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State of the trace element iodine and environmental pollutants in Portuguese pregnant women and the anthropometric profile of the offspring at birth: results from the IoMum cohort

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Dedication

Às minhas orientadoras e a toda minha família.

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A todos o meu sincero obrigada!

Abstract

Introduction: Iodine is a critical nutrient for the production of thyroid hormones: triiodothyronine (T3) and thyroxine (T4), and iodine intake is recognized as an important factor for pregnancy and fetal health. Prenatal exposure to toxic chemicals such as organophosphate (PO) pesticides, pyrethroid pesticides and heavy metals has been associated with stillbirths, low birth weight, prematurity, congenital malformation and neurobehavioral function disorder. Nevertheless, Portuguese evidence of iodine status or environmental exposures and their association with neonatal outcomes is scarce.
Aims: To evaluate the iodine status in a cohort of Portuguese pregnant women and its correlation with the antropometric profile (weight, length and head circumference) at birth . In addition, exposure to the pesticide pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) and other chemical elements lead (Pb), cadmium (Cd), arsenic (As), selenium (Se) was also assessed.
Methods: Pregnant women who attended their routine first trimester ultrasound scan from April 2018 to April 2019 at São João Hospital Center (CHUSJoão) were invited to participate. Multiple linear regression models were used to analyze the association between exposure to iodine, Pb, Cd, As, Se and 3-PBA and the variables birth weight, length and head circumference. The level of statistical significance was set at 5%.
Results: The median (UIC) in our sample population was 91.8 µg/L and 71% of the participants were iodine insufficient. Regarding the other chemical elements (Pb, Cd, As and Se), it was found that the median levels are in the corresponding reference ranges. However, 19, 32 and 17% of women had levels of Pb, As and Se, respectively, above the corresponding reference ranges. After adjustment for the covariates: smoking, maternal age, T1 weight gain, pre-pregnancy BMI, gestational age at birth and sex of the newborn, it was found that lead exposure was positively associated with weight and length at birth (beta 180.6, 0.676, respectively) and selenium exposure was positively associated with birth weight and head circumference at birth (beta 175.3, 0.592, respectively). Analysis of 3-PBA results in pregnant women showed concentrations above the detection limit (LOD) in 52% of the population. However, no correlation was found between the pyrethroid pesticide metabolite levels and the anthropometric profile of newborns.
Conclusion: The present study characterized iodine status in a cohort of Portuguese pregnant women, pointing to an iodine-insufficient status. Moreover, this study evidenced that pregnant women are exposed to a variety of environmental toxics that may affect fetal growth trajectories. Further studies should be performed to confirm this data and to analyze the impact of these exposures on longer term childhood outcomes.

Key words: Iodine, 3-PBA, Lead, Cadmium, Arsenic, Selenium, Pregnancy, Newborns, Anthropometry, Supplementation.

Resumo

Introdução: O iodo é um nutriente crítico para a produção de hormonas da tiróide: triiodotironina (T3) e tiroxina (T4). A ingestão de iodo é reconhecida como um fator importante para a gravidez e para a saúde fetal. A exposição pré-natal a produtos químicos tóxicos, como pesticidas organofosforados (PO), pesticidas piretróides e metais pesados, tem sido associada a natimortos, baixo peso ao nascer, prematuridade, malformação congênita e distúrbio da função neurocomportamental. A evidência portuguesa do estado de iodo e de exposições ambientais e sua associação com os resultados neonatais são escassas.

Objetivo: Avaliar o estado de iodo numa cohort de mulheres grávidas portuguesas e a sua correlação com o perfil antropométrico do bebé à nascença (peso, comprimento e perímetro cefálico). Além disso, a exposição ao metabolito do pesticida piretróide ácido 3-fenoxibenzóico (3-PBA) e a outros elementos químicos chumbo (Pb), cádmio (Cd), arsénio (As), selénio (Se) foi também avaliada.

Métodos: Foram convidadas a participar as grávidas que compareceram entre abril de 2018 e abril de 2019, ao Centro Hospitalar São João (CHUSJoão) para a ecografia de rotina do primeiro trimestre. Foram utilizados modelos de regressão linear múltipla para analisar a associação entre a exposição ao iodo, Pb, Cd, As, Se e 3-PBA e peso ao nascimento, comprimento e perímetro cefálico. O nível de significância estatística foi estabelecido em 5%.

Resultados: A mediana (UIC) em nossa população amostral foi de 91,8 µg/L e 71% das participantes tinham níveis de iodo abaixo do limite mínimo recomendado pela Organização Mundial de Saúde. Em relação aos outros elementos químicos (Pb, Cd, As e Se), verificou-se que os níveis medianos estão dentro dos intervalos de referência correspondentes. No entanto, 19, 32 e 17% das mulheres apresentaram níveis de Pb, As e Se, respectivamente, acima dos intervalos de referência correspondentes. Após ajuste para as covariáveis tabagismo, idade materna, ganho de peso T1, IMC pré-gestacional, idade gestacional ao nascer e sexo do recém-nascido, verificou-se que a exposição ao chumbo associou-se positivamente ao peso e comprimento ao nascimento (beta 180,6, 0,676, respetivamente) e exposição ao selênio associou-se positivamente com o peso ao nascer e o perímetro cefálico ao nascer (beta 175,3, 0,592, respetivamente). A análise dos resultados do 3-PBA em mulheres grávidas mostrou concentrações acima do limite de detecção (LOD) em 52% da população. No entanto, não foi encontrada correlação entre os níveis de metabolitos dos pesticidas piretróides e o perfil antropométrico dos recém-nascidos.

Conclusão: O presente estudo caracterizou o estado de iodo numa coorte de grávidas portuguesas, apontando para um estado insuficiente em iodo. Além disso, evidenciou que as grávidas estão expostas a uma variedade de tóxicos ambientais que podem afetar as trajetórias de crescimento fetal. Mais estudos devem ser realizados para confirmar estes dados e analisar o impacto destas exposições ambientais nos resultados da infância a longo prazo.

Palavras-Chave: Iodo, 3-PBA, Chumbo, Cádmio, Arsênio, Selénio, Gravidez, Recém-nascidos, Antropometria, Suplementação.

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List of Abbreviations

2-PBA, 2-phenoxybenzoic acid

3-PBA, 3-phenoxybenzoic acid

ADHD, Attention Deficit Hyperactivity Disorder

AGA, Appropriate for gestational age

As, Arsenic

ASD, Autism spectrum disorders

BMI, Body mass index

Cd, Cadmium

CDC, Centers for Disease Control and Prevention

CHUSJoão, São João Hospital Center

CSE, Superior Council of Statistics

DGS, Direção Geral de Saúde

DIC, Diisopropylcarbodiimide

DIT, Diiodotyrosine

EU, European Union

GC-MS, Gas chromatography with mass spectrometry

GPx, Antioxidant enzyme glutathione peroxidase

H_2O, Deionized water

H_2O_2, Hydrogen peroxide

HFIP, Hexafluoro-2-propanol

I^-, Iodide

ICP-MS, Inductively Coupled Plasma Mass Spectrometry

ID, Iodine deficiency

INE, I.P, Instituto Nacional de Estatisticas

IO_3^-, Iodate

IRMM, Institute for Reference Materials and Measurements

LGA, Large for gestational age

Li, Lithium

LOD, Limit of Detection

LOQ, Limit of Quantification

MeOH, Methanol

MIT, Monoiodotyrosine

Mn, Manganese

NHMRC, National Council for Health and Medical Research

NIS, Sodium/iodide symporter

OP, Organophosphate pesticides

OR, Odds Ratios

Pb, Lead

RDA, Recommended Daily Allowance

RN, Newborn

Se, Selenium

SES, Socioeconomic status

SGA, Small for gestational age

SPE, Solid Phase Extraction

SPs, Synthetic pyrethroid pesticides

SR, Self-reported

T1, Time point 1

T2, Time point 2

T3, Tri-iodothyronine

T4, Thyroxine

Tg, Thyroglobulin

TH, Thyroid Hormones

THD, TH disruptors

TPO, Thyroperoxidase

UIC, Urinary iodine concentration

WHO, World Health Organization

Zn, Zinc

Introduction

1. The importance of iodine during pregnancy

Iodine (atomic weight 126.9) is a critical nutrient for the production of thyroid hormones: triiodothyronine (T3) and thyroxine (T4). The total amount of iodine in an healthy adult is 15-20 mg [1].

T3 and T4 are necessary for several brain development and functional events, such as neuronal migration, myelination and transmission and for synaptic plasticity. As such, hypothyroxinemia in fetal and early postnatal life may result in brain damage, mental retardation and neurological abnormalities [2].

The mechanisms of iodine uptake and production of thyroid hormones in the thyrocyte are described in figure 1.

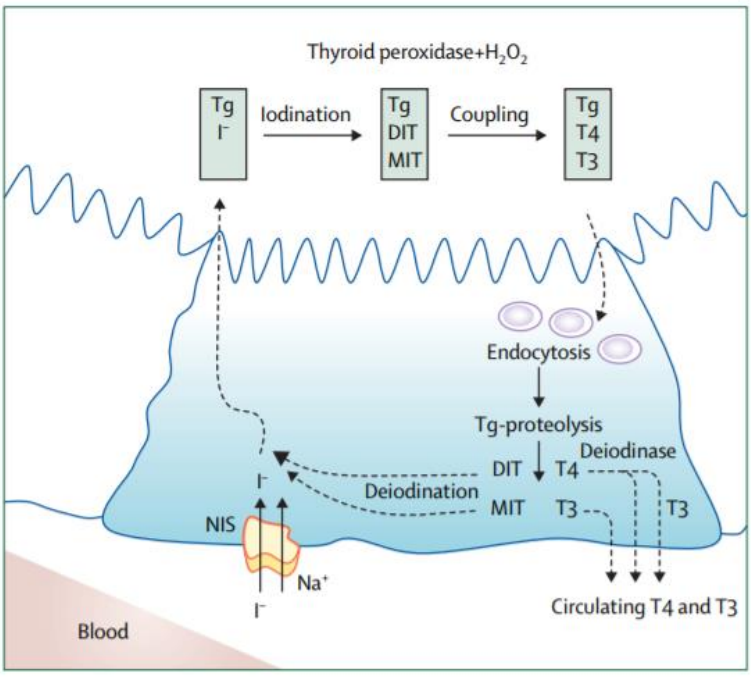

Figure 1 - Iodine uptake pathway in the thyroid cell **[3]**
Legend: DIT, diiodotyrosine; H_2O_2, hydrogen peroxide; I^-, iodide; MIT, monoiodotyrosine; T3, triiodothyronine; T4, thyroxine; Tg, thyroglobulin; TPO, thyroperoxidase

Iodide (I^-) is taken up by the thyrocyte through the sodium/iodide symporter (NIS) which localizes at the blood-facing membrane. Iodide then

migrates to the apical luminal-facing membrane being passively transported into the iodine-rich follicular lumen of the thyroid gland. Before being incorporated into thyroid hormones, iodine is oxidized by the thyroperoxidase (TPO):hydrogen peroxide (H_2O_2) system and is then attached to thyroglobulin (Tg), producing monoiodotyrosine (MIT) and diiodotyrosine (DIT). T4 and T3 are then formed by MIT and DIT coupling with the Tg molecule in the follicular lumen. After this, Tg is endocytosed by the thyrocyte and digested to release T4 and T3 which are then secreted into the bloodstream.

Much of the iodides are naturally present in the oceans 50 μg/L, and they run the iodine cycle between sea water, the atmosphere, the soil returning again to sea water. However, these iodine cycles may in some regions be slow and incomplete, turning the soils and groundwater deficient in this trace element. Cultures grown on these soils will have low iodine concentrations, and consequently, humans and animals dependent on food grown on these soils will ultimately become iodine deficient [3].

Indeed, iodine is an essential micronutrient, being obtained exclusively from exogenous food sources such as fish, crustaceans, algae, vegetables, meat, milk and its derivatives and iodized salt [4]. Iodine is present in food sources in several chemical forms such as iodide (I^-), iodate (IO_3^-), the form used for salt iodization, and iodine bound to organic molecules [5]. Iodide is promptly absorbed throughout the gastrointestinal tract in a process mediated by the NIS [5]. On the other hand, iodate is reduced to iodide in the intestine before it is absorbed.

Once in the plasma, iodide is taken up mainly by the thyroid, for thyroid hormone synthesis (Figure 1), and by the kidney. Chronic severe iodine

deficiency results in inability to synthesize thyroid hormone which leads to hypothyroidism and its impact in the organism [1].

The daily recommendations for iodine and the maximum tolerable levels of iodine intake, according to the World Health Organization (WHO) are presented in Table 1 and Table 2, respectively. As it can be observed in Table 1, daily iodine requirements vary throughout the life cycle [6], with pregnant women and infants on the top of the daily recommendations.

Table 1- Daily recommendations of iodine according to WHO. [7]

Age groups (years)	µg iodine/day
0-0,5	40
0,5-1	50
1-3	70
4-6	90
7-10	120
(Men)> 10	150
(Women)> 10	150
Pregnant	175
Breastfeeding	200

Table 2- Maximum daily iodine intake level. [7]

Ages (years)	Tolerable (UL) (µg iodine/day)
1-3	200
4-6	250
7-10	300
11-14	450
15-17	500
Adults	1,100
Pregnancy and breastfeeding	600

Cretinism, characterized by deep abnormalities in physical and intellectual development is the most severe result of iodine deficiency. Nevertheless, mild to moderate iodine deficiency, which still occurs in Europe and around the world, may also compromise children's cognitive and motor function [2].

Iodine deficiency has also been suggested to increase the risk of adverse obstetric or birth outcomes, such as premature placental abruption, preeclampsia, preterm birth, low birth weight or fetal death [8].

The fetus depends on maternal iodine intake and can synthesize thyroid hormones significantly only from half of gestation and onwards [9]. When compared to the non gravidic situation, the need for iodine during pregnancy is markedly increased mainly due to: a) an increased production of T4 to maintain maternal normal thyroid function and b) the need for thyroid hormone supply to the fetus until fetal thyroid achieves self-sufficiency.

After delivery, the increased supply of iodine is still required since iodine requirements of lactating women may be even greater than during pregnancy [2, 4]. In infants with exclusive breastfeeding between the ages of 0 and 6 months, iodine intake occurs via breastmilk [9].

A study conducted in the northern region of Portugal in 2008, involving 140 pregnant women and 78 non-pregnant women of reproductive age, have shown iodine deficiency in both groups, 61%, and 76-91%, respectively [10].

In 2010, *Limbert et al*., corroborated these results in a group of 3631 pregnant women, in which only 17% of pregnant women presented iodine levels above the minimum recommended level for pregnancy (150 µg/L) [11].

Thus, since 2013, Direção Geral de Saúde (DGS) Guideline No. 011/2013 recommends supplementation with iodine in the form of potassium iodide at a

dosage of 150 to 200 µg/day for all women in the preconceptional period, in pregnancy and in breastfeeding, except women with a known history of thyroid pathology. This practice is reinforced by the WHO [9].

The criteria for assessing the adequacy of iodine intake based on urinary iodine values (reference values for pregnant women) are described in Table 3.

Table 3 - Criteria for assessing the adequacy of iodine intake based onurinary iodine values (reference values for pregnant women). [6]

Population Group	Iodine concentration in urine (iodine, µg/L)	Intake of iodine
Pregnant	<150	Insufficient
	150-249	Adequate
	250-499	Above requirements
	≥500	Excessive

Two important studies have related iodine status with pregnancy outcomes.

Specifically, a clinical study carried out in Barcelona (2009) revealed that women with a third trimester urinary iodine concentration (UIC) between 100 and 149 µg/L had the lowest risk of having a small child for gestational age (SGA), and their infants had higher mean birth weight than those with UIC of less than 50 µg/L. Birth weight of babies born to women with third trimester UIC between 50 and 99 µg/L was also significantly higher than birth weight of babies born to women with 50 µg/L UIC or less [8].

A study conducted in 2018 in Europe indicates some evidence on a negative relationship between iodine intake and placental size suggesting an interference of iodine status with intrauterine growth dynamics [12]. However, more research between the accumulation of iodine and placental growth is

needed.

Although iodine intake is recognized as an important factor for pregnancy and fetal health, evidence of iodine status in the Portuguese population and its association with neonatal outcomes is still lacking.

2. Environmental toxic exposures in pregnancy

There is a concern that humans are exposed to many toxic chemicals, such as organophosphorus pesticides (OP), pyrethroid pesticides and heavy metals. Exposure to these compounds is dangerous since some of them have the potential for endocrine disruption and may also be neurotoxic [13].

Pregnancy is a critical window of time for noxious stimuli such as environmental pollutant exposure since fetuses may be more susceptible than adults to the harmful effects of chemicals. Prenatal exposure to chemical environments has been associated with stillbirths, low birth weight, preterm birth, congenital malformation, and disturbance of neurobehavioral function [13].

The levels and consequences of exposure to these chemicals during pregnancy vary with time, season and geographical location. In Portugal, studies on the exposure of pregnant women to pollutants and their consequences for fetal health have not been published yet.

The next sections explore the state of the art in relation to exposure to two specific classes of pollutants: trace elements - lead (Pb), cadmium (Cd), arsenic (As) and selenium (Se) – and pyrethroids that, even at low levels, can act as toxic compounds.

2.1 Trace elements, heavy metals and metalloid levels in pregnancy

The term heavy metals apply to metals which are relatively high in density and are considered toxic to living organisms and to the environment. They can intoxicate humans through contamination of water and soil, contaminated foods when exposed to pesticides and/or fertilizers, or through paints specifically in the case of heavy metals: Pb, Cd, and the metalloid As. The latter require greater attention because of their high toxicity and large participation in the daily life of the population [14].

The major routes of human exposure to heavy metals are ingestion and inhalation of contaminated food and dust. Exposure to these can be associated with cardiometabolic diseases, including cardiovascular disease, diabetes, kidney disorders and some cancers [15].

Exposure to trace elements and heavy metals varies for different countries and regions, specifically Germany [16], the USA [17], Canada [18] and Brazil [19]. Evidence suggests that several environmental exposures are related to factors such as socioeconomic status (SES), eating habits, lifestyles and environmental conditions.

For example, in the USA, millions of people are suffering from exposure to As from drinking water [20]. The concentration of As in drinking water is above the standard of the country's Environmental Protection Agency (10 µg/L) [21].

Exposure to Pb and Cd may cause changes in the thyroid (hypothyroidism and hyperthyroidism) [22]. Also, the hypothalamic-pituitary-ovarian axis can be affected directly or indirectly by heavy metals, which modifies the secretion of

prolactin, adrenocortical or thyroid hormones [14].

Heavy metals are deposited in the adrenal glands of women and may cause the blockage of several enzymes necessary for the normal functioning of the body, causing for example hyperandrogenism and polycystic ovary syndrome [14]. The accumulation of these metals alters the production of estradiol and progesterone, interfering with the normal development of the oocyte and causing embryonic chromosomal changes [14].

In addition, heavy metals cause hormonal dysregulations that can result in infertility, not only in women but also in man [14].

Birth outcomes such as weight, length, head circumference and, to some extent, the apgar score at 5 minutes after birth may be influenced by exposure of pregnant women to environmental toxics [23].

In addition, some heavy metals may interfere with placental transport systems. However further studies on the characteristics and mechanisms involved in the toxicokinetic effects of heavy metals on placental function are needed [21].

Despite the known toxicity of heavy metals, some other trace elements are biologically important, as the cases of iodine and also manganese (Mn), zinc (Zn) and the trace element Se and the intake of adequate amounts of these are essential to maintain optimal health. However, even these can turn hazardous in excessive exposure. For example, Mn is an essential element that acts as a toxic agent for neurodevelopment at supra-optimal concentrations [13].

Iodine and Se are important for thyroid function. One study investigated the intake of iodine and Se in healthy 50 to 70 years old women (n = 97) from three New Zealand cities after mandatory fortification of bread with iodized salt.

The authors concluded that iodine intake improved after mandatory fortification, however selenium intake remained low [24].

Although there are studies that show the risks of contamination in the human organism, due to excess of some trace elements, there are still few studies that associate them with neonatal results.

2.2 3-phenoxybenzoic acid (3-PBA) and pregnancy

Pesticides belong to a large family of compounds used to control insect (insecticides, insect repellents), weeds (herbicides), microbe (fungicides, disinfectants), or mice and rat (rodenticides) pests [25].

Synthetic pyrethroids belong to the class of insecticides and are commonly used. These insecticides derive from natural insecticides produced by some species of chrysanthemum flowers, the so called pyrethrins. 3-PBA results from environmental degradation of several synthetic pyrethroid pesticides (SPs) [26].

Exposure to pesticides in the population is widespread, especially via dermal, ingestion and inhalation routes [27]. After absorption into the human body, pyrethroids are metabolized rapidly and excreted in the urine. Many pyrethroids are enzymatically oxidized to the 3-PBA metabolite. Like many other insecticide compounds, pyrethroids are known to be neurotoxic [27].

Some studies have evaluated urinary pyrethroid levels among pregnant women, namely in USA [11], Mexico [28], France [29], Japan [29, 30], China [31] and Caribbean countries [25, 32].

Pesticide monitoring studies conducted in the European Union and US, with 3421 pregnant women, from January 2002 to February 2006, have indicated that in recent decades there has been an increase in pyrethroid insecticides home

usage and a decrease in the use of organophosphorus pesticides (OP), resulting in detectable amounts of pyrethroid metabolites in population samples [27].

Human fertility rates are known to be decreasing both in developed and developing countries [33]. This reduction has been associated with socioeconomic changes and adverse lifestyle factors [34]. However, pesticide environmental contaminants have attracted international attention and recently came to be considered as possible contributors to human infertility [35].

Environmental exposure to pyrethroids can also adversely impact on pregnancy outcomes and offspring health, including size at birth, neurodevelopment and behavioral problems [30].

A study of exposure to pyrethroid sprays during pregnancy has shown associations of this exposure with autism spectrum disorders (ASD) and developmental delay [36]. Cross-sectional studies also implicate pyrethroids in ASD [37] and Attention Deficit Hyperactivity Disorder (ADHD) [38].

Interestingly, pyrethroid pesticides have structural similarities with thyroid hormones. Nevertheless, in a cohort of women in the first trimester of pregnancy, *Zhang et al.* could not show an association between chemical exposure to pyrethroid pesticides during the early gestation period and maternal thyroid function [22].

Pyrethroid compounds are known to be transported by the placenta, since detectable levels of permethrin were found in cord blood samples collected upon delivery [39].

Two studies were carried out to quantify the pyrethroid metabolite, 3-PBA, in agricultural soil samples from northern Portugal [26, 40].

However, to date, there have been no studies evaluating the levels of

exposure of Portuguese pregnant women to this pesticide neither its impact on neonatal outcomes.

Aims

Due to the lack of relevant studies on pregnancy outcomes associated with iodine levels in Portugal, the main objective of this study was to evaluate the UIC in Portuguese pregnant women in the IoMum cohort and the correlation with pregnancy and birth outcomes (weight, length, and head circumference of the newborn). In addition, exposure to the other chemical elements Pb, Cd, As, Se and pyrethroid pesticide metabolite 3-PBA, was also monitored and associated with the same outcomes.

Materials and Methods

1. Ethical approval

This study was performed according to the protocol approved by the Ethics Committee of São João University Hospital Center (CHUSJoão) / Faculty of Medicine of the University of Porto. Informed written consent was obtained from all study participants.

2. Study design and participants

A prospective observational study was carried out from the IoMum cohort:pregnant women attending their first trimester routine ultrasound scan at CHUSJoão between April 2018 and April 2019 were invited to participate. All women who had a routine ultrasound scan between 10 and 13 weeks plus 6 days of gestation with confirmed fetal vitality and who signed the informed consent form, were included in the study. Exclusion criteria were: levothyroxine intake, twin pregnancy, declaration of informed consent for use of the data of the newborn not approved by the mother and delivery after the 6th of June 2019.

Gestational age was determined from the measurement of the fetal crown-rump length.

At the time of enrollment (timepoint 1, T1) and after informed consent, information was collected on various demographic and lifestyle factors, including: age, area of residence, education, weight and height of the pregnant, gestational age, smoking habits, use of medicines, supplementation with iodine and folic acid and maternal intake of natural sources of iodine (seafood, milk and dairy products). The lifestyle questionnaire was developed specifically for this study

and it was applied in a pilot study to correct for inconsistencies, to assess applicability and to validate data collection protocols.

At this time point T1, a spot urine sample was also collected, and women were invited to a second contact with the IoMum team, from 35 weeks until the end of gestation (time point 2, T2), for additional demographic and lifestyle information collection, spot urine collection and a finger prick blood spot. The urine samples were refrigerated upon collection and transported to the laboratory within the following 24 hours for aliquot creation and freezing at -80 ºC for future analysis.

Information collected at T2 falls outside the scope of this work and so, it will not be further detailed.

In addition, information regarding both maternal and the newborn's clinical details were obtained from the clinical records, including: pregnancy outcomes and complications, mode of delivery, gestational age at delivery, newborn's anthropometric and vitality parameters.

The study design and recruitment flow of the participants are described in figure 2.

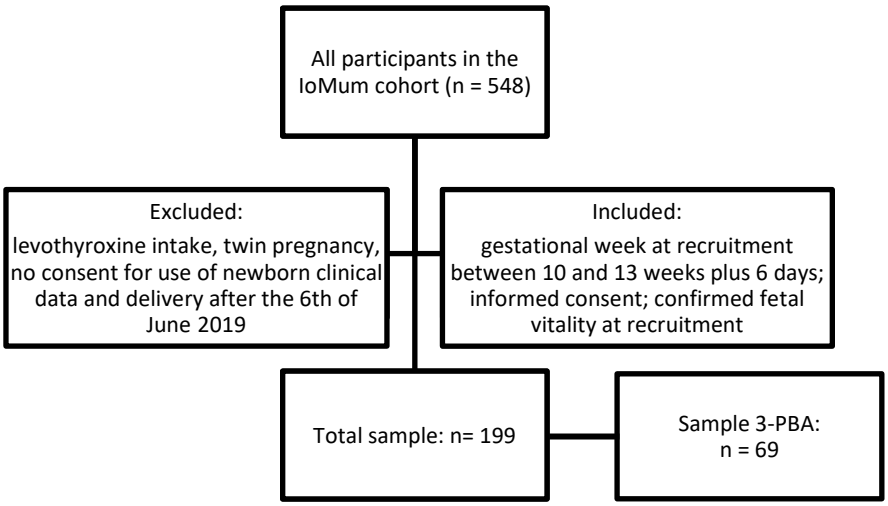

Figure 2- Recruitment flow of the participants

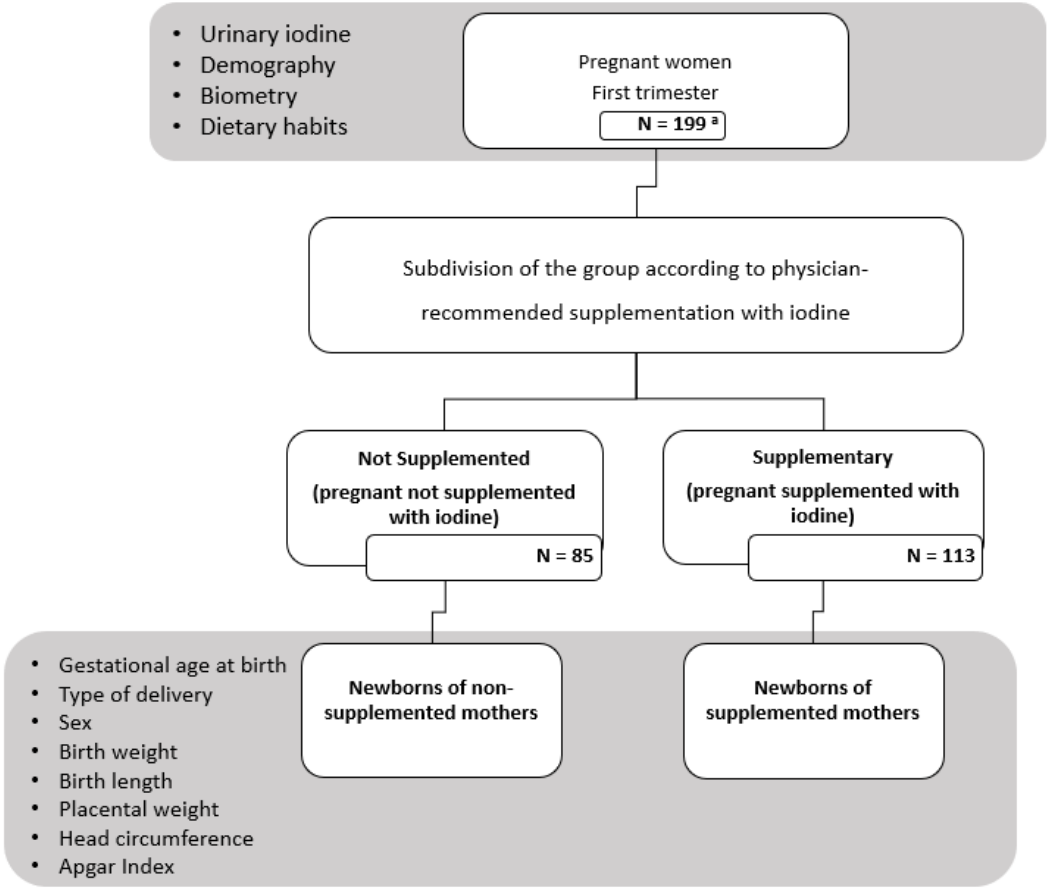

Figure 3 - Study experimental design
[a] The sum of sample sizes of supplemented and non-supplemented participants is 198. Of the 199 participants, one did not give information on iodine supplementation.

3. Biochemical Analysis

3.1 Iodine quantification

Urinary iodine excretion was measured by inductively coupled plasma-mass spectrometry (ICP-MS), according to the method developed by the Centers for Disease Control and Prevention (CDC), as previously described by us [41]. ICP-MS analyses were performed using an iCAP™ Q instrument (Thermo Fisher Scientific, Bremen, Germany), equipped with a MicroMist™ nebulizer, a baffled cyclonic spray chamber (Peltier-cooled), a standard quartz torch, and a two-cone design (nickel sample and skimmer cones).

High purity (99.9997%) argon (Gasin, Portugal) was used as a nebulizar

and as a plasma gas source.

The ICP-MS instrument operational parameters were as described by Costa Leite et al [41]: RF power (1550 W); plasma gas flow (14 L/min); auxiliary gas flow (0.8L/min); nebulizer flow rate (0.95 L/min). The equipment control and data acquisition were made through the Qtegra software (Thermo Fisher Scientific). The iodine (127 I) isotope was monitored for analytical determination, and the tellurium (125 Te) isotope was monitored as an internal standard. The instrument was tuned daily for maximum signal sensitivity and stability as well as for low oxides and doubly charged ion formation using the Tune B iCAP Q solution (Thermo Fisher Scientific; 1 µg/L of Ba, Bi, Ce, Co, In, Li, and U in 2% HNO_3 + 0.5% HCl). All the solutions were prepared with 1.0% (v/v) tetramethylammonium hydroxide, TMAH 25% w/w (Alfa Aesar, Karlsruhe, Germany), 0.01% Triton™ X-100 (Sigma-Aldrich, St. Louis, MO, USA), and 10µg/L Te (Sigma-Aldrich, St. Louis, MO, USA). The calibration curve was obtained with six solutions of iodine concentrations within the 25–1000 µg/L range. The calibration standard solutions were prepared by adequate dilution of the iodine standard (Plasma CAL, SCP Science, Quebec H9X 4B6, Canada). The internal standard solution was added to all samples and to the standard solutions in order to obtain a 10 µg/L final concentration. Urine samples were diluted (10-fold) before analysis. The base urine used in this method was synthetic urine (IRMM- Institute for Reference Materials and Measurements) [42]. For analytical quality control purposes, certified reference materials, Trace Elements Urine, L1 and L2 (Seronorm™, Sero, Billingstad, Norway), were analyzed under the same conditions as the study participants' samples [41].

3.2 Creatinine quantification

Urinary creatinine was quantified as follows: creatinine was enzymatically (creatininase) converted to creatine which was then hydrolyzed by creatinase to produce sarcosine, and this decomposed by the sarcosine oxidase to form glycine, formaldehyde and hydrogen peroxide. The hydrogen peroxide formed produces a blue pigment through the action of peroxidase and by quantitative oxidative condensation with N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) and 4-aminoantipyrine. The creatinine concentration was obtained by measuring the absorbance of the blue color at 596/694 nm [43].

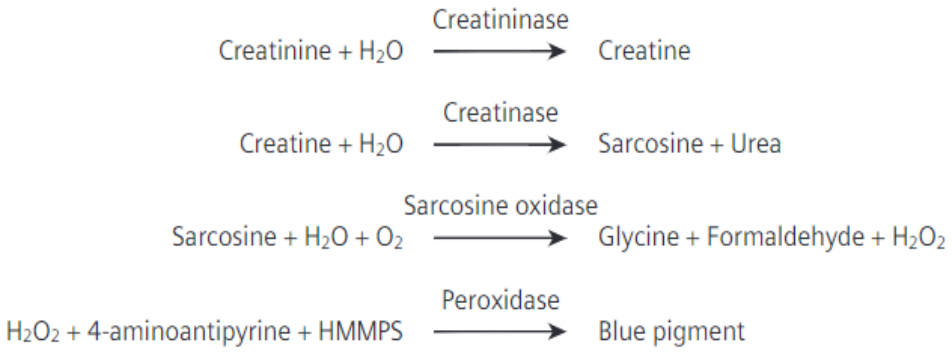

Figure 4- Reaction Equation of creatinine [43]

3.3 Chemical elements quantification

Urinary chemical elements excretion was measured by ICP-MS, according to the method developed by the CDC (Urine Multi-element ICP-DRC-MS: Method 3018.3).

ICP-MS analyses were performed using an iCAP™ Q instrument (Thermo Fisher Scientific, Bremen, Germany), equipped with a MicroMist™ nebulizer, a baffled cyclonic spray chamber (Peltier-cooled), a standard quartz torch, and a two-cone design (nickel sample and skimmer cones). High purity (99.9997%)

argon (Gasin, Portugal) was used as a nebulizar and as a plasma gas source. The ICP-MS instrument operational parameters were as follow: RF power (1550 W); plasma gas flow (14 L/min); auxiliary gas flow (0.8 L/min); nebulizer flow rate (1.02 L/min). The equipment control and data acquisition were made through the Qtegra software (Thermo Fisher Scientific). Four chemical elements were analysed such as lead (208 Pb), cadmium (111 Cd), arsenic (75 As) and selenium (82 Se). The instrument was tuned daily for maximum signal sensitivity and stability as well as for low oxides and doubly charged ion formation using the tune B iCAP Q solution (Thermo Fisher Scientific; 1 µg/L of Ba, Bi, Ce, Co, In, Li, and U in 2% HNO_3 + 0.5% HCl).

Urine samples were diluted (1:10) with a 2% (v/v) HNO_3 solution (prepared with the TraceSELECT® HNO_3, ≥ 69% w/w, Fluka, France). The calibration curve solutions were prepared in 2% (v/v) HNO_3 by proper dilution of the multi-element stock calibration standard solution PlasmaCAL SCP-33-MS (SCP Science, Baie-d'Urfé, Quebec, Canada). The calibration curves included seven points in the range 1-100 µg/L. The internal standard solution was added to all samples and standards solutions in order to obtain a final concentration of 10 µg/L. The base urine used in this method was a synthetic urine (IRMM). Before the ICP-MS analysis, the samples were homogenized using a vortex and centrifuged at 4500 rpm for 3 min. For analytical quality control purposes, certified reference materials (Trace Elements Urine L-1 and L-2 from Seronorm™, Sero, Billingstad, Norway) were analyzed under the same conditions as the samples. Creatinine analysis was also performed in order to adjust analytes concentrations.

3.4 3-PBA quantification

The analysis of 3-PBA was performed by gas chromatography with mass spectrometry (GC-MS), as described [26] 1 µL of sample was injected onto a Thermo Trace-Ultra gas chromatography, coupled to an ion trap mass detector Thermo Polaris, operated in the electron impact ionization at 70 eV. The ion source and the MS transfer temperature were set at 250ºC. Operating in the splitless mode (0.5 min), the helium was used as carrier gas at a constant flow rate of 1.3 mL min^{-1}. The temperature of the injector was 240ºC. The column, a 30 m ZB-5MSi (0.25 mm i.d., 0.25 µm film thickness Zebron-Phenomenex), oven temperature was programmed as described [26]. The analysis was developed in the SIM mode, based on the detection of selected ions for 3-PBA (141, 196 and 364).

Sample preparation was performed by solid-phase extraction (SPE). Briefly, the urine samples were thawed at room temperature. Then a solution of urine in 1.5 ml of deionized water (H_2O), 150 µL of sodium hydroxide (NaOH) (Merck) and 100 µL of the internal standard 2-phenoxy benzoic acid (2-PBA) (Sigma Aldrich) (1.5 ml + 1.5 ml + 150 µL + 100 µL, respectively) of were incubated at 37°C for 15 minutes. After chemical deconjugation, the samples were transferred to the preconditioned SPE columns (Strata-X) (Phenomenex) with 5 mL methanol (MeOH) (Riedel de Haen) and 5mL ammonium acetate (Merck). The columns were then immediately washed with 5mL (MeOH): (H2O) (30/70 V/V). Following a short vacuum pulse to remove excess wash solution, the columns were dried under vacuum for 40 min using the SPE vacuum manifold. Elution was carried out with 5mL of acidified MeOH (2% formic acid) (Carlo Erba), directly into a glass vial. Subsequently, the eluates were concentrated to 50µL

under a gentle stream of nitrogen.

3-PBA derivatization procedure was necessary prior to GC/MS analysis. The derivatization was performed by addition of 30μL hexafluoro-2-propanol (HFIP) (Sigma Aldrich), 20 μL (N, N'-Diisopropylcarbodiimide (DIC) (Sigma Aldrich) and 400 μL of n-hexane to the 50μL of the eluate obtained from the SPE extraction and vortex room temperature during 10 minutes. In the final step, liquid-liquid extraction was performed with 1mL of a 5% aqueous potassium carbonate solution (Panreac) (to neutralize the excess derivatizing agent), shaken 5 minutes in the vortex and finally the supernatant was removed and placed in a vial with insert for injecting into GC/MS. The calibration curves and linear ranges of the detector response for 3-PBA were evaluated by analysing the working standard solutions (15–200 μg L^{-1}, 8 concentrations) in triplicate. In this study, the limit of Detection (LOD) and limit of Quantification (LOQ) were calculated as the minimum amount of analyte detectable with a signal-to-noise ratio (S/N) of 3 and 10, respectively. The linearity of the method was established by setting calibration curves using linear regression analysis over the concentration range. Selectivity was verified by comparing the chromatograms of the standards dissolved in n-hexane, the standards extracted from the spiked urine and the matrix blanks.

4. Categorization of anthropometric data

For the categorization of variables of weight, head circumference and length of the newborn, percentile classification was used [44]. As a result the newborns were classified into 3 categories regarding weight, head circumference and length at birth:

SGA: Small for gestational age (below 10th percentile)

AGA: Suitable for gestational age (between 10th percentile and 90th percentile)

LGA: Great for gestational age (above the 90th percentile).

5. Categorization of professions

The categorization of professions used in this study followed the protocol elaborated by the Portuguese Classification of Professions 2010, abbreviated as CPP/2010, elaborated from the International Classification of Professions 2008 (CITP / 2008) by the Instituto Nacional de Estatisticas, I.P. (INE, I.P.), with the collaboration of the attached entities.

CPP/2010, whose Structure was approved by the 14th Resolution of the Superior Council of Statistics (CSE), of May 5, 2010, later published in the II Series of the Republic Diary No. 106, of June 1, 2010, establishes the new most relevant professions, tasks and functions as part of CITP / 2008, a classification recommended to Member States to produce and disseminate statistics by occupation at European Union (EU) level by the Commission Recommendation of 29 October 2008.

Based on this classification, we further clustered the professions in low exposure and high exposure, according to table A1 (annex 1).

6. Statistical analysis

Descriptive statistics are presented as absolute and relative frequencies for categorical variables, mean and standard deviation (SD) or median and interquartile range (IQR - 25th percentile-75th) for continuous variables,

depending on the symmetry of their distribution.

When testing hypotheses about continuous variables we used non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) considering the hypotheses of non-normality and number of groups. When testing hypotheses on categorical variables, the chi-square test and the Fisher's exact test were used, as appropriate.

Multiple linear regression models were used to analyze the association between exposure to Iodine, Pb, Cd, As, Se and 3-PBA (independent variables) and birth weight, length and head circumference (dependent variables). In these models the following co-variates were considered: smoking habits, maternal age, weight gain in T1, pre-gravid body mass index (BMI), gestational age at delivery and newborn sex. For each model coefficient values (beta) and the corresponding 95% confidence intervals are presented.

The level of statistical significance was set at 5%, so the differences were considered statistically significant whenever $P < 0.05$. Statistical analyzes were performed using SPSS® v.26.0 (Statistical Package for the Social Sciences).

Results

1. Sociodemographic data

Table 4 - Characteristics of the study population.

Residence Area (n, %)			
Maia	59	30.6	
Porto	33	17.1	
Valongo	45	23.3	
Gondomar	18	9.3	
Matosinhos	11	5.7	
Outros	27	14.0	
Maternal education level (n, %)			
Low (≤ 12 years)	94	50.3	
Medium (13 - 15 years)	66	35.3	
High (≥ 16 years)	27	14.4	
Age (n, mean ± SD, years)	198	32.0	± 5.4
Pre-pregnancy BMI (n, median (P25; P75), kg/m^2)	194	23.4	(21.1; 25.7)
minimum (kg/m^2)		15.4	
maximum (kg/m^2)		50.0	
Pre-pregnancy BMI categories (n, %)			
Low weight	10	5.2	
Normal	126	64.9	
Overweight	32	16.5	
Obesity	26	13.4	
Gestational age at recruitment (n, median (P25; P75), weeks)	199	12.0	(12.0; 13.0)
Primiparous (%)	73	39.2	
Preterm (n, %)			
No	187	94.4	
Yes	11	5.6	
Newborn sex (n, %)			
Male	99	49.7	
Female	100	50.3	
Birth weight (n, mean ± SD, grams)	199	3155.5	± 476
Birth Weight classification (n, %)			
SGA[a]	18	9.2	
AGA[a]	174	88.8	
LGA[a]	4	2.0	
UIC (n, median (P25; P75), µg/L)	197	91.8	(51.8; 164.6)
Pb (n, median (P25; P75), µg/L)	189	1.2	(0.4; 2.7)
Cd (n, median (P25; P75), µg/L)	197	0.0	(0.0; 0.1)
As (n, median (P25; P75), µg/L)	189	17.8	(8.4; 44.8)
Se (n, median (P25; P75), µg/L)	188	24.9	(12.8; 44.0)
3-PBA (n, median (P25; P75), µg/L)	69	0.4	(0.2; 0.4)

Table 4 (cont.)

Iodine supplementation, (n, %)		
No	85	42.9
Yes	113	57.1
Beginning of iodine supplementation (n, %)		
Before the 6th week of pregnancy	55	54.5
between the 6th and 12th week of pregnancy	46	45.5
Knowledge about iodized salt, (n, %)		
No	97	50.3
Yes	96	49.7
Preparation of meals with iodized salt, (n, %)		
No	88	82.2
Yes	19	17.8
Folic acid supplementation, (n, %)		
No	14	7.1
Yes	182	92.9
Smoking habits, (n, %)		
Non-Smoking	136	69.0
Smoker	29	14.7
Former smoker	32	16.2

[a] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age
[b] Missing's: between 1 and 4%
[c] Difference between weight on the day of recruitment (T1) and 6 months before, based on self-reported data.

The sociodemographic characteristics of the study population are presented in table 4. Most of the population resides in the municipalities of Maia (30.6%) and Valongo (23.3%), which are the main coverage areas of CHUSJoão.

50.3% of the population has a low level of education (≤ 12 years) while around 14.4% of the population has higher degrees of education equivalent to a Master or PhD degree. The mean age of the participants was 32 years old with the youngest and the oldest participants being 17 and 46 years old, respectively. The median pre-pregnancy BMI was 23.4 kg/m^2 and 64.9% of women were within the normal weight range (18.5 - 25 kg/m^2). This calculation was based on self-reported weight and height of pregnant women 6 months before the day of recruitment.

Regarding number of pregnancies, 39.2% of women were primiparous. Only 5.6% of the pregnancies resulted in preterm births, and in total this cohort gave birth to 99 boys and 100 girls.

The average weight at birth was 3155.5 g and 88.8% of newborns had adequate weight for gestational age. This variable was evaluated by the calculation of percentiles stipulated by the WHO.

The median urinary concentrations of the trace elements were: for iodine 91.75 µg/L, below the WHO recommended iodine adequacy range; for Pb 1.23 µg/L, within the tolerable limit of ≤4 µg/L [45]; for As 17.8 µg/L, within the tolerable limit ≤35 µg/L [45]; for Se 24.95 µg/L, within recommended suitability range 15 to 50 µg/L [45]; and for 3-PBA 0.37 µg/L, above of limit of detection LOD.

Given that most samples were null for cadmium, the respective median is 0.00µg/L and it was not possible to proceed to further analyses with this heavy metal.

Regarding micronutrient supplementation, 42.9% of pregnant women reported not taking iodine supplements and 57.1% confirmed iodine supplementation. Of these, 54.5% started supplementation before the 6th week of pregnancy and 41% reported taking iodine in a multivitamin formulation.

Analysis of knowledge about iodized salt showed that 50.3% of pregnant women reported having heard about iodized salt, while only 17.8% of women reported preparing meals with iodized salt.

Nevertheless, this evidence regarding iodized salt knowledge is weak because further questions about iodized salt products retrieved many incorrect answers (data not shown).

Still, regarding micronutrient supplementation, a great majority of women (92.9%) reported taking a folic acid supplement.

Finally, only 14.7% of women reported being an actual smoker, 16.2% a

former smoker and 69.0% reported being non-smoker. This fact may underlie de low levels of cadmium found in our samples.

Table 5 - Urinary levels of iodine, trace elements and 3-PBA by participant characteristics.

	UIC (µg/l)						Pb (µg/L)						As (µg/L)					
	n	**(%)**	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**P25**	**Median**	**P75**	***P***
Residence Area [a]																		
Gondomar	18	(9)	33.1	96.2	159.0		17	(9)	0.89	1.18	3.03		16	(9)	5.74	9.12	20.30	
Maia	58	(30)	57.8	82.6	123.4		55	(30)	0.45	1.17	3.24		54	(30)	8.82	16.43	37.86	
Matosinhos	10	(5)	34.0	68.0	102.8	0.262 [d]	10	(5)	0.23	1.25	1.94	0.724 [d]	9	(5)	6.90	11.09	42.11	0.075 [d]
Porto	33	(17)	64.0	107.6	215.4		32	(17)	0.44	1.57	3.91		32	(17)	11.71	29.49	53.56	
Valongo	45	(24)	58.0	113.0	184.8		43	(23)	0.44	0.72	2.13		45	(25)	8.27	16.74	47.06	
Other	27	(14)	39.7	75.4	155.5		26	(14)	0.24	0.96	2.09		27	(15)	6.69	22.19	45.04	
Maternal education level,																		
Low (≤ 12 years)	94	(51)	51.4	90.6	172.2		92	(52)	0.47	1.24	2.46		90	(51)	7.63	15.30	36.03	
Medium (12 - 15 years)	64	(35)	44.1	86.2	154.9	0.565 [d]	60	(34)	0.34	1.07	1.97	0.549 [d]	62	(35)	10.19	21.41	47.06	0.326 [d]
High (≥ 15 years)	27	(15)	69.0	100.3	172.0		25	(14)	0.30	1.29	3.91		25	(14)	9.34	15.49	48.34	
Occupation [b]																		
Low exposure	na	na	na	na	Na	Na	140	(74)	0.41	1.23	3.10	0.848 [e]	141	(75)	9.07	17.80	45.04	0.193 [e]
High exposure	na	na	na	na	Na	Na	49	(26)	0.48	1.17	2.13		48	(25)	5.88	16.14	41.18	
Smoking habits																		
Non-Smoker	134	(69)	52.3	87.5	154.3		129	(69)	0.35	1.12	2.29		128	(68)	8.12	16.73	44.31	
Smoker	29	(15)	57.8	118.7	184.7	0.593 [d]	27	(14)	0.60	1.50	1.92	0.318 [d]	28	(15)	7.30	16.76	53.89	0.404 [d]
Former smoker	32	(16)	49.7	93.1	197.7		31	(17)	0.72	1.45	5.65		31	(17)	11.09	26.76	50.24	
Pre-pregnancy BMI categories																		
Underweight	10	(5)	42.8	87.3	155.5		10	(5)	0.54	1.07	1.45		10	(5)	5.61	8.60	37.86	
Normal	124	(65)	57.5	96.7	183.9	0.531 [d]	116	(63)	0.36	1.27	4.02	0.398 [d]	116	(63)	8.69	18.83	46.67	0.535 [d]
Overweight	32	(17)	33.6	82.9	148.3		32	(17)	0.44	1.04	1.51		32	(17)	6.03	15.79	51.07	
Obesity	26	(14)	51.3	90.6	116.6		26	(14)	0.40	1.32	2.14		26	(14)	10.27	19.34	36.03	
Weight gain T1 (kg, mean ± SD)[c]																		
No	43	(22)	47.03	69.1	106.6	0.026 [e]	42	(23)	0.71	1.26	2.66	0.272 [e]	42	(23)	9.00	17.47	44.43	0.971 [e]
Yes	150	(78)	55.22	102.9	172.2		143	(77)	0.35	1.20	2.67		143	(77)	8.22	17.80	45.04	

	Se (µg/L)						3PBA (µg/L)					
	n	**(%)**	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**P25**	**Median**	**P75**	***P***
Residence Area [a]												
Gondomar	17	(9)	6.93	17.57	35.88		6	(9)	0.00	0.29	0.38	
Maia	56	(31)	18.05	30.85	45.87		22	(33)	0.18	0.28	0.39	
Matosinhos	9	(5)	12.79	28.76	44.05	0.334 [d]	4	(6)	0.28	0.38	0.41	0.702 [d]
Porto	32	(17)	11.81	23.15	49.44		13	(20)	0.00	0.34	0.37	
Valongo	43	(23)	11.16	22.94	32.65		13	(20)	0.31	0.37	0.43	
Other	26	(14)	15.15	25.20	39.32		8	(12)	0.27	0.34	0.40	
Maternal education level,												
Low (≤ 12 years)	91	(51)	11.86	23.69	41.25		28	(44)	0.28	0.37	0.40	
Medium (12 - 15 years)	59	(33)	12.79	25.52	38.09	0.945 [d]	23	(37)	0.00	0.28	0.42	0.282 [d]
High (≥ 15 years)	27	(15)	13.12	25.69	40.69		12	(19)	0.19	0.27	0.37	
Occupation [b]												
Low exposure	139	(74)	12.93	25.52	44.85	0.887 [e]	54	(78)	0.22	0.37	0.41	0.285 [e]
High exposure	49	(26)	12.71	23.27	39.16		15	(22)	0.00	0.29	0.37	
Smoking habits												
Non-Smoker	126	(68)	13.12	25.46	40.69		44	(65)	0.18	0.35	0.41	
Smoker	28	(15)	12.46	23.31	39.13	0.625 [d]	13	(19)	0.27	0.37	0.38	0.930 [d]
Former smoker	32	(17)	11.22	26.59	45.82		11	(16)	0.16	0.37	0.39	
Pre-pregnancy BMI categories												
Underweight	10	(5)	8.61	18.81	25.66		2	(3)	0.27	0.33	0.38	
Normal	118	(64)	11.77	24.42	44.85	0.405 [d]	50	(74)	0.22	0.37	0.39	0.918 [d]
Overweight	30	(16)	16.82	31.61	54.71		9	(13)	0.00	0.28	0.39	
Obesity	25	(14)	17.57	25.27	33.64		7	(10)	0.00	0.34	0.52	
Weight gain T1 (kg, mean ± SD)[c]												
No	41	(22)	14.00	25.27	45.60	0.568 [e]	14	(21)	0.12	0.36	0.42	1.000 [e]
Yes	143	(78)	12.79	24.74	44.05		54	(79)	0.18	0.37	0.39	

[a] Classification the residence Area based on Instituto Nacional de Estatística de Portugal I.P., 2014.

[b] Instituto Nacional de Estatística de Portugal I.P., Classificação Portuguesa das Profissões 2010. [c]Calculated difference between the weight on the date of recruitment and the weight 6 months before (both values self-reported).

[d] Kruskal-Wallis; [e] Mann-Whitney; na, Not applicable

Table 5 explores the association between urinary concentrations of iodine, Pb, As, Se or the pyrethroid pesticide metabolite 3-PBA with sociodemographic characteristics.

Median UIC seems to be the highest in Valongo and the lowest in Matosinhos, although the observed differences were not statistically significant.

Maternal education level did not seem to consistently affect iodine status, neither did smoking habits or BMI categories.

Interestingly, women that had gained weight from 6 months before T1 had higher UIC when compared to women that maintained or lost weight in the same period.

Professional occupation categorized by the likelihood of exposure (see annex 1) did not reflect differences in environmental pollutant concentrations. Additionally, pesticide metabolite concentration seemed to be independent of smoking habits, pre-gravid BMI and weight gain.

2. Status of iodine and other chemical elements

Table 6 - Proportion of women by urinary iodine and other chemical elements groups.

			n	%
UIC [a]				
	<150µg/l	Insufficient	142	72.1
	150-249µg/l	Adequate	27	13.7
	250-499µg/l	above requirements	23	11.7
	≥500µg/l	Excessive	5	2.5
Pb [b]				
	≤4µg/l	reference range	153	81.4
	>4µg/l	above reference range	35	18.6
Cd [b]				
	≤1,3µg/l	reference range	195	99.0
	>1,3µg/l	above reference range	2	1.0
As [b]				
	≤35 µg/L	reference range	129	68.3
	≥ 35,1 µg/L	above reference range	60	31.7
Se [b]				
	≤14,99µg/l	below the reference range	55	29.4
	15 - 50µg/l	reference range	100	53.5
	≥ 50,1µg/l	above reference range	32	17.1
3-PBA [c]				
	<0,364µg/l	<LOD	33	47.8
	>0,364µg/l	>LOD	36	52.2

[a] Organization, W.H., Assessment of iodine deficiency disorders and monitoring their elimination, 2007.
[b] Clinic, M. 2019 [cited 2019; Available from: https://www.mayoclinic.org/about-mayo-clinic.
[c] The Fitness for Purpose of Analytical Methods 2014, A Laboratory Guide to Method - Validation and Related Topics: Europa.[46]

Table 6 presents the proportion of women by groups of urinary concentration of iodine and other chemical elements.

For the UIC reference values, most women presented values below the adequate threshold with only 13.7% reflecting adequate iodine status.

It was possible to verify that 52.2% of the mothers presented levels of 3-PBA above the limit of detection for our analytical method.

3. Iodine and other chemical elements and newborn outcomes

Table 7 - Maternal first trimester urinary Iodine status and newborn outcomes.

			UIC (µg/L)				Iodine-to-creatinine (µg/g)				UIC<150 µg/L		UIC≥150 µg/L		
Birth size categories	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**n**	**(%)**	***P***
Birth weight															
SGA[c]	18	(9)	39.2	86.0	195.6		73.8	135.4	240.9		11	(8)	7	(13)	
AGA[c]	172	(89)	51.8	90.8	156.4	0.648 [a]	56.3	96.4	193.3	0.220 [a]	127	(91)	45	(83)	0.315 [b]
LGA[c]	4	(2)	99.6	149.7	176.7		124.4	171.9	248.8		2	(1)	2	(4)	
Birth head circumference															
SGA	12	(7)	52.3	114.4	193.5		98.1	125.0	312.9		8	(6)	4	(8)	
AGA	164	(89)	47.8	82.6	156.4	0.506 [a]	54.4	96.7	198.7	0.250 [a]	121	(91)	43	(84)	0.308 [b]
LGA	8	(4)	76.8	129.2	175.8		66.9	75.7	107.1		4	3	4	(4)	
Birth length															
SGA	12	(6)	73.9	104.7	158.6		60.4	101.7	148.0		9	(6)	3	(3)	
AGA	181	(93)	51.3	91.6	163.8	0.765 [a]	58.6	105.9	209.9	0.265 [a]	130	(93)	51	(51)	0.801 [b]
LGA	1	(1)	66.6	66.6	66.6		29.0	29.0	29.0		1	(1)	0	(0)	

[a] Kruskal-Wallis; [b] Chi-Square

[c] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age

Table 7 presents the variation of urinary iodine levels with neonatal anthropometry percentiles. UIC was not associated with anthropometric categories at birth. Nevertheless, there seems to be a trend for the increase in median UIC with the increase in birth weight percentile, as UIC consistently increases for higher birth weight percentile categories. Also, there seems to be an inverse relationship between the percentiles of length at birth and UIC, as the highest UIC medians are observed for SGA newborns.

When iodine levels were adjusted to creatinine excretion, the observed trends were attenuated.

Table 8 - Maternal first trimester urinary Lead (Pb) levels and newborn outcomes.

			Pb (µg/L)				Pb-to-creatinine (µg/g)				Pb ≤4 µg/L		Pb≥4,1 µg/L		
Birth size categories	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**N**	**(%)**	***P***
Birth weight															
SGA[c]	17	(9)	0.25	0.58	1.25		0.54	0.74	1.67		17	(11)	0	(0)	
AGA[c]	165	(89)	0.45	1.24	3.17	0.061 [a]	0.52	1.29	3.00	0.151 [a]	132	(87)	32	(97)	0.118 [b]
LGA[c]	4	(2)	0.88	1.71	3.57		1.53	3.04	4.66		3	(2)	1	(3)	
Birth head circumference															
SGA[c]	12	(6)	0.32	0.83	1.25		0.39	1.21	3.31		10	(7)	2	(6)	
AGA[c]	158	(89)	0.43	1.25	1.25	0.654 [a]	0.52	1.24	2.82	0.905 [a]	130	(90)	27	(87)	0.686 [b]
LGA[c]	6	(5)	0.47	0.77	5.00		0.54	0.62	3.48		4	(3)	2	(6)	
Birth length															
SGA[c]	12	(6)	0.38	0.66	1.55		0.32	0.74	1.46		11	(7)	1	(3)	
AGA[c]	173	(93)	0.43	1.23	2.66	0.139 [a]	0.53	1.29	3.00	0.122 [a]	141	(93)	31	(94)	0.093 [b]
LGA[c]	1	(1)	11.82	11.82	11.82		5.14	5.14	5.14		0	(0)	1	(3)	

[a] Kruskal-Wallis; [b] Chi-Square

[c] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age [44]

Table 8 presents the variation of urinary Pb levels with neonatal outcomes. This heavy metal was not associated with anthropometric percentile categories at birth. Nevertheless, there was a trend for a positive association between percentiles of birth weight or length with urinary Pb levels which is also observed when Pb levels are adjusted to urinary creatinine excretion.

Table 9 - Maternal first trimester urinary arsenic (As) levels and newborn outcomes.

	As (µg/L)						As-to-creatinine (µg/g)				As ≤35 µg/L		As ≥ 35,1 µg/L		
Birth size categories	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**n**	**(%)**	***P***
Birth weight															
SGA[c]	15	(9)	5.61	13.35	68.90		5.71	34.90	105.37		9	(7)	6	(10)	
AGA[c]	167	(89)	8.54	17.80	44.43	0.550 [a]	9.58	21.72	41.26	0.413 [a]	116	(91)	51	(86)	0.569 [b]
LGA[c]	4	(2)	15.69	36.38	58.08		25.29	40.67	95.30		2	(2)	2	(3)	
Birth head circumference															
SGA[c]	10	(6)	5.64	6.49	29.81		8.27	16.06	88.66		8	(7)	2	(4)	
AGA[c]	158	(89)	8.57	18.04	44.51	0.042 [a]	10.48	22.58	43.38	0.721 [a]	108	(91)	50	(88)	0.142 [b]
LGA[c]	8	(5)	13.46	56.24	99.05		9.22	35.87	103.97		3	(3)	5	(9)	
Birth length															
SGA[c]	12	(6)	1.96	10.02	28.28		2.20	10.03	19.13		10	(8)	2	(3)	
AGA[c]	173	(93)	8.82	18.18	47.06	0.173 [a]	11.04	22.98	45.96	0.014 [a]	116	(91)	57	(97)	0.552 [b]
LGA[c]	1	(1)	11.29	11.29	11.29		4.91	4.91	4.91		1	(1)	0	(0)	

[a] Kruskal-Wallis; [b] Chi-Square

[c] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age

Table 9 presents the variation of urinary As levels with neonatal anthropometric percentiles at birth. As levels were positively associated with birth head circumference percentile categories, as LGA babies are born from mothers that had higher urinary As levels in the first trimester of pregnancy. When adjusted for urinary creatinine, this positive association was also observed, although with no statistical significance.

In addition and surprisingly, birth length percentile categories were associated with arsenic-to-creatinine levels, with the adequate length for gestational age with the highest levels of As-to-creatinine (22.9%).

Finally, birth weight percentiles tended to be positively associated with As levels, although with no statistical significance.

Table 10 - Maternal first trimester urinary Selenium (Se) levels and newborn outcomes.

			Se (µg/L)				Se-to-creatinine (µg/g)				Se≤14,99µg/L		Se15 - 50µg/L		Se ≥50,1µg/L		
Birth size categories	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	***P***
Birth weight																	
SGA[c]	16	(9)	10.18	21.51	45.20		19.75	30.01	61.08		5	(9)	9	9	1	(3)	
AGA[c]	165	(89)	13.05	25.39	40.69	0.777 [a]	16.14	26.44	45.64	0.627 [a]	47	(87)	88	90	30	(94)	0.068 [b]
LGA[c]	4	(2)	9.71	30.69	54.67		20.39	48.93	79.12		2	(4)	1	1	1	(3)	
Birth head circumference																	
SGA[c]	12	(6)	12.01	24.29	32.22		17.45	26.06	53.55		4	(8)	8	9	0	(0)	
AGA[c]	155	(89)	12.93	24.48	41.25	0.125 [a]	15.12	27.86	44.93	0.863 [a]	46	(88)	81	89	27	(87)	0.068 [b]
LGA[c]	8	(5)	26.50	51.48	81.81		12.27	42.47	80.21		2	(4)	2	2	4	(13)	
Birth length																	
SGA[c]	10	(6)	16.50	22.93	24.05		16.61	22.94	29.80		2	(4)	7	7	1	(3)	
AGA[c]	174	(93)	12.93	25.61	44.85	0.269 [a]	16.23	28.44	47.57	0.205 [a]	51	(94)	91	93	31	(97)	0.475 [b]
LGA[c]	1	(1)	7.74	7.74	7.74		3.37	3.37	3.37		1	(2)	0	0	0	(0)	

[a] Kruskal-Wallis; [b] Chi-Square

[c] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age

Table 10 presents the variation of urinary Se levels with neonatal outcomes; overall, this trace element was not associated with anthropometric categories at birth.

Nevertheless, there is a trend for a positive association between maternal Se levels and birth weight percentile categories. Indeed, median urinary Se tends to increase for increasing categories of birth weight. This relationship is devoid of statistical significance.

Additionally, there seems to be a relationship between head circumference and Se levels, as the median urinary Selenium (and Se-to-creatinine) increases considerably for LGA babies.

4. 3-PBA and newborn outcomes

Table 11 - Maternal first trimester urinary 3-PBA levels and newborn outcomes.

			3-PBA (µg/L)				3-PBA-to-creatinine (µg/g)				≤LOD		>LOD		
Birth size categories	**n**	**(%)**	**P25**	**Median**	**P75**	***P***	**P25**	**Median**	**P75**	***P***	**n**	**(%)**	**n**	**(%)**	***P***
Birth weight															
SGA[d]	7	(10)	0.00	0.27	0.37		0.00	0.31	0.49		4	(13)	3	9	
AGA[d]	57	(85)	0.22	0.37	0.40	0.430 [a]	0.17	0.30	0.56	0.777 [a]	26	(81)	31	89	0.676 [c]
LGA[d]	3	(4)	0.00	0.31	0.39		0.00	0.27	0.63		2	(6)	1	3	
Birth head circumference															
SGA[d]	4	(7)	0.14	0.33	0.41		0.25	0.73	0.99		2	(7)	2	6	
AGA[d]	55	(90)	0.22	0.37	0.40	0.794 [a]	0.15	0.30	0.56	0.550 [a]	25	(93)	30	88	0.433 [c]
LGA[d]	2	(3)	0.37	0.38	0.39		0.17	0.22	0.27		0	(0)	2	6	
Birth length[e]															
SGA[d]	3	(4)	0.00	0.43	0.54		0.00	0.26	0.31		1	(3)	2	0.1	
AGA[d]	64	(96)	0.18	0.37	0.39	0.419 [b]	0.15	0.31	0.58	0.346 [b]	31	(97)	33	0.9	0.609 [c]

[a] Kruskal-Wallis; [b] Mann-Whitney; [c] Chi-Square

[d] SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age

[e] LGA category had a sample size of 0 and thus it was not considered for this analysis.

Table 11 presents the variation of urinary 3-PBA levels with neonatal outcomes; the pyrethroid pesticide metabolite described is not associated with anthropometric categories at birth.

5. Multiple linear regression models

Table 12 -Multiple linear regression models for the association between anthropometric measures at birth and categories of maternal first trimester urinary concentrations of iodine, lead, arsenic, selenium and 3-PBA.[e]

	Birth weight			Head circumference			Birth length		
	beta	**95 % CI**	***P***	**beta**	**95 % CI**	***P***	**Beta**	**95 % CI**	***P***
UIC [a]									
<150 µg/L	62.9	(-48.4, 174.2)	0.268	0.123	(-0.293, 0.539)	0.562	0.178	(-0.317, 0.674)	0.480
≥150 µg/L	Ref			0			0		
Pb [b]									
≤4 µg/L	Ref			Ref					
>4 µg/L	180.6	(51.6, 309.6)	0.006	0.337	(-0.145, 0.818)	0.171	0.676	(0.098, 1.253)	0.022
As [b]									
≤35 µg/L	Ref			Ref					
>35 µg/L	14.0	(- 90.1,118.2)	0.792	0.028	(-0.359, 0.415)	0.888	0.132	(-0.345, 0.608)	0.588
Se [b]									
<15 µg/L	17.811	(-93.739, 129.361)	0.754	-0.127	(-0.548, 0.294)	0.555	0.037	(-0.457, 0.530)	0.884
15 - 50 µg/L	Ref			Ref			Ref		
>50 µg/L	175.394	(41.854, 308.935)	0.010	0.592	(0.089, 1.095)	0.021	0.491	(-0.100, 1.082)	0.103
3-PBA [c]									
<0,364µg/l	Ref			Ref					
>0,364µg/l	36.7	(-111.9,185.3)	0.629	0.301	(-0.28, 0.883)	0.309	-0.349	(-0.993, 0.294)	0.287

[a] Organization, W.H., Assessment of iodine deficiency disorders and monitoring their elimination, 2007.
[b] Clinic, M. 2019 [cited 2019; Available from: https://www.mayoclinic.org/about-mayo-clinic.
[c] The Fitness for Purpose of Analytical Methods 2014, A Laboratory Guide to Method - Validation and Related Topics: Europa
[e] Covariates: smoking, maternal age, T1 weight gain, pre-pregnancy BMI, gestational age at birth and sex of the newborn

Table 12 presents multiple linear regression analyzes for the association between anthropometric measurements at birth and categories of maternal urinary concentrations in the first trimester of iodine, Pb, As, Se and 3-PBA, with adjustment for the covariates: smoking, maternal age, T1 weight gain, pre-pregnancy BMI, gestational age at birth and sex of the newborn. It was possible to verify that the trends observed in the previous association analyses gained statistical significance, namely, regarding the association of maternal Pb and Se levels and anthropometric measures at birth. Indeed, the group of babies born to mothers with Pb levels above the reference value (4 µg/L) had at birth on average more 180.6 grams or 0.676 centimeters of length than babies born to mothers with low levels of Pb in the urine ($p = 0.006$ and $p = 0.022$ respectively).

Additionaly, the group of babies born to mothers with Se levels above the reference value (50 µg/L) had at birth on average more 175 grams or 0.592 centimeters of head circumference than babies born to mothers with low Se levels in the urine ($p = 0.010$ and $p = 0.021$ respectively).

Discussion

The present study evaluated the UIC in Portuguese pregnant women in the IoMum cohort and the association with neonatal outcomes (weight, length, and head circumference at birth). In addition, exposure to the pyrethroid pesticide metabolite 3-PBA or to the trace elements Pb, Cd, As and Se was also monitored and associated with the same outcomes.

Median UIC in our sample population was 91.8 µg/L, which points to an inadequate iodine status. In fact, analysis of iodine levels according to the WHO criteria for iodine status in pregnant women showed that a majority (72.1%) of women had insufficient iodine intake, and only 29% presented with UIC values above the lower threshold for adequacy.

The data reported by Limbert in a study conducted between 2005 and 2007 in Portugal, showed that only 17% of women had values of UIC above 150 µg/L, reflecting that in the last 12 – 15 years the panorama has undergone few changes with a slight increase in the proportion of iodine sufficiency in pregnant women. That study determined UIC values in 3631 pregnant Portuguese women across the country, and it pointed to widespread insufficient iodine intake in mainland Portugal, with a median value of 84.9 µg/L, and presented a median UIC of 77.2 µg/L, for 222 participants who were attended at the Santo Antônio Hospital in Porto [11]. Although there seems to be an increase in the median UIC in our study when compared to Limbert's, we cannot establish direct comparisons since the population analysed may not have the same characteristics and the methods for iodine quantification are different in the two studies.

Indeed, the Limbert study [11] used a method of rapid colorimetry for UIC

determination, which reduces the robustness of the analysis when compared to our study, which used the ICP-MS, the reference method for its reliability and sensitivity to determine urinary iodine concentration in the population.

Importantly however, the Limbert study [11] was conducted before the implementation of the DGS guideline for iodine supplementation in pregnant women, in 2013 [9], and this could explain the slight increase in the proportion of sufficient iodine intake (from 17% in the Limbert`s study to 27.9% in the present study). Also of note, we could observe that more than half of our sample population (57.1%) reported taking iodine supplements. In this context, a proportion of 72.1% of insufficiency is surprising.

When we further analyze the 57.1% of the population reporting iodine supplementation, we can observe that of these, only 62.0% reported having taken the supplement on the same day of urine collection. Moreover, 41.0% reported taking multivitamin supplements containing iodine, instead of iodine only supplements. These facts may account for the discrepancy between the proportion of iodine supplemented (57.1%) and of iodine sufficient (27.9%) pregnant women. Indeed, median UIC of the group of women reporting having taken the supplement in the same day of urine collection was 153.5 µg/L while the median UIC of the other portion of the population who reported taking iodine supplements but who had not taken the supplement in the day of urine collection was 81.8 µg/L.

Supplementation of mothers with iodine before conception or early pregnancy may result in children with better cognitive performance. If iodine supplementation is started before pregnancy in women with maternal iodine deficiency, better thyroid function will be observed [47].

At first, supplementation may not be as effective in an iodine-deficient population. The beneficial effects of iodine on offspring development may be lost if supplementation begins after 10 to 20 weeks gestation [47].

In our study, 17.8% of women reported using iodized salt for meal preparation at home, and these women revealed a median UIC of 104.9 µg/L when compared to 82.6 µg/L in women reporting no use of iodized salt.

It is thus not surprising that in Portugal, where iodized salt use is one of the lowest in Europe, a high prevalence of iodine deficiency among pregnant women is observed, as already suggested by Costa Leite, *et al.* [41].

In view of better nutritional guidance on iodine supplementation Guess K., *et al.* found that while some health professionals recommend women with iodine supplements during pregnancy, less than half of them were aware of the dose recommended by the National Council for Medical and Health Research (NHMRC). As a result, women may not be getting adequate amounts of iodine from their supplements, and this may increase the risk of inadequate or excessive intake and potential adverse health consequences [48]. We cannot exclude that similar situation may be happening in Portugal.

Regarding anthropometric parameters at birth, we found no association between iodine status and categories of birth weight, head circumference or length. These results corroborate studies performed in other countries showing that UIC does not affect these parameters. For example, Snart et al. have collected spot urines from 541 pregnant women before 15 weeks of gestation, in three major cities of the UK and they found no association between UIC and birth weight centile [49]. Also, a recent systematic review failed to show a clear effect of iodine adequacy on growth parameters of the offspring [50].

Nevertheless, some studies did show an association between UIC and anthropometric parameters at birth. A prospective cohort study in Bangladesh with 1617 women, found that maternal UIC up to 1.0 µg/L in early pregnancy was positively associated with birth weight, length and head circumference in male newborns. For boys, an increase of 0.5 µg/L in maternal UIC corresponded to an increase in fetal weight at birth, body length and head circumference of 70 g ($p = 0.019$), 0.41cm ($p = 0.013$) and 0.28cm ($p = 0.031$), respectively [51].

In another population-based study with 657 pregnancies, conducted in the city of Sabadell, Spain, women with UIC in the 3rd trimester in the range of 100 to 149 µg/L had a lower risk of having a SGA newborn when compared to women with UIC below 50 µg/L [8].

So, given the diversity of reported results, the effect of iodine on neonatal anthropometric outcomes should be further explored.

In relation to the other chemical elements Pb, Cd, As and Se, median levels were found to fall in correspondent reference ranges. Nevertheless, 18.6 31.7 and 17.1% of women were found to have levels of Pb, As and Se respectively, above the correspondent reference ranges. Trace element exposures are usually associated with consumption of contaminated foods, professional occupation or area of residence. In this study, we could not find a variation of trace element exposure with the occupation, most likely because of difficulties in categorizing levels of exposure through professional occupation. On the other hand, and despite the absence of statistical significance median urinary concentration of these trace elements seemed to vary according to the municipality of residence. For example, As was found to be 3 times higher in Porto than in Gondomar which had the lowest median. These facts deserve

further attention.

On the other hand, exposure to cadmium was found to be very low, as the median was 0,00 µg/L and only 1% of the population fell above the reference range. As such, no association was found between Cd exposure and its main determinant – smoking habits.

In fact a small proportion of the population (14.7%) was found to be an actual smoker an this fact may explain at least in part the low levels of cadmium found in our population

Bocca B., *et al*. suggest that early determination of essential and toxic elements and antioxidant status in newborns, by measuring serum and urinary levels, is essential to help prevent consequences for postnatal health through diet and/or micronutrients [52].

In this study, crude analysis between exposures and anthropometric percentiles at birth showed no associations, except for maternal urinary As concentration which showed to increase with increasing birth percentiles of head circumference. To date, no studies were found correlating arsenic levels and macrocephaly.

Nevertheless, when association analyses were performed with adjustment to smoking, maternal age, T1 weight gain, pre-pregnancy BMI, gestational age at birth and sex of the newborn, lead showed positive associations with birth weight and length and selenium showed a positive association with head circumference at birth.

These results were unexpected. Indeed, a study conducted in Korea in 2017 that assessed the relationship of lead with birth outcomes and explored 782 newborn infants found a significant negative association between Pb pregnancy

blood levels and birth weight and head circumference [53]. Additionally, in 2017 a study in Japan designed to examine the effects of prenatal exposure to hazardous chemicals failed to show an association with birth weight in a cohort of 489 mother and newborn pairs [54].

In the framework of our results, we cannot exclude the possibility that lead could contribute to weight gain above normality. Nevertheless, a greater sample size is needed to confirm this suspicion.

Regarding selenium exposure, Wai et al. failed to show an association between third trimester maternal selenium urinary concentration and birth weight [54].

On the other hand, a systematic review published in 2014 reported that preterm infants with low birth weight presented the lowest Se levels [55]. Our study adds to this evidence, proposing that high levels of Selenium are associated with higher birth weight and head circumference. Alltogether, these evidence reinforce the importance of Se for proper growth.

Analysis of results for 3-PBA samples in pregnant women showed concentrations above LOD in 52.2% of the sample population. However, no significance was found for associations between pyrethroid pesticide metabolite levels and the anthropometric profile of newborns at birth.

A prospective cohort study conducted in northern China, started in 2010, aimed at linking pesticides and other environmental exposures with the health of pregnant women and their children. Surprisingly, it was observed that the total levels of pyrethroid metabolites in the mother's urine maternal urine were positively associated with birth weight and head circumference [30].

Berkowitz et al. (2004) found no significant association between newborn

anthropometric measures (birth weight, length and head circumference) and maternal 3-PBA urinary in early pregnancy [56].

The creatinine corrections of all samples were presented, however, we mainly focus the present discussion on UIC that is the WHO reference, and there were no major changes in results when compared to those adjusted for creatinine. Indeed, in most studies, assessments are made with UIC rather than with iodine-to-creatinine.

One main limitation of this study was sample size. Although iomum cohort in CHUSJoão has a total valid sample size of 485, this particular substudy was limited to 199 participants corresponding to the mother-newborn pairs obtained until June 2019. In addition, the total sample size for pyrethroid exposure analyses (n = 69) was generated by samples collected during the summer of 2018. This period was selected because at this time the population may be more exposed to pesticides such as mosquito insecticides and tend to ingest more fresh vegetables which may be contaminated by pesticides used in agriculture.

Another limitation refers to our difficulty in categorizing professional occupation as a determinant of environmental exposures. In fact, information of professional occupation was extracted from clinical registries with no possible association with the likelihood of exposure.

Several strengths of our study should be mentioned. First of all, we simultaneously evaluated the concentrations of iodine, Pb, Cd, As, Se and 3-PBA in maternal urine as biomarkers of prenatal exposure. There are few studies in the literature reporting such broad analyses.

Additionally, although the recruitment was performed at CHUSJoão, our study design elicited the study of pregnant women coming from routine ante-natal

care surveillance, and it was not restricted to pregnant women in hospital consultation and thus with pregnancy pathologies.

In conclusion, the present study characterized iodine status in a cohort of Portuguese pregnant women, showing that slightly more than half of the women were iodine supplemented and, despite that, a great proportion had insufficient iodine intake.

Moreover, this study evidenced that pregnant women are exposed to a variety of environmental toxics that may affect fetal growth trajectories.

Further studies should be performed to confirm this data and to analyze the impact of these exposures on longer term childhood outcomes.

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Annex

1. Annex 1 - Categorization of professions [a]

Representatives of the legislative and executive bodies, directors, managers and executive managers	LE[b]
Specialists in intellectual and scientific activities	LE
Middle level technicians and professions	LE
Administrative staff	LE
Personal, security and safety personnel and vendors	HE[c]
Farmers and skilled workers in agriculture, fisheries and forestry	HE
Skilled workers in industry, construction and craftsmen	LE
Plant and machine operators and assembly workers	LE
Unskilled workers	HE
Unemployed	LE
Student	LE
Outros	LE

[a]Adapted, Instituto Nacional de Estatística de Portugal I.P., Classificação Portuguesa das Profissões 2010.
[b] Low exposure; [c] High exposure

2. Annex 2 - Classification of variables used in the present study

Cardinal variables
• (3-PBA) to creatinine ratio at T1, in µg/g - calculated using Creatinine T1 g/L and Levels 3-PBA • As to creatinine at T1, in µg/g - calculated using Creatinine T1 g/L and As T1 • birth length percentile for gestational age and gender, calculated using the Peditools tool • birth length z-score at birth, for gestational age and gender, calculated using the Peditools tool • birth weight percentile for gestational age and gender, calculated using the Peditools tool • birth weight z-score for gestational age and gender, calculated using the Peditools tool • birth weight, in grams, obtained by clinical record whenever possible. When the registry was missing, it was obtained by online form or by telephone • concentration of 111Cd in µg/L • concentration of 208Pb in µg/L • concentration of 3PBA in µg/L • concentration of 65As in µg/L • concentration of 82 Se in µg/L • gestational age at birth obtained by clinical record whenever possible. When there wasn´t available data (births occurring outside CHSJoão), gestational age at birth was calculated from gestational age at T1 and date of birth obtained by online form or by telephone • gestational age at T1, obtained from the clinical records • head circumference at birth, in centimeters, obtained by clinical record whenever possible. When the registry was missing, it was obtained by online form or by telephone • head circumference percentile at birth for gestational age and gender, calculated using the Peditools tool • head circumference z-score at birth for gestational age and gender, calculated using the peditools tool • iodine to creatinine ratio at T1, in µg/g - calculated using Creatinine T1 g/L and UIC T1 • ioduria (UIC) in µg/L • maternal age at T1, calculated in years from date of birth (self-reported) and the date of recruitment • maternal height in cm • maternal weight in kg at T1 (self-reported) • maternal weight in kg, 6 months before T1 (self-reported) • mother's age • number of pregnancies including the current one • Pb to creatinine at T1, in µg/g - calculated using Creatinine T1 g/L and Pb T1 • pre-pregnancy maternal body mass index calculated using maternal weight 6 months before T1 (in kg) and maternal height (in m); • Se to creatinine a T1, in µg/g - calculated using Creatinine T1 g/L and Se T1 • weight gain in T1 corresponding to the difference between maternal weight at T1 and maternal weight 6 months before T1

Ordinal variables
• 111Cd µg/L level categories • 208Pb µg/L level categories • 3-PBA level categories • 65As µg/L level categories • 82Se µg/L level categories • categories of birth head circumference percentiles • categories of birth length percentile • categories of birth weight percentiles • categories of UIC • Iodine supplementation initiation • maternal area of residence • maternal BMI categories (self-reported) • maternal educational level (self-reported) • maternal educational level category

Nominal variables
• folic acid supplementation • iodine supplementation • knowledge of iodized salt • maternal smoking habits • preparation of meals with iodized salt

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