48	-7.81	-21.6, 8.43	0.32
3	61	-9.94	-21.5, 3.34	0.14	46	-9.54	-23.3, 6.63	0.23
4	69	-3.40	-15.5, 10.4	0.61	54	-7.42	-21.4, 9.06	0.35
5	56	1.20	-12.1, 16.5	0.87	46	-2.34	-17.9, 16.2	0.79
Coconut meat consumption								
Never	241	ref	ref	ref	181	ref	ref	ref
Ever	70	-1.59	-11.3, 9.22	0.76	61	-3.71	-14.4, 8.34	0.53
Poultry meat consumption								
Never	41	ref	ref	ref	33	ref	ref	ref
Ever	270	-9.61	-20.5, 2.76	0.12	209	-13.4	-25.7, 0.94	0.07
Beef/mutton/pork meat consumption								
Never	66	ref	ref	ref	49	ref	ref	ref
Ever	245	8.63	-2.30, 20.8	0.13	193	10.6	-2.76, 25.9	0.12
Organ meat consumption								
Never	179	ref	ref	ref	134	ref	ref	ref
Ever	132	-0.67	-9.04, 8.49	0.88	108	5.67	-4.82, 17.3	0.30
Large fish consumption								
Never	57	ref	ref	ref	47	ref	ref	ref
Ever	254	11.7	-0.11, 24.9	0.05	195	7.60	-5.97. 23.1	0.29
Vitamin D supplementation group								
Placebo	67	ref	ref	ref	47	ref	ref	ref
4200 IU/week	70	7.01	-6.17, 22.0	0.31	50	15.2	-1.94, 35.3	0.10
16800 IU/week	62	4.75	-8.52, 19.9	0.50	50	8.72	-7.63, 28.0	0.32
28000 IU/week	112	0.81	-10.5, 13.5	0.89	95	7.67	-56.5, 23.9	0.32

[a] Separate univariate models were run for each covariate and serum creatinine relationship

[b] Multivariable model adjusted for: gravidity, postpartum maternal BMI (kg/m^2), blood Pb (μg/dL), blood Se (μg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/mL), maternal education (no schooling or primary incomplete), asset index (1-5), coconut, poultry, organ, beef/mutton/pork meat or fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 16800 IU/week, 28000 IU/week)

[c] $p < 0.05$ is considered significant, significant results are marked with a *

[d] Variable scaled to ng/ 10 mL

5.3.6 The association of maternal blood Cd and urine ACR concentrations at delivery, adjusted for presence of blood

5.3.6.1 Unadjusted model

The univariate linear regression model of blood Cd concentrations and urine ACR at delivery did not show a significant relationship (Table 21).

5.3.6.2 Multivariable model

The multivariable model of the association of blood Cd concentrations at delivery on urine ACR concentrations at delivery was adjusted for the following covariates: gravidity, maternal BMI, blood Pb and Se concentrations at delivery, gestational age at birth, maternal age, urine cotinine concentrations, maternal education, asset index, presence of blood, coconut meat consumption, poultry meat consumption, beef/mutton/pork meat consumption, organ meat consumption, large fish consumption, and vitamin D supplementation group (Table 21). As with serum creatinine, the categorical variable maternal education was collapsed from five groups into four because of a low sample size in the ‘no schooling’ group. The model was not found to be significant.

Table 21: Separate univariate models of covariates and urine ACR at delivery, and the association of maternal blood Cd and urine ACR at delivery, adjusted for covariates.

	Univariate model[a]				*Multivariate model*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Blood Cd (μg/L)	444	28.8	-11.4, 87.1	0.18	351	12.0	-26.4, 70.2	0.60
Gravidity	444	6.51	-6.00, 20.7	0.32	351	24.3	3.21, 49.6	0.02*
Maternal BMI (kg/m^2)	412	-1.75	-4.80, 1.39	0.27	351	-1.27	-4.90, 2.50	0.50
Blood Pb (μg/dL)	444	2.67	-0.55, 6.00	0.11	351	2.14	-1.48, 5.90	0.25
Blood Se (μg/L)	444	0.38	-0.34, 1.11	0.30	351	0.35	-0.46, 1.16	0.40
Gestational age at birth (weeks)	440	-1.62	-9.37, 6.78	0.70	351	-0.87	-9.86, 9.03	0.86
Maternal age (years)	444	-0.62	-3.75, 2.61	0.70	351	-1.95	-6.29, 2.60	0.39
Urine cotinine (ng/10 mL)[d]	394	0.00	-0.03, 0.03	0.99	351	-0.14	-0.68, 0.39	0.60
Maternal education								
No schooling or primary incomplete	113	ref	ref	ref	87	ref	ref	ref
Primary complete	58	-11.8	-43.6, 38.0	0.58	50	0.43	-38.3, 63.5	0.99
Secondary incomplete	167	-10.7	-36.3, 25.1	0.51	141	25.4	-13.7, 82.4	0.23
Secondary complete or higher	106	-17.2	-43.1, 20.4	0.32	85	29.6	-16.3, 101	0.24
Asset index (quintiles)								
1 (lowest)	89	ref	ref	ref	72	ref	ref	ref
2	87	-15.7	-44.4, 27.8	0.42	73	-17.5	-47.4, 29.3	0.40
3	92	-32.1	-55.0, 2.27	0.06	75	-38.6	-61.0, -3.25	0.04*
4	89	-26.7	-51.5, 10.8	0.14	72	-30.2	-56.7, 12.6	0.14
5	86	-33.2	-56.0, 1.35	0.06	71	-33.5	-58.8, 7.51	0.10
Presence of blood								
Negative	142	ref	ref	ref	129	ref	ref	ref
Trace	41	152	59.5, 298	0.00*	39	131	41.5, 276	0.00*
Small	40	143	52.8, 285	0.00*	38	126	38.1, 270	0.00*
Moderate	67	194	101, 331	0.00*	61	224	113, 395	0.00*
Large	94	406	259, 614	0.00*	84	421	257, 662	0.00*
Coconut meat consumption								
Never	341	ref	ref	ref	272	ref	ref	ref
Ever	103	-8.90	-33.3, 24.3	0.56	91	9.60	-21.0, 52.0	0.58
Poultry meat consumption								
Never	54	ref	ref	ref	46	ref	ref	ref
Ever	390	2.77	-31.2, 53.6	0.89	317	8.41	-29.9, 67.6	0.72
Beef/mutton/poultry meat consumption								
Never	90	ref	ref	ref	72	ref	ref	ref
Ever	354	39.6	0.84, 93.2	0.04*	291	68.2	16.8, 142	0.01*
Organ meat consumption								
Never	255	ref	ref	ref	205	ref	ref	ref
Ever	189	-9.96	-31.0, 17.4	0.44	158	-5.79	-30.0, 26.8	0.69
Large fish consumption								
Never	92	ref	ref	ref	75	ref	ref	ref
Ever	352	11.5	-19.3, 54.2	0.51	288	12.4	-22.3, 62.6	0.53
Vitamin D supplementation group								
Placebo	88	ref	ref	ref	66	ref	ref	ref
4200 IU/week	99	-11.5	-41.0, 32.8	0.56	77	-27.1	-53.6, 14.7	0.17
16800 IU/week	83	-12.7	-42.8, 33.3	0.53	71	-36.9	-60.7, 1.46	0.06
28000 IU/week	174	-26.1	-48.5, 6.10	0.10	149	-37.2	-57.7, -6.69	0.02*

[a] Separate univariate models were run for each covariate and ACR relationship

[b] Multivariable model adjusted for: gravidity, postpartum maternal BMI (kg/m^2), blood Pb (μg/dL), blood Se (μg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut, poultry, beef/mutton/pork, organ meat or fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[c] $p<0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10mL

5.3.7 Considerations of potential confounders

Vitamin D supplementation group, food sources of Cd, and tobacco exposure were considered as potential confounders of the association of maternal blood Cd concentrations with renal function. To understand the empirical relationships of these confounders with the outcomes of interest in this cohort, we used univariate linear regression models to estimate the associations of each variable with each renal outcome (serum creatinine at delivery and urine ACR at delivery).

5.3.7.1 Vitamin D

In separate unadjusted models, we analyzed the effect of vitamin D supplementation group on serum creatinine and urine ACR measured at delivery. Vitamin D supplementation was not found to have an effect on maternal serum creatinine concentrations (Table 22 Model A), nor on urine ACR (Table 22 Model B).

5.3.7.2 Food items

In separate unadjusted models, we examined the relationships of food sources that were found to be significantly associated with maternal blood Cd (coconut, poultry, beef/mutton/pork, organ meat, and large fish intake) and serum creatinine and urine ACR measured at delivery (Table 22 Models C-L). No foods were found to be associated with serum creatinine or urine ACR, except for beef/mutton/pork consumption, with ever consumption of beef/mutton/pork being associated with a 39.6% increase in ACR (CI: 0.84, 93.2; p=0.04).

5.3.7.3 Tobacco exposure

In separate unadjusted models, we assessed the relationship between tobacco exposure (quantified using urine cotinine) and serum creatinine and urine ACR measured at delivery.

Urine cotinine was not found to be associated with either renal biomarker (Table 22, Models M-N).

Table 22: Associations of potential confounders and renal outcomes.

		Serum creatinine[a]				*Urine ACR*[b]			
		N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A						**Model B**			
Vitamin D treatment group									
	Placebo	67	ref	ref	ref	88	ref	ref	ref
	4200 IU/week	70	7.01	-6.17, 22.0	0.31	99	-11.5	-41.0, 32.8	0.56
	16800 IU/week	62	4.75	-8.52, 19.9	0.50	83	-12.7	-42.8, 33.3	0.53
	28000 IU/week	112	0.81	-10.5, 13.5	0.89	174	-26.1	-48.5, 6.10	0.10
Model C						**Model D**			
Food sources									
Poultry meat consumption									
	Never	41	ref	ref	ref	54	ref	ref	ref
	Ever	270	-9.61	-20.5, 2.76	0.12	390	2.77	-31.2, 53.6	0.89
Model E						**Model F**			
Coconut meat consumption									
	Never	241	ref	ref	ref	341	ref	ref	ref
	Ever	70	-1.59	-11.3, 9.22	0.76	103	-8.90	-33.3, 24.3	0.56
Model G						**Model H**			
Beef/mutton/pork consumption									
	Never	66	ref	ref	ref	90	ref	ref	ref
	Ever	245	8.63	-2.30, 20.8	0.13	354	39.6	0.84, 93.2	0.04*
Model I						**Model J**			
Organ meat consumption									
	Never	179	ref	ref	ref	255	ref	ref	ref
	Ever	132	-0.66	-9.04, 8.49	0.88	189	-9.96	-31.0, 17.4	0.44
Model K						**Model L**			
Large fish consumption									
	Never	57	ref	ref	ref	92	ref	ref	ref
	Ever	254	11.7	-0.11, 24.9	0.05	352	11.5	-19.3, 54.2	0.51
Model M						**Model N**			
Tobacco exposure									
Urine cotinine (ng/10 mL)[d]		247	0.03	-0.05, 0.11	0.42	394	0.00	-0.27, 0.28	0.99

[a] Separate models were run for vitamin D treatment group (Model A), consumption of poultry (Model C), coconut meat (Model E), beef/mutton/pork (Model G), organ meat (Model I), large fish (Model K) and urine cotinine (ng/10 mL, Model M) and the association with serum creatinine

[b] Separate models were run for vitamin D treatment group (Model B), consumption of poultry (Model D), coconut meat (Model F), beef/mutton/pork (Model H), organ meat (Model J), large fish (Model L) and urine cotinine (ng/10 mL, Model N) and the association with ACR

[c] A $p<0.05$ is considered significant, significant results are marked with a *

[d] Variable is scaled to ng/10 mL

5.3.8 Considerations of iron, vitamin D supplementation, and Se as modifiers of the association of maternal blood Cd concentrations on renal function

5.3.8.1 Univariate associations of Fe, vitamin D supplementation, and Se with serum creatinine at delivery

There were no significant associations of serum ferritin, or Se with serum creatinine at delivery. Plasma CRP was significantly associated with increased serum creatinine in models adjusted for serum ferritin (Table 23).

Table 23: Univariate regression of serum ferritin, vitamin D supplementation group and whole blood Se with serum creatinine at delivery.

Variable[a]	n	% difference	(% difference) 95% CI	P[b]
Serum ferritin (ng/10 mL)[c]	305	0.14	-0.17, 0.45	0.38
Plasma CRP (mg/L)	305	0.15	0.05, 0.26	0.00*
Whole blood Se (μg/L)	311	0.08	-0.16, 0.33	0.50
Vitamin D supplementation				
Placebo	67	ref	ref	ref
4200 IU/week	70	7.00	-6.17, 22.0	0.31
16800 IU/week	62	4.75	-8.52, 19.9	0.50
28000 IU/week	112	0.81	-10.5, 13.5	0.89

[a] Separate models were run for each variable-serum creatinine relationship
[b] $p<0.05$ is considered significant; significant results are marked with a *
[c] Variable is scaled to ng/10 mL

5.3.8.2 The association of blood Cd concentrations with serum creatinine at delivery with Fe, vitamin D supplementation, and Se as interaction terms

Including the variables serum ferritin (adjusted for plasma CRP, Table 24, Model A), vitamin D supplementation (Table 25, Model A) and maternal blood Se (Table 26 Model A) as interaction terms with maternal blood Cd concentrations showed no effect modification.

5.3.8.3 The association of blood Cd concentrations with urine ACR at delivery with Fe, vitamin D supplementation and Se as interaction terms

Including the variables serum ferritin (adjusted for CRP, Table 24, Model B), vitamin D supplementation (Table 25, Model B) and maternal blood Se (Table 26 Model B) as interaction terms with maternal blood Cd concentrations showed no effect modification.

Table 24: Association of maternal blood Cd and renal biomarkers with serum ferritin (adjusted for plasma CRP) as an interaction term with maternal blood Cd.

	Serum creatinine[a]				*Urine ACR*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Blood Cd (µg/L)	240	0.35	-24.7, 33.8	0.98	324	11.2	-44.6, 123	0.77
Serum ferritin (ng/10 mL)[d]	240	-0.14	-3.83, 3.69	0.94	324	0.14	-7.40, 8.30	0.97
Interaction								
Blood Cd*Ferritin	240	-0.08	-4.68, 4.75	0.97	324	-0.41	-10.2, 10.4	0.94
Plasma CRP (mg/L)	240	0.18	0.06, 0.30	0.00*	324	0.53	0.16, 0.91	0.01*
Gravidity	240	1.15	-5.24, 7.98	0.73	324	25.6	3.70, 52.2	0.02*
Maternal BMI (kg/m^2)	240	0.28	-1.11, 1.69	0.69	324	0.04	-3.84, 4.07	0.98
Blood Pb (µg/dL)	240	1.26	-0.07, 2.61	0.06	324	3.34	-0.59, 7.43	0.10
Blood Se (µg/L)	240	0.04	-0.26, 0.34	0.81	324	0.31	-0.57, 1.20	0.49
Gestational age at birth (weeks)	240	2.64	-1.26, 6.70	0.19	324	-1.06	-10.3, 9.14	0.83
Maternal age (years)	240	0.27	-1.36, 1.93	0.74	324	-3.53	-8.02, 1.18	0.14
Urine cotinine (ng/10 mL)[d]	240	0.05	-0.03, 0.13	0.25	324	0.54	-0.10, 0.19	0.10
Maternal education								
No schooling or primary incomplete	58	ref	ref	ref	76	ref	ref	ref
Primary complete	31	11.4	-6.55, 32.7	0.23	44	16.3	-30.7, 95.0	0.57
Secondary incomplete	88	17.2	2.51, 34.0	0.02*	126	27.1	-14.0, 87.7	0.23
Secondary complete or higher	63	16.8	0.70, 35.2	0.04*	78	40.0	-11.2, 121	0.15
Asset index (quintiles)								
1 (lowest)	48	ref	ref	ref	64	ref	ref	ref
2	47	-12.2	-25.5, 3.43	0.12	64	-12.1	-42.2, 41.0	0.59
3	46	-10.1	-23.7, 5.96	0.20	66	-33.4	-58.3, 6.53	0.09
4	54	-8.29	-22.1, 7.92	0.30	65	-26.1	-54.9, 21.1	0.23
5	45	-3.93	-19.2, 14.3	0.65	65	-31.5	-58.1, 12.1	0.13
Presence of blood								
Negative	-	-	-	-	121	ref	ref	ref
Trace	-	-	-	-	37	126	36.2, 273	0.00*
Small	-	-	-	-	32	132	37.3, 263	0.00*
Moderate	-	-	-	-	57	205	97.4, 370	0.00*
Large	-	-	-	-	77	415	249, 664	0.00*
Coconut meat consumption								
Never	179	ref	ref	ref	238	ref	ref	ref
Ever	61	-2.98	-13.8, 9.15	0.61	86	9.03	-22.3, 52.9	0.62
Poultry meat consumption								
Never	32	ref	ref	ref	40	ref	ref	ref
Ever	208	-13.9	-26.2, 0.48	0.06	284	15.8	-26.2, 81.6	0.52
Beef/mutton/pork consumption								
Never	49	ref	ref	ref	63	ref	ref	ref
Ever	191	8.86	-4.22, 23.7	0.19	261	68.1	14.3, 147	0.01*
Organ meat consumption								
Never	134	ref	ref	ref	181	ref	ref	ref
Ever	106	6.33	-4.22, 18.0	0.25	143	-8.10	-32.7, 25.5	0.59
Large fish consumption								
Never	47	ref	ref	ref	62	ref	ref	ref
Ever	193	7.32	-6.10, 22.6	0.30	262	21.2	-18.2, 79.7	0.34
Vitamin D supplementation group								
Placebo	47	ref	ref	ref	62	ref	ref	ref
4200 IU/week	49	12.4	-4.28, 32.0	0.15	69	-27.9	-54.5, 14.3	0.16
16800 IU/week	49	9.56	-6.87, 28.9	0.27	61	-33.0	-58.7, 8.57	0.10
28000 IU/week	95	6.67	-7.20, 22.6	0.36	132	-34.0	-55.9, 12.43	0.04*

[a] A separate multivariable model (Model A) was run for blood Cd and serum creatinine with serum ferritin (adjusted for CRP) as an effect modifier; multivariable model adjusted for: serum ferritin (ng/10 mL), plasma CRP (mg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), blood Se (µg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[b] A separate multivariable model (Model B) was run for blood Cd and ACR with serum ferritin (adjusted for CRP) as an effect modifier; multivariable model adjusted for: serum ferritin (ng/10 mL), plasma CRP (mg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), blood Se (µg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[c] $p<0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10 mL

Table 25: Association of maternal blood Cd and renal biomarkers with vitamin D supplementation group as an interaction term with maternal blood Cd.

	Serum creatinine[a]				*Urine ACR*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Blood Cd (µg/L)	242	-4.31	-38.6, 49.1	0.85	351	50.8	-57.7, 437	0.53
Vitamin D supplementation group								
Placebo	47	ref	ref	ref	63	ref	ref	ref
4200 IU/week	50	31.2	-13.4, 99.0	0.20	74	-13.6	-73.1, 178	0.81
16800 IU/week	50	-1.44	-35.6, 50.9	0.94	67	-32.9	-79.4, 119	0.51
28000 IU/week	95	0.89	-31.5, 48.6	0.96	147	-10.6	-68.8, 156	0.83
Interaction								
Blood Cd*Placebo	47	ref	ref	ref	63	ref	ref	ref
Blood Cd*4200 IU/week	50	-16.4	-50.3, 40.6	0.50[d]	74	-22.0	-82.6, 249[e]	0.74
Blood Cd*16800 IU/week	50	14.2	-33.5, 96.1	0.63[d]	67	-10.4	-80.1, 304[e]	0.89
Blood Cd*28000 IU/week	95	8.93	-34.1, 80.0	0.74[d]	147	-39.5	-84.9, 143[e]	0.48
Gravidity	242	1.11	-5.43, 8.10	0.75	351	24.9	3.58, 50.7	0.02*
Maternal BMI (kg/m^2)	242	0.40	-0.99, 1.82	0.57	351	-1.19	-4.88, 2.64	0.54
Blood Pb (µg/dL)	242	1.17	-0.16, 2.53	0.09	351	2.08	-1.58, 5.86	0.27
Blood Se (µg/L)	242	-0.00	-0.30, 0.30	0.98	351	0.37	-0.45, 1.19	0.38
Gestational age at birth (weeks)	242	2.78	-1.16, 6.86	0.17	351	-0.79	-9.83, 9.15	0.87
Maternal age (years)	242	0.83	-0.81, 2.50	0.32	351	-2.13	-6.50, 2.44	0.35
Urine cotinine (ng/10 mL)[f]	242	0.04	-0.04, 0.12	0.31	351	-0.14	-0.67, 0.40	0.62
Maternal education								
No schooling or primary incomplete	58	ref	ref	ref	81	ref	ref	ref
Primary complete	32	13.0	-5.16, 34.7	0.17	49	0.24	-38.6, 63.7	0.99
Secondary incomplete	88	17.8	2.85, 34.9	0.02*	139	27.5	-12.6, 85.8	0.21
Secondary complete or higher	64	15.6	-0.30, 34.2	0.05	82	31.1	-15.6, 103	0.23
Asset index (quintiles)								
1 (lowest)	48	ref	ref	ref	69	ref	ref	ref
2	48	-7.84	-21.7, 8.44	0.32	73	-17.7	-47.6, 29.3	0.40
3	46	-9.81	-23.5, 6.39	0.22	71	-39.5	-61.7, -4.51	0.03*
4	54	-8.37	-22.3, 8.05	0.30	69	-31.2	-57.5, 11.3	0.13
5	46	-3.08	-18.6, 15.4	0.72	69	-34.4	-59.5, 6.45	0.09
Presence of blood								
Negative	-	-	-	-	129	ref	ref	ref
Trace	-	-	-	-	39	128	39.4, 273	0.00*
Small	-	-	-	-	38	127	38.0, 272	0.00*
Moderate	-	-	-	-	61	222	110, 393	0.00*
Large	-	-	-	-	84	417	253, 659	0.00*
Coconut meat consumption								
Never	181	ref	ref	ref	260	ref	ref	ref
Ever	61	-3.89	-14.6, 8.18	0.51	91	9.47	-21.2, 52.0	0.59
Poultry meat consumption								
Never	33	ref	ref	ref	43	ref	ref	ref
Ever	209	-13.2	-25.7, 1.35	0.07	308	7.12	-31.0, 66.2	0.74
Beef/mutton/pork meat consumption								
Never	49	ref	ref	ref	70	ref	ref	ref
Ever	193	11.2	-2.34, 26.6	0.11	281	69.9	17.5, 146	0.00*
Organ meat consumption								
Never	134	ref	ref	ref	197	ref	ref	ref
Ever	108	6.00	-4.53, 17.7	0.27	154	-6.30	-30.5, 26.3	0.67
Large fish consumption								
Never	47	ref	ref	ref	70	ref	ref	ref
Ever	195	7.08	-6.56, 22.7	0.32	281	11.8	-22.8, 62.0	0.55

[a] A separate multivariable model (Model A) was run for blood Cd and serum creatinine with vitamin D supplementation as an effect modifier; multivariable model adjusted for: gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), blood Se (µg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[b] A separate multivariable model (Model B) was run for blood Cd and ACR with vitamin D supplementation as an effect modifier; multivariable model adjusted for: gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), blood Se (µg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[c] p<0.05 is considered significant, significant results are marked with a *

[d] Wald test for interaction term is non-significant (p=0.43)

[e] Wald test for interaction term is non-significant (p=0.83)

[f] Variable scaled to ng/10 mL

Table 26: Association of maternal blood Cd and renal biomarkers with Se as an interaction term with maternal blood Cd.

	Serum creatinine[a]				*Urine ACR*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Blood Cd (µg/L)	242	45.2	-48.0, 306	0.48	351	-84.4	-99.0, 131	0.18
Blood Se (µg/L)	242	0.19	-0.44, 0.83	0.56	351	-0.72	-2.36, 0.94	0.39
Interaction								
Blood Cd*Se	242	-0.30	-1.07, 0.47	0.44	351	1.51	-0.53, 3.60	0.15
Gravidity	242	0.85	-5.62, 7.75	0.80	351	25.3	4.06, 50.9	0.02*
Maternal BMI (kg/m^2)	242	0.44	-0.95, 1.84	0.54	351	-1.03	-4.67, 2.75	0.59
Blood Pb (µg/dL)	242	1.10	-0.24, 2.46	0.11	351	2.06	-1.56, 5.81	0.27
Gestational age at birth (weeks)	242	2.96	-0.97, 7.04	0.14	351	-1.12	-10.1, 8.74	0.82
Maternal age (years)	242	0.68	-0.94, 2.33	0.41	351	-2.03	-6.36, 2.51	0.37
Urine cotinine (ng/mL)[d]	242	0.04	-0.04, 0.12	0.35	351	-0.11	-0.64, 0.43	0.70
Maternal education								
No schooling or primary incomplete	81	ref	ref	ref	81	ref	ref	ref
Primary complete	41	11.9	-6.04, 33.2	0.21	49	-1.50	-39.5, 60.3	0.95
Secondary incomplete	106	18.3	3.28, 35.6	0.02*	139	23.5	-15.1, 79.6	0.27
Secondary complete or higher	83	15.4	-0.49, 33.9	0.06	82	27.9	-17.4, 98.1	0.27
Asset index (quintiles)								
1 (lowest)	64	ref	ref	ref	69	ref	ref	ref
2	60	-7.31	-21.2, 9.10	0.36	73	-19.4	-48.6, 26.4	0.35
3	61	-9.29	-23.1, 6.96	0.24	71	-38.0	-60.6, -2.47	0.04*
4	69	-7.40	-21.4, 9.09	0.36	69	-30.2	-56.7, 12.6	0.14
5	56	-2.20	-17.8, 16.4	0.80	69	-33.2	-58.6, 7.85	0.10
Presence of blood								
Negative	-	-	-	-	129	ref	ref	ref
Trace	-	-	-	-	39	126	38.7, 268	0.00*
Small	-	-	-	-	38	129	39.7, 274	0.00*
Moderate	-	-	-	-	61	221	111, 390	0.00*
Large	-	-	-	-	84	417	254, 655	0.00*
Coconut meat consumption								
Never	241	ref	ref	ref	260	ref	ref	ref
Ever	70	-3.55	-14.3, 8.54	0.55	91	9.52	-21.0, 51.8	0.58
Poultry meat consumption								
Never	41	ref	ref	ref	43	ref	ref	ref
Ever	270	-13.4	-25.7, 0.97	0.07	308	9.04	-29.4, 68.5	0.42
Beef/mutton/pork consumption								
Never	49	ref	ref	ref	70	ref	ref	ref
Ever	193	11.3	-2.26, 26.8	0.11	281	63.1	13.1, 135	0.01*
Organ meat consumption								
Never	134	ref	ref	ref	197	ref	ref	ref
Ever	108	5.00	-5.54, 16.7	0.36	154	-3.78	-28.6, 29.6	0.80
Large fish intake								
Never	47	ref	ref	ref	70	ref	ref	ref
Ever	195	7.43	-6.13, 23.0	0.30	281	16.3	-19.8, 68.5	0.42
Vitamin D supplementation group								
Placebo	67	ref	ref	ref	63	ref	ref	ref
4200 IU/week	70	14.4	-2.59, 34.4	0.10	74	-28.2	-54.3, 12.8	0.15
16800 IU/week	62	8.45	-8.01, 27.9	0.33	67	-35.8	-60.0, 3.16	0.07
28000 IU/week	112	6.85	-7.16, 23.0	0.35	147	-37.2	-57.7, -6.80	0.02*

[a] A separate multivariable model (Model A) was run for blood Cd and serum creatinine with Se as an effect modifier; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[b] A separate multivariable model (Model B) was run for blood Cd and ACR with Se as an effect modifier; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut meat consumption (never/ever), poultry meat consumption (never/ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever) vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week)

[c] $p<0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10 mL

5.3.9 Sensitivity analyses

5.3.9.1 Association of maternal blood Cd and eGFR at delivery

Multivariate linear regression models were run with delivery eGFR estimated using the CKD-EPI equation [183]. The equation used for the calculation is as follows:

CKD-EPI (mL/min/1.73 m^2): 141x min $(S_{Cr}/\kappa,1)^{\alpha}$ x max$(S_{Cr}/\kappa,1)^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if Black]

Where S_{Cr}= serum creatinine (mg/dL), κ= 0.7 (if female) or 0.9 (if male), min= minimum of S_{Cr}/κ or 1, max: maximum of S_{Cr}/κ or 1 & age=years. No differences were found between associations in the Cd-serum creatinine model and the Cd-eGFR model (Appendix, Table 3).

5.3.9.2 Including month of enrolment and month of delivery as covariates in Aim 2 models

Adjusting for month of enrolment and month of delivery in Aim 2 models did not significantly change model inferences (Appendix Table 12a and Appendix Table 12b).

Chapter 6 Discussion

High levels of cadmium (Cd) exposure in low-middle income countries, such as Bangladesh, are an ongoing public health problem [70, 159]. This study aimed to assess if vitamin D supplementation affected the absorption of Cd, leading to higher blood Cd concentrations, and potentially influencing the occurrence of renal dysfunction in a vulnerable pregnant population. The MDIG trial provided an ideal setting to test this hypothesis, as this study leveraged the randomized, dose ranging, placebo-controlled design of the MDIG trial to assess the causal effect of vitamin D supplementation on maternal blood Cd concentrations at delivery.

Contrary to our hypothesis, there was no increase in maternal blood Cd concentrations following supplementation with increasing amounts of vitamin D, as compared to placebo. Surprisingly, vitamin D supplementation was associated with an increased risk of measuring detectable concentrations of Cd in cord blood, a finding which has not been previously reported. In this cohort, maternal blood Cd concentrations were not found to be associated with serum creatinine concentrations or urine ACR concentrations. In light of these results, it does not appear that vitamin D supplementation had an effect on maternal delivery blood Cd concentrations of our study population, nor were increasing maternal blood Cd concentrations associated with renal dysfunction.

6.1 Aim 1

6.1.1 No effect of vitamin D on maternal blood Cd concentrations

There was no evidence that increasing vitamin D supplementation had an effect on blood Cd concentrations at delivery, contrary to our hypothesis. This hypothesis has never before been established in a human trial and has only been tested in an animal model using chicks. The study in which the effect was established was published in 1961, with no further published research on

the topic [15]. There were various reasons why we were unable to make strong inferences from our data, such as the discrepancies occurring between Cd concentrations tested in two separate laboratories. In addition, we may have not observed an effect as the women in the MDIG study were supplemented with Fe, Ca and folic acid, and thus may have had adequate intakes of minerals. This could have prevented the increased gastrointestinal uptake of Cd which commonly occurs in malnourished individuals [49]. Moreover, women were supplemented with vitamin D from 17-24 weeks gestation up until delivery, when blood was drawn for Cd measurements. This may not have been a long enough supplementation period to fully observe the effect of vitamin D on upregulating Cd absorption. In the MDIG trial, a dose-dependent effect of vitamin D supplementation on concentrations of maternal delivery and cord 25(OH)D, and maternal intact PTH concentrations were observed. Vitamin D supplementation also led to a modest increase in maternal delivery serum Ca concentrations [162]. However, the biodynamics between vitamin D and Cd are not well understood and could take a long duration of time to take effect.

It would have been advantageous to have measured participants blood Cd concentrations at baseline, in order to comprehend whether a difference in absorption was occurring during the period of supplementation. As this study question was very novel, it is difficult to understand entirely whether the hypothesis was not supported due to experimental factors, or whether in fact there was no true effect of vitamin D supplementation on Cd absorption. The maternal Cd concentrations in our study population (median: 0.68 μg/L) are somewhat higher than the blood concentrations previously observed in a sample of pregnant rural Bangladeshi women (median: 0.5 μg/L) [70]. However, pregnant women in other regions, such as former Yugoslavia, Saudi Arabia, and China have been found to have substantially higher Cd concentrations than in Bangladesh [100], with healthy pregnant Chinese women having geometric mean delivery blood

Cd concentrations of 2.26 μg/L [184]. As Cd is a non-essential metal, it is evident that Cd exposure is prevalent in many regions in the world.

We did not find that serum ferritin, daily Ca intake, or maternal blood selenium (Se) concentrations were modifiers on the effect of vitamin D supplementation on blood Cd concentrations at delivery. The increased gastrointestinal absorption of Cd in the presence of low iron (Fe) stores has been described extensively in observational research [24, 75]. However, in our study, Fe status (quantified using serum ferritin) was not strongly associated with maternal blood Cd concentrations in univariate models. We may not have observed a relationship between ferritin and Cd due to women in the MDIG trial being supplemented with Fe from enrolment up until delivery. The increased Fe intake may have decreased the amount of Cd being absorbed during this period. As blood Cd is a biomarker of recent Cd exposure (with a half-life of 2-3 months) [24], it may have captured this time period of decreased Cd absorption due to increased Fe stores, and not been a reflection of lifetime Cd body burden. However, as 10% of our participants had serum ferritin concentrations at delivery of less than 15 ng/mL, which is the World Health Organization's cut-off for Fe deficiency in adults, we can hypothesize that at baseline, our study population was quite Fe deficient [181]. It appears that even after being supplemented with a considerably high amount of Fe (66 mg/day), some women still remained Fe deficient, pointing to potentially severe Fe deficiency at baseline, or poor Fe absorption. It would have been beneficial to have collected serum ferritin data at enrolment, in order to assess the relationship of the nutrient and metal before supplementation. In addition, criteria for participants in the MDIG trial excluded women with severe anemia (hemoglobin concentrations <70 g/L), which could have caused study participants to be less likely to be Fe deficient than the general Bangladeshi population [162]. If this study included Bangladeshi women who were not

prenatally supplemented with Fe and were not excluded due to being severely anemic, we may have seen a stronger relationship between vitamin D and Cd.

It has been shown that Cd may displace the uptake of calcium (Ca) by utilizing Ca transporters, with low dietary intake of Ca being associated with increased Cd concentrations [40]. In our study, Ca intake was quantified using a relative Ca intake measure generated from the MDIG food frequency questionnaire and supplemented with Ca content and serving size information from the Bangladeshi Food Guide, Canadian Nutrient File and Indian Food Guide. Portion sizes and Ca content of foods was deemed to be constant across women in the trial. The relative Ca intake generated thus is not an exact measure and may not accurately capture individual Ca intake. In our trial, blood Cd was not found to be associated with relative daily Ca intake. Women were also supplemented with Ca during the trial period (500 mg/day), and while adherence to supplementation data was not collected during the trial, an audit showed that most participants (85%) reported a high adherence of over 85%. While the measure of relative Ca intake showed many women did not ingest enough Ca from food sources, the additional Ca supplement may have increased gastrointestinal Ca absorption to sufficient levels. Vitamin D's impact on Cd concentrations most likely occurs at the site of Ca absorption, via transporters such as CaT1. As women received Ca supplementation during the trial, Cd may not have been preferentially absorbed by Ca channels at the same rate as in a Ca deficient state. However, Cd may have still been absorbed through other nutrient transporters (such as zinc (Zn) [52]), which could explain why Cd concentrations were still quite elevated in the population.

Cd and Se have proposed similar mechanisms of gastrointestinal absorption. Higher Se status may be protective against Cd accumulation by acting as an antagonist and by alleviating oxidative stress [185]. However, we did not find an association between the two elements in our

study. Blood Se concentrations showed most of the study participants were within the range of Se sufficiency (>100μg/L) [179]. However, serum Se and selenoprotein-1 (SEPP1) concentrations of our study participants point to insufficient bioavailable Se (unpublished). We may have not observed a clear relationship between Cd and Se as participants possibly did not have sufficient Se concentrations to displace Cd absorption.

We can speculate that while the supplementation of participants with Fe and Ca during the trial period was a limitation to our study, as it may have attenuated our results, it also may have inadvertently decreased participants Cd absorption during the period of supplementation.

6.1.2 The effect of vitamin D on detectable venous cord blood Cd

We were surprised to see that vitamin D supplementation had an effect on the detectability of cord blood Cd, despite the absence of a significant effect on maternal concentrations. Although many of the pregnant women had elevated blood Cd concentrations, a substantial proportion had venous cord blood Cd concentrations below the LOD. Therefore, is was evident that the placenta is serving as a partial barrier to transfer of Cd from the mother to the fetus. This is in line with previous literature which has shown that the transfer of Cd from maternal stores to the fetus is inefficient [39, 184]. The present findings further suggest that transplacental Cd transfer may be directly or indirectly regulated by vitamin D. We can speculate that it may be due to a transplacental Ca transporter, which Cd could use as a site of uptake. There are a variety of potential Cd transporters on the placenta, such as T type voltage-dependent Ca channels (CaV3 and CaV1.2) [33]. A study conducted on pregnant women in Thailand also found upregulated expressions of placental CACNA1C mRNA in the high Cd exposed group, as compared to low Cd exposed women. The high Cd exposed women were found to have higher placental Cd and metallothionein (MT) expression, and lower placental Ca in comparison to the low Cd exposed

group [45]. It has been shown that Cd binding to MT in the placenta could lead to decreased transfer of Cd to the fetal circulation [131]. In the last trimester of pregnancy, transplacental Ca movement increases substantially [186]. It is possible that placental specific Ca transporters were preferentially binding Cd as opposed to Ca. While maternal Ca stores may have been approaching sufficiency due to participants being Ca supplemented during pregnancy, it is conceivable that Ca transporters in the placenta, such as CACNA1C, were not completely saturated with Ca. Thus, Cd could have competed for CACNA1C binding sites at the placenta and been transferred to the fetal circulation in the presence of vitamin D supplementation. Currently, little is known about the placenta and reproduction toxicology, namely the organs ability to transport, and store toxic substances [187]. As this is a novel finding, it would be beneficial to see this result replicated in another vitamin D supplementation trial of a Cd exposed population. We did not observe a dose-response relationship with increasing vitamin D supplementation, nor were all supplementation groups founds to be statistically significant. Therefore, we can conclude that while this was an interesting finding, more work needs to be done to confirm it. As in the case with maternal whole blood, it does not appear that Fe, Se or Ca were associated with cord blood Cd.

We found that the uneven distribution of participants in month of enrolment, and consequently month of delivery could have confounded the effect of vitamin D supplementation on the detectability of cord blood Cd. It would be pertinent to further explore whether month of delivery could have an impact on the detectability of cord blood Cd, perhaps through increased Cd in the environment due to seasonality.

6.1.3 Discrepancies between the CDC and NBL Cd datasets

We tried multiple approaches to explain the discordant results but ultimately, we were not able to identify a clear reason. As previously mentioned, we decided that the CDC dataset gave us the most robust and reliable results, which is the reason why we chose this dataset for our analyses. The CDC dataset had a median blood Cd concentration (0.68 μg/L) that was most similar to previously observed Cd concentrations in pregnant women in Bangladesh (0.5 μg/L at 14 weeks gestation) [70], whereas the NBL dataset had a considerably higher mean of 2.0 μg/L. However, we cannot be certain that the CDC results are the true absolute Cd concentrations in maternal blood, as we did notice a further decline in concentrations when re-testing samples at the Nutritional Biochemistry Laboratory in Dhaka. As Cd is known to be freeze-thaw stable [166], we considered that the decrease in concentrations may have been due to a potential unidentified inhibitor or Cd chelator in the blood.

6.2 Aim 2

6.2.1 Inability to proceed with modelling the association of maternal blood Cd on B2MG concentrations at delivery

Unfortunately, we were unable to use our β_2-microglobulin (B2MG) dataset in our analyses, due to unexplainably high and potentially inaccurate urine B2MG concentrations, and issues with missing data. In addition, we were not able to find an appropriate method of analysis of the dataset. Urine B2MG has infrequently been measured during pregnancy, so our results could have added vital information to the literature on concentrations during pregnancy. It appears that B2MG concentrations are significantly higher in pregnant women as compared to non-pregnant women [126], and preliminary results of urine B2MG showed similar elevated results in our pregnant cohort. In retrospect, measuring pH and presence of blood in urine immediately after

collection and excluding samples with pH<6 and with blood present could have refined our samples to be as accurate as possible for urine B2MG collection. While we observed weak relationships between presence of blood in urine and B2MG concentrations, it is possible that concentrations were somehow affected, especially considering the instability of the biomarker in urine. As urine creatinine used to standardize the measurements was also measured in delivery samples, it is possible that presence of blood in urine also had an effect on these results.

6.2.2 No association of maternal blood Cd concentrations with markers of renal function

We did not find that blood Cd was associated with serum creatinine at delivery in a univariate model, nor in a multivariable model. A study found that increased Cd exposure was associated with higher serum creatinine (or reduced eGRF) [188], but the findings have not always been consistent. A study on Pb and Cd exposed workers found urine Cd was associated with decreased serum creatinine and higher eGFR. Some proposed mechanisms for these findings are Cd-induced hyperfiltration, or Cd modulating proximal tubule creatinine secretion [189]. Almost all studies model the Cd/kidney relationship using urine Cd excretion as a marker of Cd body burden, while we utilized blood Cd, which is a marker of more recent Cd exposure. Cd exposure levels in our study population may not have been elevated enough to cause renal dysfunction; animal studies have found Cd concentrations above 200 μg/g in the renal cortex led to the development of proteinuria in rats [28].

During pregnancy, serum creatinine decreases in the second trimester, and then rises again approaching term [190]. A reference range for normal serum creatinine during pregnancy is not well established, but a recent systematic review concluded that a serum creatinine of >77 μmol/L could point to potential kidney dysfunction [191]. In our study cohort, 25% of women had

serum creatinine values over this threshold. It's important to note that serum creatinine can be affected by a varying factors, such as hydration status, exercise, and protein intake at the time of measurement [192]. However, if we are to take our measured serum creatinine values as the true reflection of participants kidney function, then it can be postulated that ¼ of our study cohort had signs of renal dysfunction. A recently published cross-sectional study on urban populations in Bangladesh found that 31% of female participants had renal insufficiency (in this study, defined as >97 μmol/L). In our study population, only 9% of women have serum creatinine concentrations over 97 μmol/L. As well, participants had a mean eGFR of 114 mL/min/1.73 m^2, while in the cross-sectional Bangladeshi study, pre-menopausal women had a mean eGFR of 103 mL/min/1.73 m^2. The investigators of the study postulated that the use of toxic metal contaminated herbal medications, over-usage of drugs, and consumption of food products adulterated with chemicals and heavy metals may have led to renal damage in this population [193]. As we saw a positive relationship between maternal delivery blood Pb and serum creatinine concentrations in unadjusted models, it is possible that Pb exposure is a more likely cause of renal dysfunction. It is well known that arsenic (As) exposure is prevalent in Bangladesh and could also be a potential cause of renal dysfunction in the population [194, 195]. However, we did not measure As concentrations in our study cohort. Both our findings and the study on urban Bangladeshi populations point to renal insufficiency being a prevalent problem in Bangladeshi women. Whether this dysfunction is associated with Cd exposure, we cannot be certain. Our data does not point to Cd being a likely reason behind the potentially high prevalence of renal dysfunction in this cohort.

While we were unable to find a validated eGFR equation for pregnant women, we did adjust for age and BMI in our multivariable models, which should give us equivalent estimates to an eGFR

calculation. When we calculated an estimated eGFR using the CKD-EPI equation, we found no differences between our serum creatinine and eGFR model in associations with blood Cd (Appendix Table 4). This was not surprising as serum creatinine and eGFR data were highly correlated (r=-0.96).

We did not see an association of blood Cd and urine ACR, in both univariate and multivariate models. Albuminuria (albumin/creatinine concentrations > 30 mg/g), occurs due to an increase in albumin excretion via the glomeruli due to endothelial damage, or a decrease in the reabsorption of albumin in proximal tubules [196]. Observational studies have found associations between Cd exposure and albuminuria, such as a study on the general American population in which blood Cd was found to be an independent risk factor for albuminuria, as well as reduced eGFR [197]. Another study conducted in the United States found a correlation between urine albumin and urine Cd concentrations, but the relationship did not remain significant after adjusting for urine creatinine and additional confounders [198]. As with the aforementioned study, most research on the Cd/renal dysfunction relationship has been conducted using urine Cd measures. It's possible that maternal blood Cd concentrations did not reflect the overall body burden of Cd, especially if Cd was being absorbed at a decreased amount during the study period due to Ca and iron supplementation.

We found a distinct positive relationship between presence of blood in urine and albumin concentrations. One prior study found a strong relationship between blood in urine and increased urine ACR in patients with Type 2 diabetes. The study found that hematuria (presence of blood in urine) did not contribute to the level of albuminuria, and was probably not increasing the amount of albumin in urine [83]. While we first considered that albumin in blood and serum may be contributing to the increased urine albumin concentrations, it is also possible that albuminuria is

associated with the occurrence of true hematuria, in which case, the blood found in urine may have been of renal origin. To account for the association of blood in urine and increased urine albumin concentrations, we adjusted all our models with urine ACR concentrations as an outcome for level of blood in urine. In our study population, a significant number of women had urine ACR levels >30 μg/mg, which signifies microalbuminuria (37% of our study population with urine albumin results). A smaller portion of women had values >300 μg/mg (2% of the study population). From these results, we can postulate that a significant number of the women in our study are displaying signs of impaired glomerular filtration capacity of the kidneys. A study on urine albumin as a predictor of preeclampsia in pregnant Bangladeshi women found some indication of elevated urine albumin concentrations in women with preeclampsia [199]. Elevated urine albumin concentrations may be prevalent in pregnant Bangladeshi women. It is possible that if urine specimens in our study did not have blood in them, we could have seen a stronger association between blood Cd and urine ACR.

6.2.3 Cadmium exposure from food and tobacco intake

We found the intake of a few food items to be associated with blood Cd concentrations at delivery. While we were expecting to see strong associations with rice intake, the low variability of intake in our study population made it difficult to decipher this relationship. All participants in our study consumed rice at least once a day, with 99.8% reporting consuming it at least twice daily. As we know rice is a source of Cd in Bangladesh, it is possible that the Cd content of rice differed between brands purchased by our study participants, or areas in which the rice is grown. Alternatively, individual factors may have altered the Cd absorption from rice, such as consumption of Ca or Se rich foods. The foods that were associated with delivery Cd concentrations were poultry, coconut, beef/mutton/pork, and organ meat consumption, as well as

large fish consumption. Ever eating these foods was associated with decreased blood Cd concentrations, as compared to never eating these food products. We believe that consumption of these foods may have displace the consumption of other Cd containing food products, such as additional servings of rice. As a higher proportion of coconut, large fish, and beef/mutton/pork consumers appeared to belong to the higher asset index quintiles, this suggests that perhaps these individuals were able to afford to purchase and consume higher quality protein products. This is also corroborated by the finding that study participants that belonged to the higher asset index quintiles had lower cadmium concentrations, as compared to individuals in the lowest asset index quintile (results not shown). We found a positive association between ever consumption of beef/mutton/pork products and urine ACR concentrations, which may be due to a higher protein intake impacting glomerular hyperfiltration. Our associations of food intake and blood Cd concentrations may be limited by our analysis, as food intakes were dichotomized into never/ever consumed categories. It is possible if we utilized a different methodology, such as Spearman rank correlation, we would have observed different associations.

We did not find that delivery urine cotinine, a biomarker of tobacco exposure, and blood Cd were strongly associated with each other. We postulate that tobacco consumption is not a prevalent source of Cd exposure in our cohort, with women mostly absorbing Cd from food products or additional unmeasured sources.

6.3 Study strengths and limitations

6.3.1 Study strengths

A major strength of our study is that it leveraged the randomized, dose-ranging, placebo-controlled design of the MDIG trial, which allowed us to make causal interpretations on the effect of vitamin D supplementation on maternal delivery blood Cd concentrations. The study

location was an appropriate setting for our study, as Cd exposure is known to be high in Bangladesh, especially among reproductive aged women. Due to study participants being vitamin D deficient at baseline, we observed a clear dose-response effect of supplementation on maternal serum 25(OH)D concentrations at delivery which allowed us to discern the effect of vitamin D supplementation on maternal blood Cd.

6.3.2 Study limitations

As with all research, there were various limitations to our study. The potential insufficient variation in Fe and Ca status due to supplementation may have led to a decrease in Cd concentrations during pregnancy, and thus minimized the effect of vitamin D supplementation on blood Cd concentrations. We were also not able to control for all sources of Cd exposure, such as sikor consumption during pregnancy, potentially because this practice is more prevalent in rural communities, or because women were ashamed to admit to it. It may have been valuable to measure blood Cd and serum ferritin at baseline, as well as urine Cd in order to gain a better understanding of the metal's association with renal markers. It would have also been beneficial to look at the relationship of Cd and Zn concentrations, however we were not able to obtain Zn results due to contamination of samples from specimen vials. Unfortunately, due to challenges with the laboratory data, specifically urine biomarkers and Cd, we were unable to make strong inferences with our dataset.

While we did not see a relationship between blood in urine and B2MG, creatinine and cotinine, it is impossible to rule out some potential modulation of concentrations in samples with blood present. Thus, there is some doubt as to the validity of all of our urine biomarkers. Due to time constraints, we were unable to look at the association of blood Cd concentrations and maternal blood pressure data. As a significant amount of research on Cd exposure in pregnancy focuses on

hypertension, preeclampsia and blood pressure as an outcome of exposure, this could have added significant findings to our results. The significant differences between month of enrolment between the MDIG trial and our sub-study may have led to the occurrence of selection bias. Cd concentrations in the December-February months were found to be significantly different from other time periods, however this difference may have mostly been due to the low participant numbers in the December-February period. Month of enrolment was not found to differ significantly between vitamin D supplementation groups and adjusting for the covariate in separate models did not change inferences of the effect of vitamin D supplementation of maternal blood Cd, however it attenuated the effect of vitamin D on detectability of cord blood Cd. Our food frequency questionnaire analysis (dichotomizing intake into never/ever consumption) may have led to a potentially inaccurate association of particular food items and blood Cd concentrations. Lastly, our results may not be generalizable to populations outside of Bangladesh, nor to pregnant women who did not receive vitamin D, Fe, Ca, and folic acid supplementation during pregnancy.

6.4 Conclusions

Contrary to our hypothesis, we did not see an effect of vitamin D supplementation on maternal blood Cd concentrations. We also did not observe an association between maternal blood Cd concentrations and markers of renal function, namely delivery urine ACR and serum creatinine. However, in secondary analyses we did see an effect of vitamin D supplementation on the detectability of Cd in cord blood. This finding suggests vitamin D plays a potentially harmful role in the preferential uptake of Cd by the fetus from maternal stores. This was the first study to investigate the effect of vitamin D on Cd concentrations, and the mitigatory relationship of Se, Fe and Ca on the effect of vitamin D on Cd in a human trial setting. This study adds to the

limited body of research on the effect of vitamin D on the uptake of divalent cations, such as Cd. While our results do not indicate any detrimental effects of vitamin D supplementation on maternal blood Cd concentrations, it is possible that the effect was attenuated due to prenatal nutrient supplementation in an otherwise malnourished population. Our results are relevant to recommendations of vitamin D supplementation in pregnancy in Cd exposed individuals, as it may lead to increased Cd exposure in infants. However, this effect needs to be further researched before any changes to recommendations are made. To the knowledge of the authors, it is also one of a very limited number of studies that have looked at the association of Cd and renal consequences in pregnant women. While Bangladeshi Cd concentrations appear to be elevated as compared to Cd concentrations in high income countries, it does not appear that Cd exposure is associated with the adverse renal function of pregnant women. Our research did find a high prevalence of renal dysfunction in our study population, indicating the need to further investigate the factors which could cause high prevalence of renal insufficiency in this population.

6.5 Future directions

It would be beneficial to see the results of our study replicated in additional vitamin D supplementation trials during pregnancy, specifically ones conducted in low middle-income settings with known Cd exposure. This would provide us with substantiated evidence that vitamin D supplementation does not in fact increase maternal Cd absorption during pregnancy, as we have seen in our study. In addition, it is pertinent to further assess the effect of vitamin D on cord blood Cd concentrations. It would be valuable to test this effect in animal studies in order to understand the mechanism underlying this effect. While we did not find that Cd had an association with renal markers in our study, it would be beneficial to analyze this relationship further using additional renal outcomes, such as blood pressure data and urine NAG, an enzyme

often measured in studies on Cd and renal function [200]. As well, further study of the underlying factors behind high rates of renal insufficiency in females in Bangladesh would be advantageous for future public health policy making.

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Chapter 7 Appendices

Appendix Table 1: Associations of food items from the FFQ with maternal blood Cd at delivery.

Food item	Never consumed n (%)	Ever consumed n (%)	% difference[a]	(% difference) 95% CI	P[b]
Milk (goat and cow, whole and skim)	156 (25)	463 (75)	-3.41	-11.4, 5.25	0.43
Milk (condensed and powdered)	462 (75)	157 (25)	-4.08	-12.0, 4.50	0.34
Yogurt and curd	354 (57)	265 (43)	-4.85	-11.8, 2.59	0.29
Ice cream	274 (44)	345 (56)	-3.38	-10.4, 4.16	0.37
Cheese (cottage)	575 (93)	44 (7)	-5.35	-18.1, 9.44	0.46
Rice, white, boiled[c]	0 (0)	619 (100)	-	-	-
Rice, popped and puffed	139 (22)	480 (78)	-4.27	-12.5, 4.68	0.34
Rice, flaked	536 (87)	83 (13)	9.01	-2.28, 21.6	0.12
Barley	595 (96)	24 (4)	-8.74	-24.8, 10.7	0.35
Ruti	102 (16)	517 (84)	-4.56	-13.7, 5.54	0.36
Bread, white	285 (46)	334 (54)	-3.33	-10.3, 4.18	0.37
Biscuits, not fortified	325 (53)	294 (47)	4.71	-2.82, 12.8	0.23
Biscuits, Ca fortified	350 (57)	269 (43)	-4.05	-11.0, 3.44	0.28
Peanuts (groundnuts)	395 (64)	224 (36)	-2.60	-9.88, 5.27	0.51
Other nuts	568 (92)	51 (8)	-11.4	-22.6, 1.45	0.08*
Pumpkin and squash seeds	485 (78)	134 (22)	-6.52	-14.6, 2.33	0.14
Sesame seeds	596 (96)	23 (4)	-8.97	-25.3, 10.9	0.35
Chickpeas (bengal gram)	337 (55)	281 (45)	1.51	-5.83, 9.42	0.70
Lentils (red and yellow)	29 (5)	590 (95)	-4.79	-20.2, 13.6	0.59
Beans (mung, adzuki, jack, lima, navy, velvet) and cowpeas (seeds and pods)	474 (77)	145 (23)	0.76	-7.74, 10.0	0.87
Gram (red, green, black) and other peas	516 (83)	103 (17)	2.27	-7.48, 13.1	0.66
Coconut meat	489 (79)	130 (21)	-10.2	-18.0, -1.61	0.02*
Potato	10 (2)	609 (98)	-8.10	-31.6, 23.6	0.58
Sweet potato (orange, yellow and white flesh)	468 (76)	151 (24)	-4.32	-12.3, 4.36	0.32
Amaranth (spiny) and agathi leaves	608 (99)	5 (1)	-4.43	-36.8, 44.5	0.83
Amaranth (red and green), cowpea, drumstick, colocasia and fenugreen leaves	292 (47)	327 (53)	1.28	-6.02, 9.14	0.74
Other leafy greens (spinach, Indian spinach, jute, turnip, and radish leaves	238 (38)	381 (62)	2.62	-4.96, 10.8	0.51
Okra (lady fingers)	159 (26)	460 (74)	-5.36	-13.1, 3.07	0.21
Other vegetables (bitter, bottle, teasel, pointed gourds, carrots, brinjal, cabbage)	30 (5)	589 (95)	-5.13	-20.3, 12.9	0.55
Wood apple	595 (96)	24 (4)	20.9	-8.20, 46.7	0.05*
Tamarind	381 (62)	238 (38)	-0.87	-8.20, 7.03	0.82
Other fresh fruits (mango, pineapple)	73 (12)	546 (88)	-1.57	-12.3, 10.5	0.79
Eggs	66 (11)	553 (89)	-5.82	-16.5, 6.28	0.33
Poultry	92 (15)	527 (85)	-11.3	-20.1, -1.54	0.02*
Beef, mutton, pork	140 (23)	479 (77)	-8.42	-16.2, 0.10	0.05*
Organ meats (all animals: liver, brain etc.)	375 (61)	244 (39)	-6.25	-13.1, 1.18	0.10*
Shrimp	201 (32)	417 (68)	-3.86	-11.2, 4.12	0.33
Small fish (bata, bacha, guru, mola, pomfret, poa/bele and fry fish)	207 (33)	412 (67)	1.91	-5.84, 10.3	0.64
Medium fish (scorpion, aire, folui, chapila, khalshe, butter, climbing, tengra, catfish)	182 (29)	437 (71)	-2.40	-10.1, 5.92	0.56
Large fish (carp, ruhit, mrigal, sar, magur, dragon, bocha, hilsha, flat fish)	124 (20)	495 (80)	-8.54	-16.7, 0.37	0.06*
Dried fish	252 (41)	365 (59)	-3.00	-10.1, 4.61	0.43

[a] Separate pairwise regression models were run for each covariate expressed as a never/ever variable and blood Cd concentrations

[b] $p<0.10$ is considered significant; significant results are marked with a *

[c] Omitted due to collinearity

Appendix Table 2: Effect of vitamin D supplementation on maternal blood Cd at delivery, adjusted for serum ferritin, and with serum ferritin as an effect modifier.

	Adjusted model[a]				*Interaction model*[b]			
	N	% difference	(% difference) 95% CI	*P*[f]	N	% difference	(% difference) 95% CI	*P*[f]
Model A					**Model B**			
Serum ferritin (ng/10 mL)[c]	548	-0.35	-1.16, 0.47	0.40	548	0.04	-2.07, 2.19	0.97
Plasma CRP (mg/L)	548	-0.02	-0.12, 0.09	0.74	548	-0.02	-0.23, 0.09	0.71
Vitamin D treatment group								
Placebo	114	ref	ref	ref	114	ref	ref	ref
4200 IU/week	121	5.97	-6.04, 19.5	0.34	121	8.18	-11.2, 31.8	0.44
16800 IU/week	105	1.63	-10.3, 15.1	0.80	105	6.72	-15.2, 34.3	0.58
28000 IU/week	208	0.23	-9.97, 11.6	0.97	208	3.13	-14.7, 24.7	0.75
Interaction								
Placebo	-	-	-	-	114	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	121	-0.33	-2.76, 2.15	0.79[g]
16800 IU/week*ferritin	-	-	-	-	105	-0.78	-3.82, 2.35	0.62[g]
28000 IU/week*ferritin	-	-	-	-	208	-0.45	-2.89, 2.05	0.72[g]
Model C					**Model D**			
Serum ferritin (ng/10 mL)[d]	608	-0.43	-1.17, 0.31	0.25	608	-0.17	-2.22, 1.94	0.88
Vitamin D treatment group								
Placebo	117	ref	ref	ref	117	ref	ref	ref
4200 IU/week	136	7.90	-3.90, 21.2	0.20	136	8.32	-10.8, 31.5	0.42
16800 IU/week	119	3.89	-7.83, 17.1	0.53	119	9.56	-12.3, 36.8	0.42
28000 IU/week	236	1.30	-0.12, 12.4	0.81	236	3.69	-13.9, 24.8	0.70
Interaction								
Placebo	-	-	-	-	117	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	136	-0.07	-2.46, 2.39	0.96[h]
16800 IU/week*ferritin	-	-	-	-	119	-0.85	-3.77, 2.16	0.58[h]
28000 IU/week*ferritin	-	-	-	-	236	-0.36	-2.73, 2.06	0.77[h]
Model E					**Model F**			
Serum ferritin (ng/10 mL)[e]	610	-0.32	-0.64, 0.00	0.05	610	-0.26	-0.64, 0.11	0.17
Vitamin D treatment group								
Placebo	118	ref	ref	ref	118	ref	ref	ref
4200 IU/week	137	7.66	-4.07, 20.8	0.21	137	8.61	-4.82, 23.9	0.22
16800 IU/week	119	3.79	-7.89, 17.0	0.54	119	8.89	-9.13, 30.5	0.36
28000 IU/week	236	1.17	-8.79, 12.2	0.83	236	3.07	-9.86, 17.8	0.66
Interaction								
Placebo	-	-	-	-	118	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	137	-0.11	-0.96, 0.74	0.80[i]
16800 IU/week*ferritin	-	-	-	-	119	-0.75	-2.89, 1.43	0.49[i]
28000 IU/week*ferritin	-	-	-	-	236	-0.27	-1.50, 0.99	0.68[i]

[a] Separate adjusted models were run with serum ferritin as a covariate in the model, variable scaled to ng/10 mL
[b] Separate adjusted models were run with serum ferritin as an interaction term in the model, variable scaled to ng/10 mL
[c] Serum ferritin with influential outliers removed, adjusted for CRP (Model A & B)
[d] Serum ferritin with influential outliers removed, unadjusted (Model C & D)
[e] Serum ferritin with influential outliers, unadjusted (Model E & F)
[f] p<0.05 is considered significant
[g] Significance of interaction tested using Wald test (p=0.97)
[h] Significance of interaction tested using Wald test (p=0.92)
[i] Significance of interaction tested using Wald test (p=0.89)

Appendix Table 3: Effect of vitamin D supplementation on detectability of cord blood Cd at delivery, adjusted for serum ferritin, and with serum ferritin as an effect modifier.

	Adjusted model[a]				*Interaction model*[b]			
	N	Risk Ratio	95% CI	*P*[f]	N	Risk Ratio	95% CI	*P*[f]
Model A					**Model B**			
Serum ferritin (ng/10 mL)[c]	464	1.01	0.98, 1.05	0.48	464	0.96	0.84, 1.09	0.49
Plasma CRP (mg/L)	464	1.00	1.00, 1.01	0.21	464	1.00	1.00, 1.01	0.13
Vitamin D treatment group								
Placebo	94	ref	ref	ref	94	ref	ref	ref
4200 IU/week	90	2.13	1.23, 3.66	0.01*	90	1.93	0.75, 4.96	0.17
16800 IU/week	100	1.14	0.61, 2.12	0.69	100	0.70	0.22, 2.19	0.54
28000 IU/week	180	1.62	0.96, 2.75	0.07	180	0.91	0.36, 2.38	0.84
Interaction								
Placebo	-	-	-	-	94	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	90	1.02	0.88, 1.18	0.84[g]
16800 IU/week*ferritin	-	-	-	-	100	1.09	0.92, 1.29	0.34[g]
28000 IU/week*ferritin	-	-	-	-	180	1.10	0.96, 1.26	0.17[g]
Model C					**Model D**			
Serum ferritin (ng/10 mL)[d]	498	1.02	0.99, 1.06	0.18	498	0.96	0.84, 1.09	0.53
Vitamin D treatment group								
Placebo	95	ref	ref	ref	95	ref	ref	ref
4200 IU/week	98	2.09	1.21, 3.60	0.01*	98	1.74	0.67, 4.48	0.25
16800 IU/week	108	1.32	0.73, 2.39	0.37	108	0.71	0.24, 2.13	0.54
28000 IU/week	197	1.62	0.96, 2.72	0.07	197	1.00	0.31, 2.45	1.00
Interaction								
Placebo	-	-	-	-	95	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	98	1.03	0.89, 1.20	0.69[h]
16800 IU/week*ferritin	-	-	-	-	108	1.11	0.94, 1.30	0.21[h]
28000 IU/week*ferritin	-	-	-	-	197	1.08	0.95, 1.24	0.25[h]
Model E					**Model F**			
Serum ferritin (ng/10 mL)[e]	499	1.00	0.99, 1.01	0.86	499	0.96	0.84, 1.09	0.53
Vitamin D treatment group								
Placebo	96	ref	ref	ref	96	ref	ref	ref
4200 IU/week	98	2.10	1.21, 3.64	0.01*	98	1.74	0.67, 4.48	0.25
16800 IU/week	108	1.31	0.72, 2.39	0.38	108	0.71	0.24, 2.13	0.54
28000 IU/week	197	1.63	0.96, 2.76	0.07	197	1.00	0.41, 2.46	1.00
Interaction								
Placebo	-	-	-	-	96	ref	ref	ref
4200 IU/week*ferritin	-	-	-	-	98	1.03	0.89, 1.20	0.68[i]
16800 IU/week*ferritin	-	-	-	-	108	1.11	0.94, 1.30	0.21[i]
28000 IU/week*ferritin	-	-	-	-	197	1.08	0.95, 1.24	0.25[i]

[a] Separate adjusted models were run with serum ferritin as a covariate in the model, variable scaled to ng/10 mL
[b] Separate adjusted models were run with serum ferritin as an interaction term in the model, variable scaled to ng/10 mL
[c] Serum ferritin with influential outliers removed, adjusted for CRP (Model A & B)
[d] Serum ferritin with influential outliers removed, unadjusted (Model C & D)
[e] Serum ferritin with influential outliers, unadjusted (Model E & F)
[f] $p<0.05$ is considered significant; significant results are marked with a *
[g] Significance of interaction tested using Wald test (p=0.25)
[h] Significance of interaction tested using Wald test (p=0.42)
[i] Significance of interaction tested using Wald test (p=0.42)

Appendix Table 4: Association of maternal blood Cd and eGFR at delivery.

	Univariate model[a]				*Multivariate model*[b]			
	N	Effect size	95% CI	*P*[c]	N	Effect size	95% CI	*P*[c]
Blood Cd (μg/L)	311	-0.03	-8.82, 8.76	0.99	242	2.95	-7.88, 13.8	0.59
Gravidity	311	-2.73	-5.46, 0.00	0.05	242	-0.46	-4.99, 4.06	0.84
Maternal BMI (kg/m^2)	306	-0.73	-1.48, 0.01	0.06	242	-0.39	-1.35, 0.56	0.42
Blood lead (μg/dL)	311	-0.72	-1.49, 0.04	0.06	242	-0.77	-1.68, 0.15	0.10
Blood Se (μg/L)	311	-0.07	-0.25, 0.10	0.42	242	0.02	-0.18, 0.23	0.82
Gestational age at birth (weeks)	311	-0.84	-3.07, 1.39	0.46	242	-2.51	-5.18, 0.17	0.07
Maternal age (years)	311	-1.47	-2.20, -0.73	0.00*	242	-1.47	-2.58, -0.35	0.01*
Urine cotinine (ng/10 mL)[d]	247	-0.03	-0.08, 0.02	0.29	242	-0.03	-0.08, 0.03	0.34
Maternal education								
No schooling or primary incomplete	81	ref	ref	ref	58	ref	ref	ref
Primary complete	41	-1.35	-9.11, 11.8	0.80	32	-6.75	-18.7, 5.21	0.27
Secondary incomplete	106	-2.35	-10.4, 5.71	0.57	88	-10.4	-19.7, -1.16	0.03*
Secondary complete or higher	83	-3.00	-11.5, 5.52	0.49	64	-9.88	-20.1, 0.31	0.06
Asset index (quintiles)								
1 (lowest)	64	ref	ref	ref	48	ref	ref	ref
2	60	3.59	-6.19, 13.4	0.47	48	8.06	-3.11, 19.2	0.16
3	61	8.22	-1.52, 18.0	0.10	46	8.35	-2.98, 19.7	0.15
4	69	1.90	-7.54, 11.3	0.69	54	6.40	-4.88, 17.7	0.26
5	56	-2.00	-12.0, 7.96	0.69	46	2.24	-9.72, 14.2	0.71
Coconut meat consumption								
Never	241	ref	ref	ref	181	ref	ref	ref
Ever	70	0.12	-7.28, 7.52	0.97	61	3.45	-4.67, 11.6	0.40
Poultry meat consumption								
Never	41	ref	ref	ref	33	ref	ref	ref
Ever	270	6.08	-3.03, 15.2	0.19	209	9.92	-0.65, 20.5	0.07
Beef/mutton/pork consumption								
Never	66	ref	ref	ref	49	ref	ref	ref
Ever	245	-4.86	-12.4, 2.68	0.21	193	-5.87	-14.8, 3.02	0.19
Organ meat consumption								
Never	179	ref	ref	ref	134	ref	ref	ref
Ever	132	-0.44	-6.70, 5.81	0.89	108	-4.12	-11.3, 3.07	0.26
Large fish consumption								
Never	57	ref	ref	ref	47	ref	ref	ref
Ever	254	-8.40	-16.3, -0.47	0.04*	195	-5.22	-14.5, 4.06	0.27
Vitamin D supplementation group								
Placebo	67	ref	ref	ref	47	ref	ref	ref
4200 IU/week	70	-3.87	-13.2, 5.46	0.42	50	-9.24	-20.3, 1.84	0.10
16800 IU/week	62	-1.40	-11.0, 8.22	0.78	50	-4.70	-15.9, 6.52	0.41
28000 IU/week	112	0.07	-8.36, 8.51	0.99	95	-5.36	-15.0, 4.31	0.28

[a] Separate univariate models were run for each covariate

[b] Multivariable model adjusted for: gravidity, postpartum maternal BMI (kg/m^2), blood Pb (μg/dL), blood Se (μg/L), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never/ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 16800 IU/week, 28000 IU/week)

[c] $p < 0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10 mL

Appendix Table 5: Maternal characteristics at enrolment, comparison of clotted and un-clotted whole blood samples.

Enrolled participants, N	**Un-clotted 619**	**Clotted 181**	***P*[f]**
Age (years), median (min, max)	23 (18,40)	23 (18, 38)	0.59
Marital status, n (%)[a]			0.06
Married	617 (100)	179 (99.4)	
Not Married	0 (0)	1 (0.6)	
Level of education, n (%)			0.61
No schooling	25 (4.04)	8 (4.45)	
Primary incomplete	127 (20.5)	31 (17.1)	
Primary complete	90 (14.5)	21 (11.6)	
Secondary incomplete	232 (37.5)	77 (42.5)	
Secondary complete or higher	145 (23.4)	44 (24.6)	
Primary occupation, n (%)[b]			0.74
Homemaker	580 (94.0)	168 (93.3)	
Other	37 (6.00)	12 (6.7)	
Asset index quintiles, n (%)[c]			0.23
1 (lowest)	122 (19.8)	25 (13.9)	
2	123 (20.0)	30 (16.7)	
3	128 (20.8)	43 (23.9)	
4	123 (20.0)	39 (21.7)	
5 (highest)	120 (19.5)	43 (23.9)	
Gravidity[d], median (min, min)	2 (1, 9)	2 (1, 6)	0.52
Weight (kg), mean ± SD	54.7 ± 10.0	53.9 ± 9.94	0.36
Height (cm), mean ± SD	151 ± 5.47	151 ± 5.42	0.67
BMI (kg/m^2)	24.0 ± 4.00	23.7 ± 4.10	0.42
Month of enrolment, n (%)			0.09
March-May	269 (43.5)	76 (42.0)	
June-August	237 (38.3)	73 (40.3)	
September-November	77 (12.4)	14 (7.73)	
December-February	36 (5.82)	18 (9.94)	
Hemoglobin (g/L), mean ± SD	106.0 ± 11.2	105.4 ± 11.8	0.55
Serum 25(OH)D[e], mean ± SD	27.0 ± 13.7	28.2 ± 14.3	0.31

[a] $N_{Un\text{-}clotted}$=617, $N_{Clotted}$= 180

[b] $N_{Un\text{-}clotted}$= 615, $N_{Clotted}$= 178

[c] $N_{Un\text{-}clotted}$ = 616 for asset index 1,2,3,4, $N_{Clotted}$= 180 for asset index 1,2,3,4,5

[d] Number of pregnancies, including the current pregnancy.

[e] $N_{Un\text{-}clotted}$=617, $N_{Clotted}$=180

[f] Parametric and non-parametric tests were used to assess any differences between groups (t-test, Mann-Whitney test, chi-squared test); $p<0.05$ is considered significant

Appendix Table 6: Aim 1 variables stratified by vitamin D supplementation group.

Maternal blood Cd dataset	Vitamin D Dose (IU/week)										
	N	0	N	4200	N	16800	N	28000	P[a]	N	All participants
Blood Cd (µg/L); median (IQR)	118	0.69 (0.37)	141	0.69 (0.42)	121	0.65 (0.45)	239	0.67 (0.41)	0.56	619	0.68 (0.42)
Blood Se (µg/L); mean (SD)	118	135 (17.8)	141	135 (18.6)	121	136 (20.3)	239	135 (18.0)	0.96	619	135 (18.5)
Serum ferritin (ng/mL); median (IQR)	117	57.0 (59.4)	136	52.1 (54.1)	119	53.1 (48.4)	236	55.4 (54.4)	0.35	610	45.8 (46.1)
Plasma CRP (mg/L); median (IQR)	115	9.02 (19.6)	124	9.48 (18.3)	105	11.5 (19.4)	209	10.2 (21.0)	0.73	553	10.1 (20.2)
Daily Ca intake (mg); median (IQR)	118	461 (311)	141	422 (333)	121	438 (330)	239	441 (350)	0.43	619	441 (336)
Baseline serum 25(OH)D (nmol/L); median (IQR)	116	26.2 (18.2)	140	26.3 (16.3)	121	25.7 (16.7)	238	23.8 (16.2)	0.55	615	25.2 (16.8)
Delivery serum 25(OH)D (nmol/L); median (IQR)	73	20.5 (17.5)	82	69.0 (28.7)	73	98.6 (30.5)	141	110 (38.9)	0.00*	369	88.4 (57.2)
Cord blood Cd dataset											
Cord blood Cd; % detectable	100	15%	104	33%	111	21%	201	26%	0.15	516	24%
Cord blood Se (µg/L); mean (SD)	100	154 (21.6)	104	158 (25.4)	111	161 (22.4)	201	162 (22.7)	0.02*	516	156 (30.0)

[a] $p<0.05$ is considered significant; parametric and non-parametric tests were performed to assess between group differences (chi-squared test, ANOVA); significant results are marked with a *

Appendix Table 7: Baseline variables stratified by vitamin D supplementation group.

	Vitamin D dose				
	0	**4200**	**16800**	**28000**	***P*[f]**
Enrolled participants, N	**118**	**141**	**121**	**239**	
Age (years), median (min, max)	23 (18, 38)	23 (18, 40)	23 (18, 35)	23 (18, 35)	0.77
Marital status, n (%)[a]					0.99
Married	118 (100)	141 (100)	121 (100)	237 (100)	
Not Married					
Level of education, n (%)					0.36
No schooling	3 (2.54)	4 (2.84)	5 (4.13)	13 (5.44)	
Primary incomplete	26 (22.0)	26 (18.4)	25 (20.7)	50 (20.9)	
Primary complete	18 (15.2)	21 (14.9)	12 (9.92)	39 (16.3)	
Secondary incomplete	42 (35.6)	48 (34.0)	57 (47.1)	85 (35.6)	
Secondary complete or higher	29 (24.6)	42 (29.8)	22 (18.2)	52 (21.8)	
Primary occupation, n (%)[b]					0.76
Homemaker	113 (95.8)	133 (94.3)	112 (92.6)	222 (93.7)	
Other	5 (4.24)	8 (5.67)	9 (7.44)	17 (7.11)	
Asset index quintiles, n (%)[c]					0.65
1 (lowest)	27 (22.9)	27 (19.3)	15 (12.4)	53 (22.4)	
2	20 (17.0)	31 (22.1)	28 (23.1)	44 (18.6)	
3	25 (21.2)	25 (17.9)	30 (24.8)	48 (20.3)	
4	25 (21.2)	25 (17.9)	26 (21.5)	47 (19.8)	
5 (highest)	21 (17.8)	32 (22.9)	22 (18.2)	45 (19.0)	
Gravidity[d], median (min, min)	2 (1, 9)	2 (1, 6)	2 (1, 6)	2 (1, 7)	0.91
Weight (kg), mean ± SD	54.2 ± 9.89	54.0 ± 10.7	54.5 ± 9.03	55.4 ± 10.1	0.55
Height (cm), mean ± SD	151 ± 5.28	151 ± 5.34	151 ± 5.42	151 ± 5.69	0.99
BMI (kg/m^2)	23.7 ± 3.82	23.7 ± 4.42	23.9 ± 3.37	24.3 ± 3.96	0.50
Month of enrolment, n (%)					0.99
March-May	52 (44.1)	57 (40.4)	54 (44.6)	106 (44.4)	
June-August	45 (38.1)	58 (41.1)	45 (37.2)	89 (37.2)	
September-November	14 (11.9)	16 (11.4)	16 (13.2)	31 (13.0)	
December-February	7 (5.93)	10 (7.09)	6 (4.96)	13 (5.44)	
Hemoglobin (g/L), mean ± SD	105 ± 11.1	107 ± 11.6	106 ± 11.1	106 ± 10.9	0.50
Serum 25(OH)D[e], mean ± SD	27.2 ± 14.8	27.5 ± 13.8	28.4 ± 14.4	25.9 ± 12.6	0.38

[a] N_0=116, N_{4200}= 140, N_{28000}=238
[b] N_{4200}=140, N_{28000} = 238
[c] N_{28000}= 237
[d] Number of pregnancies, including the current pregnancy
[f] $p<0.05$ is considered significant; parametric and non-parametric tests were performed to assess between group differences (chi-squared test, ANOVA)

Appendix Table 8a: Association of maternal blood Cd and month of enrolment.

	n	% difference	(% difference) 95% CI	P[a]
Month of enrolment				
March-May	269	ref	ref	ref
June-August	237	8.38	-0.18, 17.7	0.06
September-November	77	-0.12	-11.4, 12.5	0.98
December-February	36	23.4	4.67, 45.3	0.01*

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 9a: Association of detectable cord blood Cd and month of enrolment.

	n	Risk Ratio	95% CI	P[a]
Month of enrolment				
March-May	230	ref	ref	ref
June-August	190	0.99	0.67, 1.47	0.96
September-November	48	2.72	1.86, 3.98	0.00*
December-February	48	2.07	1.33, 3.21	0.00*

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 8b: Association of maternal blood Cd and month of delivery.

	n	% difference	(% difference) 95% CI	P[a]
Month of delivery				
March-May	0	N/A	N/A	N/A
June-August	198	ref	ref	ref
September-November	234	0.31	-8.30, 9.74	0.95
December-February	172	2.42	-7.04, 12.9	0.63

[a] p<0.05 is considered significant; significant results are marked with a *

Appendix Table 9b: Association of detectable cord blood Cd and month of delivery.

	n	Risk Ratio	95% CI	P[a]
Month of delivery				
March-May	0	N/A	N/A	N/A
June-August	189	ref	ref	ref
September-November	200	0.45	0.29, 0.71	0.00
December-February	126	1.5	1.09, 2.08	0.01

[a] p<0.05 is considered significant; significant results are marked with a *

Appendix Table 10a: The effect of vitamin D supplementation group on maternal blood Cd, adjusted for month of enrolment.

	n	% difference	(% difference) 95% CI	P[a]
Treatment group				
Placebo	118	ref	ref	ref
4200 IU/week	141	6.07	-5.49, 19.0	0.32
16800 IU/week	121	5.15	-6.70, 18.5	0.41
28000 IU/week	239	1.78	-8.27, 12.9	0.74
Month of enrolment				
March-May	269	ref	ref	ref
June-August	237	8.26	-.31, 17.6	0.06
September-November	77	-0.15	-11.4, 12.5	0.98
December-February	36	23.1	4.46, 45.1	0.01*

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 10b: The effect of vitamin D supplementation group on maternal blood Cd, adjusted for month of delivery.

	n	Risk Ratio	95% CI	P[a]
Treatment group				
Placebo	100	ref	ref	ref
4200 IU/week	104	1.90	1.11, 3.25	0.02*
16800 IU/week	111	1.38	0.77, 2.46	0.28
28000 IU/week	201	1.60	0.96, 2.68	0.07
Month of enrolment				
March-May	230	ref	ref	ref
June-August	190	0.97	0.66, 1.44	0.89
September-November	48	2.47	1.68, 3.62	0.00*
December-February	48	2.04	1.32, 3.14	0.00*

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 11a: The effect of vitamin D supplementation group on maternal blood Cd, adjusted for month of delivery.

	n	% difference	(% difference) 95% CI	P[a]
Treatment group				
Placebo	115	ref	ref	ref
4200 IU/week	138	4.68	-6.95, 17.8	0.45
16800 IU/week	119	3.42	-8.45, 16.8	0.59
28000 IU/week	232	0.11	-9.99, 11.3	0.98
Month of delivery				
March-May	0	N/A	N/A	N/A
June-August	198	ref	ref	ref
September-November	234	-0.01	-8.65, 9.43	1.00
December-February	172	2.05	-7.41, 12.5	0.68

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 11b: The effect of vitamin D supplementation group on detectability of cord blood Cd, adjusted for month of delivery.

	n	Risk Ratio	95% CI	P[a]
Treatment group				
Placebo	100	ref	ref	ref
4200 IU/week	104	1.84	1.08, 3.14	0.03*
16800 IU/week	110	1.34	0.75, 2.40	0.32
2800 IU/week	201	1.58	0.94, 2.63	0.08
Month of delivery				
March-May	0	N/A	N/A	N/A
June-August	189	ref	ref	ref
September-November	200	0.46	0.30, 0.72	0.00*
December-February	126	1.45	1.03, 1.97	0.03*

[a] $p<0.05$ is considered significant; significant results are marked with a *

Appendix Table 12a: Association of maternal blood Cd and renal biomarkers, additionally adjusting for month of enrolment.

	Serum creatinine[a]				*Urine ACR*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Blood Cd (µg/L)	242	-1.42	-15.5, 15.0	0.85	351	20.4	-19.9, 81.0	0.37
Gravidity	242	1.86	-4.41, 8.55	0.57	351	23.5	3.31, 47.7	0.02*
Maternal BMI (kg/m^2)	242	0.17	-1.17, 1.53	0.80	351	-1.98	-5.46, 1.63	0.28
Blood Pb (µg/dL)	242	0.58	-0.73, 1.91	0.38	351	-0.45	-3.98, 3.20	0.80
Blood Se (µg/L)	242	-0.05	-0.34, 0.24	0.74	351	0.37	-0.41, 1.15	0.35
Gestational age at birth (weeks)	242	2.16	-1.70, 6.18	0.28	351	-1.72	-10.4, 7.82	0.71
Maternal age (years)	242	0.64	-0.93, 2.25	0.42	351	-1.91	-6.11, 2.48	0.39
Urine cotinine (ng/10 m)[d]	242	0.02	-0.06, 0.10	0.55	351	-0.22	-0.73, 0.30	0.41
Maternal education								
No schooling or primary incomplete	81	ref	ref	ref	81	ref	ref	ref
Primary complete	41	9.39	-7.57, 29.5	0.29	49	2.44	-36.0, 63.9	0.92
Secondary incomplete	106	17.0	2.56, 33.4	0.02*	139	26.5	-11.8, 81.5	0.20
Secondary complete or higher	83	17.2	1.45, 35.4	0.03*	82	33.0	-12.7, 103	0.18
Asset index (quintiles)								
1 (lowest)	64	ref	ref	ref	69	ref	ref	ref
2	60	-7.82	-21.2, 7.81	0.31	73	-14.0	-44.2, 32.7	0.50
3	61	-8.66	-22.4, 7.50	0.27	71	-30.5	-55.2, 7.86	0.10
4	69	-7.34	-21.1, 8.75	0.35	69	-22.1	-51.0, 23.9	0.29
5	56	-2.90	-18.1, 15.1	0.73	69	-22.8	-51.5, 23.1	0.28
Presence of blood								
Negative	-	-	-	-	129	ref	ref	ref
Trace	-	-	-	-	39	151	56.5, 302	0.00*
Small	-	-	-	-	38	123	38.7, 258	0.00*
Moderate	-	-	-	-	61	237	123, 407	0.00*
Large	-	-	-	-	84	415	256, 644	0.00*
Coconut meat consumption								
Never	241	ref	ref	ref	260	ref	ref	ref
Ever	70	-0.34	-11.2, 11.8	0.95	91	19.5	-12.9, 64.0	0.27
Poultry meat consumption								
Never	41	ref	ref	ref	43	ref	ref	ref
Ever	270	-12.6	-24.7, 1.52	0.08	308	12.1	-26.3, 70.8	0.59
Beef/mutton/pork consumption								
Never	49	ref	ref	ref	70	ref	ref	ref
Ever	193	6.69	-6.02, 21.1	0.32	281	52.2	6.78, 117	0.02*
Organ meat consumption								
Never	134	ref	ref	ref	197	ref	ref	ref
Ever	108	6.86	-3.54, 18.4	0.20	154	1.07	-24.1, 34.7	0.94
Large fish consumption								
Never	47	ref	ref	ref	70	ref	ref	ref
Ever	195	7.38	-5.80, 22.4	0.29	281	7.56	-24.7, 53.6	0.69
Vitamin D supplementation group								
Placebo	67	ref	ref	ref	63	ref	ref	ref
4200 IU/week	70	10.6	-5.51, 29.4	0.21	74	-35.5	-58.4, -0.16	0.05*
16800 IU/week	62	5.29	-10.3, 23.6	0.53	67	-42.8	-63.8, -9.44	0.02*
28000 IU/week	112	4.33	-8.98, 19.6	0.54	147	-38.2	-57.8, -9.54	0.01*
Month of enrolment								
March-May	92	ref	ref	ref	151	ref	ref	ref
June-August	111	15.4	2.31, 30.1	0.02*	162	125	65.8, 205	0.00*
September-November	28	43.8	21.4, 70.3	0.00*	28	122	29.0, 283	0.00*
December-February	11	23.2	-5.29, 60.2	0.12	10	98	-13.6, 354	0.11

[a] A separate multivariable model (Model A) was run for blood Cd and serum creatinine; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week), month of enrolment (March-May, June-August, September-November, December-February)

[b] A separate multivariable model (Model B) was run for blood Cd and ACR; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut meat consumption (never/ever), poultry meat consumption (never/ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever) vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week), month of enrolment (March-May, June-August, September-November, December-February)

[c] $p<0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10 mL

Appendix Table 12b: Association of maternal blood Cd and renal biomarkers, additionally adjusting for month of delivery.

	Serum creatinine[a]				*Urine ACR*[b]			
	N	**% difference**	**(% difference) 95% CI**	***P*[c]**	**N**	**% difference**	**(% difference) 95% CI**	***P*[c]**
Model A					**Model B**			
Blood Cd (µg/L)	242	1.76	-12.5, 18.4	0.82	351	23.6	-18.3, 86.8	0.32
Gravidity	242	-0.20	-6.29, 6.29	0.95	351	25.2	4.33, 50.2	0.02*
Maternal BMI (kg/m^2)	242	0.28	-1.05, 1.62	0.68	351	-1.87	-5.41, 1.81	0.31
Blood Pb (µg/dL)	242	0.22	-1.11, 1.57	0.75	351	-0.17	-3.87, 3.67	0.93
Blood Se (µg/L)	242	-0.07	-0.35, 0.22	0.64	351	0.27	-0.52, 1.07	0.50
Gestational age at birth (weeks)	242	1.51	-2.24, 5.41	0.43	351	-1.80	-10.5, 7.80	0.70
Maternal age (years)	242	0.93	-0.63, 2.50	0.24	351	-1.80	-6.07, 2.65	0.42
Urine cotinine (ng/10 mL)[d]	242	0.04	-0.33, 0.12	0.27	351	-0.22	-0.75, 0.31	0.41
Maternal education								
No schooling or primary incomplete	81	ref	ref	ref	81	ref	ref	ref
Primary complete	41	9.22	-7.48, 28.9	0.30	49	3.39	-35.9, 66.7	0.89
Secondary incomplete	106	13.0	-0.69, 28.7	0.06	139	22.6	-15.2, 77.3	0.28
Secondary complete or higher	83	10.1	-4.45, 27.0	0.18	82	25.4	-18.4, 92.8	0.30
Asset index (quintiles)								
1 (lowest)	64	ref	ref	ref	69	ref	ref	ref
2	60	-5.65	-19.2, 10.2	0.46	73	-12.5	-43.8, 36.1	0.55
3	61	-6.99	-20.6, 8.90	0.37	71	-35.5	-58.7, 0.69	0.05
4	69	-2.25	-16.5, 14.5	0.78	69	-25.5	-53.6, 19.7	0.22
5	56	-1.09	-16.3, 16.8	0.90	69	-25.4	-53.5, 19.8	0.22
Presence of blood								
Negative	-	-	-	-	129	ref	ref	ref
Trace	-	-	-	-	39	145	51.7, 296	0.00*
Small	-	-	-	-	38	131	42.1, 274	0.00*
Moderate	-	-	-	-	61	239	123, 414	0.00*
Large	-	-	-	-	84	418	257, 653	0.00*
Coconut meat consumption								
Never	241	ref	ref	ref	260	ref	ref	ref
Ever	70	0.15	-10.6, 12.2	0.98	91	13.3	-17.9, 56.4	0.45
Poultry meat consumption								
Never	41	ref	ref	ref	43	ref	ref	ref
Ever	270	-12.9	-24.8, 0.91	0.07	308	8.74	-29.0, 66.7	0.70
Beef/mutton/pork consumption								
Never	49	ref	ref	ref	70	ref	ref	ref
Ever	193	7.54	-4.98, 21.7	0.25	281	55.8	8.76, 123	0.02*
Organ meat consumption								
Never	134	ref	ref	ref	197	ref	ref	ref
Ever	108	4.50	-5.47, 15.5	0.39	154	-2.17	-27.0, 31.1	0.88
Large fish consumption								
Never	47	ref	ref	ref	70	ref	ref	ref
Ever	195	9.23	-3.96, 24.2	0.18	281	11.7	-22.2, 60.4	0.55
Vitamin D supplementation group								
Placebo	67	ref	ref	ref	63	ref	ref	ref
4200 IU/week	70	7.02	-8.45, 25.1	0.39	74	-32.7	-57.0, 5.17	0.08
16800 IU/week	62	4.74	-10.6, 22.7	0.57	67	-42.8	-64.2, -8.78	0.02*
28000 IU/week	112	3.77	-9.27, 18.7	0.59	147	-36.5	-56.9, -6.42	0.02*
Month of delivery								
March-May	0	N/A	N/A	N/A	0	N/A	N/A	N/A
June-August	78	ref	ref	ref	117	ref	ref	ref
September-November	84	-1.20	-12.7, 11.9	0.85	136	77.3	26.5, 149	0.00*
December-February	80	30.6	14.3, 49.2	0.00*	98	103	37.7, 198	0.00*

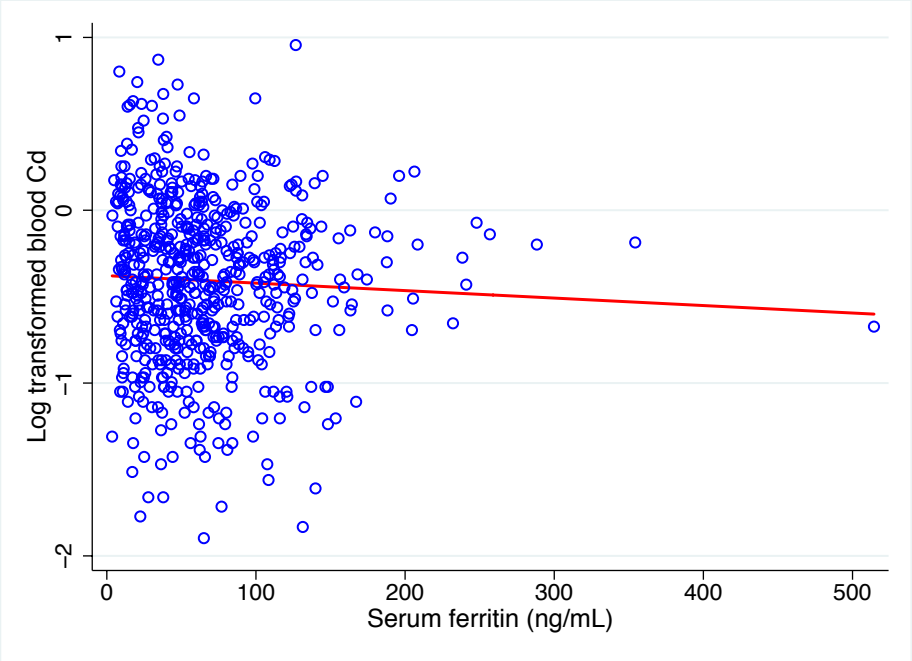
[a] A separate multivariable model (Model A) was run for blood Cd and serum creatinine; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), coconut meat consumption (never/ever), poultry meat consumption (never, ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever), vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week), month of delivery (June-August, September-November, December-February)

[b] A separate multivariable model (Model B) was run for blood Cd and ACR; multivariable model adjusted for: blood Se (µg/L), gravidity, postpartum maternal BMI (kg/m^2), blood Pb (µg/dL), gestational age at birth (weeks), maternal age (years), urine cotinine (ng/10 mL), maternal education (no schooling or primary incomplete, primary complete, secondary incomplete, secondary complete or higher), asset index (1-5), presence of blood (negative, trace, small, moderate, large), coconut meat consumption (never/ever), poultry meat consumption (never/ever), beef/mutton/pork meat consumption (never/ever), organ meat consumption (never/ever), large fish consumption (never/ever) vitamin D supplementation group (placebo, 4200 IU/week, 16800 IU/week, 28000 IU/week), month of delivery (June-August, September-November, December-February)

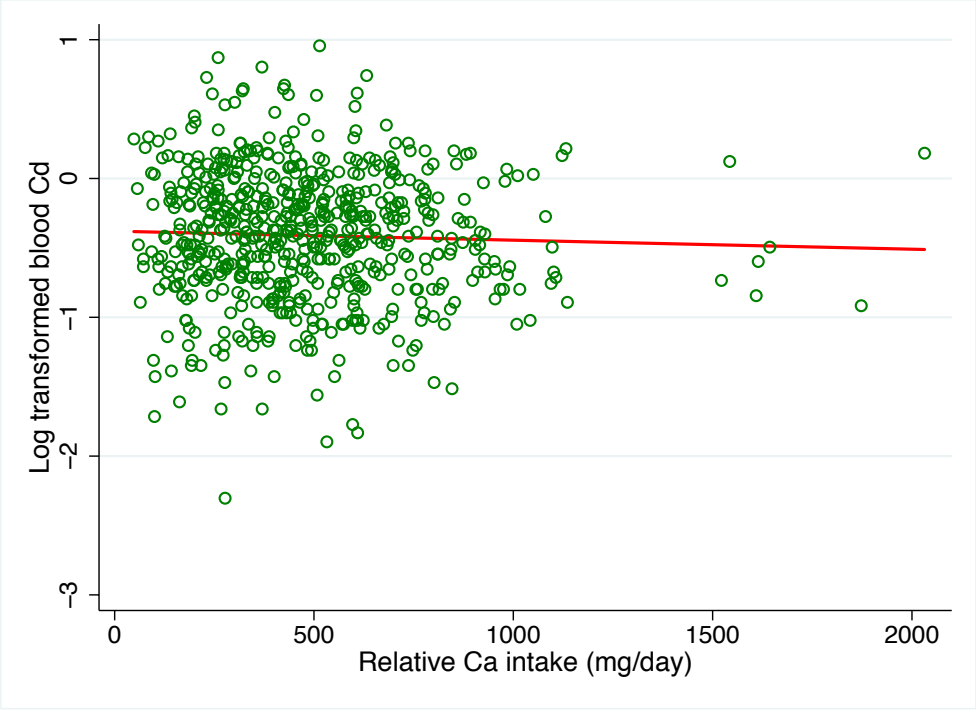
[c] $p<0.05$ is considered significant; significant results are marked with a *

[d] Variable scaled to ng/10 mL

Appendix Figure 1: Scatterplot of serum ferritin concentrations (ng/mL) and log transformed maternal blood Cd, excluding two influential observations >1000 ng/mL.



Appendix Figure 2: Scatterplot of daily Ca intake (mg) and log transformed maternal blood Cd.



Appendix Figure 3: Scatterplot of blood Se (μg/L) and log transformed maternal blood Cd.