U.PORTO

FM UP **FACULDADE DE MEDICINA**
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

Ana Emanuel Pinho Dias

Urinary levels of toxic metals and pregnancy and newborn outcomes: biomonitoring of the IoMum cohort

Março, 2023

FMUP

U.PORTO

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Ana Emanuel Pinho Dias

Urinary levels of toxic metals and pregnancy and newborn outcomes: biomonitoring of the IoMum cohort

Mestrado Integrado em Medicina

Área: Ciências médicas e da saúde

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Doutora Elisa Oliveira Braga Keating

E sob a Coorientação de:

Doutora Virgínia Maria Monteiro da Cruz Fernandes e
Doutor Agostinho Almiro de Almeida

Trabalho organizado de acordo com as normas da revista:
Biological Trace Element Research

Março, 2023

FMUP

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Eu, Ana Emanuel Pinho Dias, abaixo assinado, nº mecanográfico 201603831, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 9/01/2023

Assinatura conforme cartão de identificação:

Ana Emanuel Pinho Dias

UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Ana Emanuel Pinho Dias

NÚMERO DE ESTUDANTE	E-MAIL
201603831	anadias0197@hotmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Ciências médicas e da saúde

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Urinary levels of toxic metals and pregnancy and newborn outcomes: biomonitoring of the IoMum cohort

ORIENTADOR

Elisa Oliveira Braga Keating

COORIENTADOR (se aplicável)

Virgínia Maria Monteiro da Cruz Fernandes e Agostinho Almiro de Almeida

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 9/01/2023

Assinatura conforme cartão de identificação: _Ana Emanuel Pinho Dias_

Aos meus queridos pais,
por me darem todos os meios para que eu conseguisse alcançar o meu sonho, por me
apoiarem e amarem incondicionalmente.

Aos amigos que me acompanharam ao longo destes desafiantes e enriquecedores 6 anos,
pelas memórias de uma vida.

Urinary toxic levels and pregnancy and newborn outcomes: results of the IoMum cohort biomonitoring

Ana Dias [1], Beatriz Marques [1], Isabella Bracchi [1, 2], Juliana Guimarães [1, 2], Cláudia Matta Coelho [1, 2], Cátia Pinheiro [1, 3], Carmo Prucha [4, 11], Cristina Martins [4], Edgar Pinto [5,], Rui Azevedo [5], Cristina Delerue-Matos [6], André Moreira-Rosário [7], Luís Filipe Ribeiro de Azevedo [7], Cláudia Camila Dias [7], Conceição Calhau [2, 8], João Costa Leite [9, 11], Catarina Rodrigues [2, 10], Diogo Pestana [10], Virgínia Cruz Fernandes [2, 6], Agostinho Almeida [5, 11], Carla Ramalho [4, 12, 13,] , Elisa Keating [1, 2]

[1] Dept. Biomedicine – Unit of Biochemistry, Faculty of Medicine, University of Porto, Portugal

[2] CINTESIS@RISE, Faculty of Medicine, University of Porto

[3] Departamento de Dietética e Nutrição, Universidade do Algarve, Faro, Portugal

[4] Department of Obstetrics, Centro Hospitalar Universitário S. João, Porto, Portugal

[5] REQUIMTE/LAQV – Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

[6] REQUIMTE/LAQV – Instituto Superior de Engenharia, Politécnico do Porto, Portugal

[7] Department of Community Medicine, Information and Health Decision Sciences (MEDCIDS), Faculty of Medicine, University of Porto, Portugal

[8] Nutrition & Metabolism, NOVA Medical School│FCM, Universidade Nova de Lisboa, Portugal

[9] Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

[10] Nutrition & Metabolism, NOVA Medical School│FCM, Universidade Nova de Lisboa, Portugal

[11] CINTESIS@RISE, Nova Medical School, Universidade Nova de Lisboa

[12] Department of Ginecology-Obstetrics and Pediatrics, Faculty of Medicine, University of Porto, Portugal

[13] Instituto de Investigação e Inovação em Saúde, i3S, Universidade do Porto, Portugal

Abstract

This study aimed to evaluate the association between exposure of pregnant women to bismuth (Bi), thallium (Tl), nickel (Ni), and antimony (Sb) and sociodemographic characteristics of the study sample, pregnancy outcomes, and anthropometric parameters of the newborn.

This was a prospective study based on the IoMum cohort. Pregnant women undergoing routine first-trimester ultrasounds at Centro Hospitalar São João, from April 2018 to April 2019, were invited to participate. Inclusion criteria were gestational age ≥ 10 and < 14 weeks, confirmed foetal vitality, and informed consent signature. Spot urine samples (n=349) were analyzed using ICP-MS to quantify metals concentrations.

Median urinary metal concentrations were, in µg/L: Bi 0.02, Tl 0.02, Ni 4.3, and Sb 0.04. Tl and Bi excretion were associated with residence area, with higher values in Valongo. Maternal age was positively associated with excretions of Bi, Sb, and Tl. Bi, Ni, and Sb excretion decreased from normal weight to obese women (pre-pregnancy BMI). Occupation as a health professional was associated with higher excretion of Bi and Ni. Occupations categorized as "high exposure" (including industry workers, cooks, and housekeeping and hairdressing professionals) were associated with higher excretions of all the metals. Tl excretion increased with the frequency of fish consumption and with increasing anthropometric categories of weight, head circumference, and length at birth.

The present study showed that some professions may increase the exposure of pregnant women to the studied toxic metals. Despite this, the levels of exposure are not of concern regarding possible impact on maternal or newborn health.

Keywords: Pregnancy, Newborns, Anthropometry, Heavy metals, Occupational exposure;

List of Abbreviations

AGA, Appropriate for gestational age

BMI, Body mass index

CDC, Centers for Disease Control and Prevention

CHUSJoão, Centro Hospitalar Universitário São João

CITP/2008, International Classification of Professions 2008

CPP/2010, Portuguese Classification of Professions 2010

FDA, Food and Drug Administration

GC-MS, Gas chromatography mass spectrometry

GDM, Gestational diabetes mellitus

IARC, International Agency for Research on Cancer

ICP-MS, Inductively coupled plasma mass spectrometry

LGA, Large for gestational age

LOD, Limit of detection

UMC, Urinary metal concentration

SGA, Small for gestional age

T1, Timepoint 1

T2, Timepoint 2

WHO, World Health Organization

Introduction

Exposure to metals during pregnancy

Individuals are naturally subject to exposure to different metals[1] through daily intake of food and water, as well as inhalation of airborne particulate matter. Moreover, different occupational activities may represent an additional and specific source of exposure. An overload of these metals in the human body can have important adverse health effects [1].

There are several metals that are biochemically important and must be ingested continuously to maintain health. Some examples of these nutritionally essential metals are iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu). On the other hand, several metals, such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), have no known biological function and are toxic even when present in the human body in very low concentrations[1].

The physiological adaptations that occur during pregnancy increase the demand for essential trace elements, as well as increase the susceptibility to the harmful effects of toxic metals[2]. Pregnancy and fetal development are seen as a specific time window of vulnerability, during which the toxic effects of heavy metals can result in disturbed fetal development and poor pregnancy outcomes, such as reduced fetal growth, impaired neurological development, congenital malformation, spontaneous abortion, stillbirth, preterm birth and low birth weight [1].

Most studies on the effects of natural exposure to metals are epidemiological studies in which the degree of exposure of the general population is evaluated, together with the associated effects [1]. Studies on specific populations (e.g., pregnant women), however, are quite scarce – and particularly in the case of Portugal, there are almost no studies on the exposure to toxic metals of pregnant women, as well as the consequences on fetal health and pregnancy outcomes.

Below we review the main sources and possible known consequences of exposure regarding the four metals that were the focus of our study: bismuth, thallium, antimony, and nickel.

---

[1] Strictly speaking, some of the elements referred to throughout this text (e.g., arsenic), including one of the elements that was the subject of this study (antimony), are not metals but "metalloids" (since they present some characteristics of metals but others of non-metals). However, for the purposes of simplifying writing, the generic designation of "metals" will always be used.

[2] Often collectively referred to as "heavy metals", since physiochemically most of them are in fact heavy metals.

Bismuth

Bismuth (Bi), atomic number 83, is the element at position 69 in terms of abundance in the Earth's continental crust. It is a heavy metal, found as the free metal or in combined forms as bismuth oxide and bismuth sulfide. Since it is a naturally occurring element, individuals can be exposed to Bi through diet and environmental exposure [2]. Despite being a heavy metal, Bi is considered as having low toxicity particularly when compared with neighbor heavy metals such as mercury, thallium and lead [3].

In fact, Bi has been used for the treatment of medical conditions such as oral and upper respiratory tract infections, syphilis, diarrhea, heartburn (pyrosis), dyspepsia (indigestion), gastroesophageal reflux and peptic ulcer disease [2]. Of note is its use in the eradication of *Helicobacter pylori* from gastrointestinal tract. *H. pylori* infection remains a major health problem today, especially in developing countries and increasing resistance to antibiotics has compromised the effectiveness of standard triple therapy (a proton pump inhibitor plus clarithromycin and amoxicillin or metronidazole), and there is a need to resort to more complex therapeutic regimens, which include bismuth. Thus, quadruple therapy (with Bi) is the recommended first-line treatment in areas with a high prevalence of clarithromycin resistance [4]. The most used Bi compounds for this purpose are bismuth subsalicylate and bismuth subcitrate [2]. So, Bi-containing drugs can be an important source of exposure to this heavy metal.

On the other hand, Bi is used in several technological and industrial applications: soldering, metallurgical additives, atomic research, as a non-toxic substitute for lead in the manufacture of ceramic glazes, fishing drains, food processing equipment, free machining brass for plumbing applications, greases, crystal ware, pearlescent pigments and even cosmetic products [2].

Despite – as referred above - systemic toxic effects associated with the use of Bi compounds are rarely observed because its systemic absorption in the gastrointestinal tract is practically null, however, administration of Bi compounds in significantly high concentrations can lead to several toxic manifestations in the humans, including nephropathy, encephalopathy, osteoarthropathy, gingivitis, stomatitis, colitis, and hepatitis. In the vast majority of cases, these effects are reversible after cessation of exposure to Bi [2].

Overexposure to Bi during pregnancy is known to associate with pregnancy disorders [5]. For example, Cabrera-Rodriguez *et al.* have shown that exposure to Bi above the 95th percentile (0.04 ng/mL), measured in 471 cord blood samples, was associated with low birth weight, which is a clinical parameter considered as an indicator of various biological aggressions, both in the newborn and in adulthood [6]. Furthermore, Bi is considered possibly unsafe during breastfeeding [7].

As already mentioned, bismuth subsalicylate is very often used to treat diarrhea, particularly travelers' diarrhea [2]. It is contraindicated in pregnancy, as it has been associated with prolonged

gestation and delivery, increased perinatal mortality, decreased birth weight and neonatal hemorrhage [8]. Administered in late pregnancy, it may increase the risk of pulmonary hypertension in newborns due to ductus arteriosus constriction [7].

Thallium

Thallium (Tl), atomic number 81, is a post-transition metal, rare in nature, although more abundant than Bi [9]. It occurs in the environment mainly as monovalent cation (Tl^+) [10] and is one of the most toxic metals [11].

Exposure to Tl is a serious threat to human health and occurs mainly through contaminated food and drinking water [11]. It can also occur by skin absorption and inhalation [9]. After exposure, Tl readily reaches the bloodstream and is transported throughout the body, accumulating in the bones, kidneys, and nervous system [12]. In toxicokinetic terms, Tl toxicity takes place in three main stages: a) intravascular distribution stage – in which, during the first 4 hours after exposure, Tl is distributed to the organs through the blood; b) central nervous system (CNS) distribution stage – which occurs within 4 to 48 hours after exposure; c) elimination stage – a slow phase that begins about 24 hours after exposure and is accomplished primarily by urinary and fecal excretion [9].

Sources of environmental Tl include coal combustion, semiconductor manufacturing and vehicle exhaust emissions [13]. Tl is used as a poison for rats and ants, and since it is odorless and tasteless, it has become an accidental poisoning as well as a criminal poison in some cases [9]. To this day, Tl is seen in rodenticide and insecticide. Beyond that, in Africa, Tl has been used as a pesticide, which has led to food contamination [9].

Despite the known harmful effects of Tl, the U.S. Food and Drug Administration (FDA) has approved the use of the Tl-201 isotope as a radiopharmaceutical for some nuclear medicine exams, mainly in myocardial and parathyroid imaging. Adverse effects reported so far include severe allergic reactions and gastrointestinal disturbances [14].

Because Tl poisoning is rare, it is often misdiagnosed or diagnosed late [15]. However, acute toxic exposure is known to result in gastrointestinal, neurological, and dermatological manifestations. Chronic toxic exposure also leads to the same manifestations, but neurological symptoms persist even when Tl blood levels begin to decline after cessation of exposure. Lower limb Involvement has been most frequently reported as persistent. Patients exposed to Tl may also experience cardiovascular symptoms such as tachycardia and hypertension [9].

Exposure to Tl during early pregnancy has been directly associated with the risk of preterm birth, and there appears to be a sex-specific effect on this pregnancy outcome [16].

Additionally, an association between exposure to Tl in early pregnancy and the risk of developing gestational diabetes has also been described. This association was dependent on age [17]. On the other hand, maternal levels of Tl in urine during pregnancy has been shown to be related to low birth weight, but further studies on prenatal Tl exposure are needed to confirm this finding [18].

Nickel

Nickel (Ni) is a silver-white transition metal with high thermal and electrical conductivity. It is resistant to air, water, and alkali corrosion, but it is readily soluble at pH < 6.5 in dilute oxidizing acids. It is the 24th most abundant element in the Earth's crust, where it is found in combination with antimony, arsenic, and sulfur. Ni can exist in several oxidation states, Ni^{2+} being the most widespread in the environment and biological systems [19].

Currently, Ni is not considered an essential nutrient for humans because there is no documented biochemical function that specifically and exclusively depends on it, and low amounts of the element in the diet has not been shown to disrupt the life cycle [20]. On the other hand, many Ni compounds are important environmental and occupational toxicants [21].

Regarding its metabolism in the human body, Ni forms a complex that reacts with molecular oxygen to produce free radicals, which eventually justifies its toxicity [22].

Ni occurs naturally in the atmosphere due to volcanic activity, forest fires and rock weathering. Human exposure to this metal is mainly due to various industrial processes such as food processing, mining, and refining, as well as the use of stainless steel, jewelry, electrical devices, and medical implants. The burning of fossil fuels causes atmospheric levels of Ni to increase significantly. Tobacco smoke is also an important source of exposure. Thus, humans are exposed to Ni either by inhalation, oral ingestion and skin contact [21].

The most serious consequences of exposure to Ni compounds are nose and lung cancer, which highlights the importance of exposure to the metal by inhalation. For this reason, some Ni compounds have been classified as Group 1 Carcinogens by the International Agency for Research on Cancer (IARC) [21]. In addition, manifestations of non-malignant respiratory diseases may also occur. Ni exposure is associated with asthma, chronic bronchitis, emphysema, pulmonary edema, pulmonary fibrosis, bronchoconstriction and reduced lung function, with factory workers exposed daily to Ni fumes being at particular risk [21].

Exposure to Ni in early pregnancy has been shown to be associated with an increased risk of gestational diabetes mellitus [23]. The metabolic alterations resulting from the toxicity of this metal can lead to a decrease in embryonic vitality and, thus, affect fetal development [24].

Antimony

Antimony (Sb) is a medium-hard, silver-white metalloid [25]. It can have different oxidation states, but is usually found in the oxidation states +3 and +5 [26].

This metalloid is often combined with other metals to create hardened alloys to be used in solder, sheet metal, pipes, metal bearings, castings and ammunition [25]. It is used in many industries, most notably in plastics, fire-retardants, lead-acid batteries, polyethylene terephthalate (PET) production and pump sealants. In addition to occupational exposure, individuals are also exposed to environmental Sb through water, air and soil [26].

Sb is considered as probably carcinogenic [26]. Acute toxic exposure to Sb via oral, dermal or inhalation routes can lead to toxic side health effects, such as vomiting, diarrhea, joint and muscle pain and electrocardiogram changes [26]. Despite its known toxicity, pentavalent Sb remains the mainstay of leishmaniosis treatment [27].

Regarding the risks associated with exposure to Sb during pregnancy, it is known that higher levels of exposure increase the risk of developing gestational diabetes mellitus [28]. Exposure to Sb during pregnancy also appears to reduce fetal growth [29].

Aims

There is a lack of relevant studies on exposure to toxic metals and their consequences on fetal health and pregnancy outcomes, particularly in Portugal.

Thus, the main objectives of this study were:

(1) to evaluate the level of exposure to the four toxic metals Bi, Tl, Ni and Sb in pregnant women form the IoMum cohort, based on their urinary concentration;

(2) to investigate the correlation between exposure levels and sociodemographic characteristics of the study sample;

(3) to investigate the correlation between exposure levels and pregnancy outcomes and anthropometric parameters of newborns.

Materials and Methods

Ethical approval

The data used for the IoMum study were obtained according to the research protocol (ClinicalTrials.gov #NCT04010708) that was subsequently approved by the Ethics Committee of Centro Hospitalar Universitário de São João (CHUSJoão) / Faculty of Medicine of the University of Porto (FMUP) (ClinicalTrials.gov #NCT04010708).

Written informed consent was obtained from all participating pregnant women after they were informed about the purpose of this study as well as all the consequent procedures.

Study design and participants

Pregnant women who underwent routine first-trimester ultrasound at CHUSJoão during the period from April 2018 to April 2019 were invited to participate in the IoMum cohort study. Based on this cohort, a prospective observational study was subsequently performed.

Pregnant women with confirmed gestational age between 10 weeks and 13 weeks plus 6 days, with confirmed foetal vitality on the day of recruitment, and who agreed to sign the informed consent form were included in the study. The gestational age of each pregnant woman was assessed by measuring the crown-rump length and fetal rump length. Thus, 548 pregnant women were enrolled at baseline.

Exclusion criteria were gestational age at recruitment ≤10 and >14 weeks, unsigned standard informed consent, unsigned newborn informed consent, and twin pregnancy. After the exclusion criteria were applied, the final study sample became 352 participants, and 349 urine samples were analyzed for use in the study.

At the time of enrollment (timepoint 1, T1), participants received a questionnaire to assess various demographic and lifestyle factors. This questionnaire included questions such as age, area of residence, education level, pre-pregnancy weight, height, gestational age, primiparity, smoking habits (and the number of cigarettes smoked per day, if applicable), and frequency of some types of food (including fish and dairy) consumption.

The questionnaire was developed specifically for this study and was applied to a pilot study to correct for eventual inconsistencies, evaluate its applicability, and validate data collection protocols.

Still, at T1, a spot urine sample was collected. In addition, pregnant women were also invited to have a second contact with the IoMum team members from 35 weeks to the end of their pregnancy (timepoint 2, T2). All the information collected in Timepoint 2 is outside the scope of this study, and therefore, it will not be detailed further.

The urine samples that were collected at T1 were transported to the laboratory within the next 24 hour, where they were aliquoted and frozen at -80 ºC to allow for the possibility of using them in later analyses.

Clinical information about the newborns and pregnancy of the women who participated in the study was taken from clinical records, and included: maternal occupation, pregnancy outcomes and complications, type of delivery, gestational age at delivery, and newborn´s anthropometry.

The flowchart of recruitment and inclusion of participants in the study is described in Figure 1
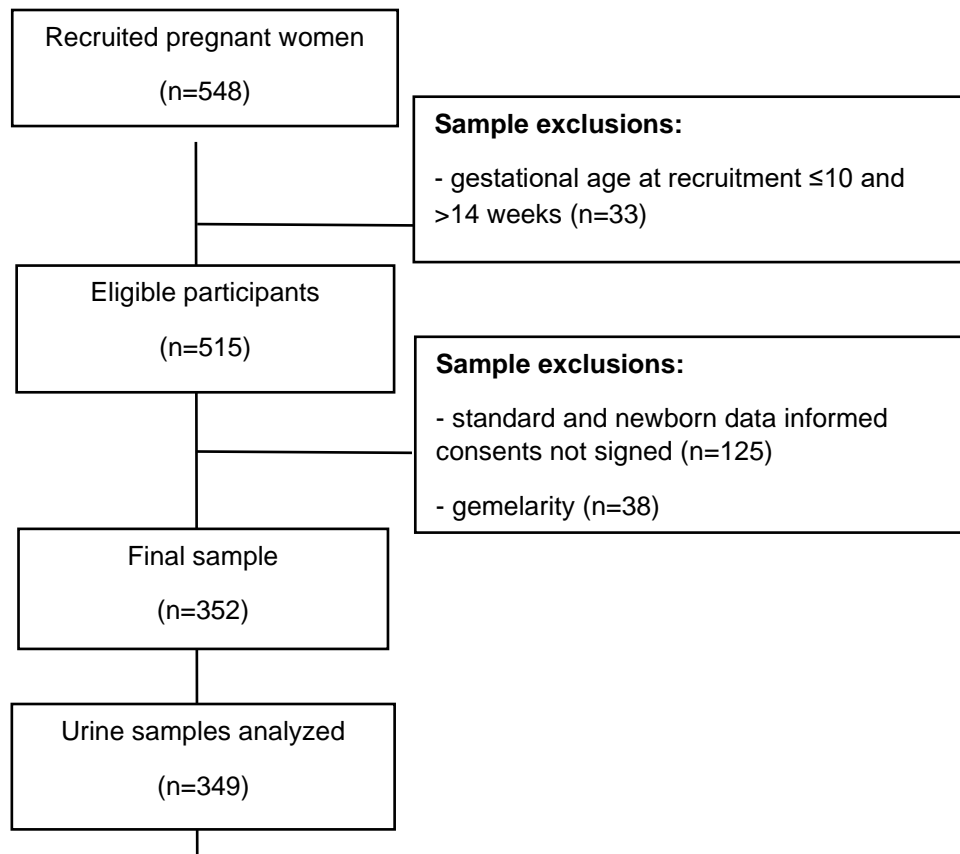
```
                    ┌─────────────────────────────────┐
                    │   Recruited pregnant women      │
                    │                                 │
                    │           (n=548)               │
                    └─────────────────────────────────┘
                                    │
                                    │          ┌──────────────────────────────────────────┐
                                    │          │ Sample exclusions:                       │
                                    ├──────────│                                          │
                                    │          │ - gestational age at recruitment ≤10 and │
                                    │          │ >14 weeks (n=33)                         │
                                    │          └──────────────────────────────────────────┘
                    ┌─────────────────────────────────┐
                    │     Eligible participants       │
                    │                                 │
                    │           (n=515)               │
                    └─────────────────────────────────┘
                                    │
                                    │          ┌──────────────────────────────────────────┐
                                    │          │ Sample exclusions:                       │
                                    │          │                                          │
                                    ├──────────│ - standard and newborn data informed     │
                                    │          │ consents not signed (n=125)              │
                                    │          │                                          │
                                    │          │ - gemelarity (n=38)                      │
                                    │          └──────────────────────────────────────────┘
                    ┌─────────────────────────────────┐
                    │         Final sample            │
                    │                                 │
                    │           (n=352)               │
                    └─────────────────────────────────┘
                                    │
                    ┌─────────────────────────────────┐
                    │     Urine samples analyzed      │
                    │                                 │
                    │           (n=349)               │
                    └─────────────────────────────────┘
                                    │
```

Figure 1. Flowchart of recruitment and inclusion of participants in the study. Sample size (n) for each group is given.

Biochemical Analysis

Urinary Creatinine

Determination of urinary creatinine concentration was performed in a certified laboratory by the measurement of the absorbance at 596/694nm of the blue pigment produced from creatinine by a cascade of reactions.

First, there is the enzymatic conversion of creatinine to creatine by creatininase. Next, creatine is hydrolyzed by creatinase to produce sarconise. Subsequently, sarconise is transformed by sarcosine oxidase to produce glycine, formaldehyde, and hydrogen peroxide.

Finally, peroxidation of hydrogen peroxide together with quantitative oxidative condensation with N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) and 4-aminoantipyrine leads to the production of the blue pigment [30].

Urinary levels of toxic metals

There is extensive evidence that urine is a suitable specimen for assessing metal exposure [31]. So, the urinary excretion of each metal was measured using inductively coupled mass spectrometry (ICP-MS), according to the method developed by the CDC´s Urine Multi-element ICP-DRC-MS: Method 3018.3 [32].

Using a CETAC ASX-520 autosampler (Teledyne CETAC Technologies), diluted urine samples were introduced into the iCAP™ Q ICP-MS instrument (Thermo Scientific) through a MicroMist™ nebulizer and a baffled cyclonic spray chamber carried by a flowing stream of high purity argon. In addition, by coupling radiofrequency energy to the high purity argon, plasma was created.

At the plasma, due to the high thermal energy, the matter is first atomized and then the atoms become ionised. The ions then enter the mass spectrometer where the individual isotopes of the metals are separated (in the quadrupole) and detected. Detection of the ions results in electrical signals that are converted into digital information.

All instrument control, including signal processing and calculation of results (concentration), was performed using the Qtegra™ ISDS Software (Thermo Scientific).

Urine samples were diluted (1:10) with a 2% (v/v) HNO_3 solution. Standard calibration solutions were prepared in 2% (v/v) HNO_3 by appropriate dilution of the multi-element stock standard solution PlasmaCAL SCP-33-MS. Also, to create the calibration curves, synthetic urine (IRMM) was used. Prior to ICP-MS analysis, diluted samples were vortexed and centrifuged at 4500 rpm for 3 min.

The parameters used in the ICP-MS were RF power of 1550 W, plasma gas flow at 14 L/min, auxiliary gas flow of 0.8 L/min, and nebulizer flow of 1.02 L/min. Using ICP-MS, the four metals were analyzed using the following isotopes (m/z ratio): bismuth (^{209}Bi), thallium (^{204}Tl), nickel (^{59}Ni), and antimony (^{122}Sb).

For analytical quality control purposes, Seronorm™ Trace Elements Urine L-1 and L-2 (from Sero, Norway) were analyzed under the same conditions as the samples.

BMI calculation and categorization

The calculation of body mass index (BMI) calculation was based on pre-pregnancy weight and height which were self-reported by pregnant women in the questionnaires and was calculated by dividing weight, in kilograms, by the square of height, in meters. Subsequently, participants were divided into 4 different BMI categories according to the WHO classification [30]:

⸺ Underweight: BMI <18.5;
⸺ Normal: BMI [18.5 – 24,9];
⸺ Overweight: BMI [25.0 – 29,9];
⸺ Obese: BMI >30.0.

Occupational exposure categorization

To categorize occupational exposure, the Portuguese Classification of Professions 2010 (CPP/2010) was used, based on the International Classification of Occupations 2008 (ISCO/2008) by the Instituto Nacional de Estatistica, I.P. (INE, I.P.). This defines the most relevant new professions, tasks, and functions as part of CIPT/2008.

Using this classification as a reference and checking all occupational sources of metals described in the literature to date, the high-exposure occupational category was defined.

This new variable includes all the health professionals, such as doctors, nurses, dentists, psychologists, health technicians, nutritionists, pharmacists, and healthcare assistants. In addition, it also includes aestheticians, hairdressing professionals, warehouse operators, , industry workers, cooks and housekeeping professionals.

Anthropometric data categorization

The Categorization of newborns´ anthropometric variables (weight, length and head circumference) was based on percentiles stipulated in the WHO child growth standards.

The newborn´s anthropometric data used in this study (weight in grams, head circumference in cm, and length in cm) were converted into percentiles adjusted for gestational age at birth based on Fenton´s 2013 growth charts [33]. Subsequently, they were categorized into 3 growth categories:

- SGA: Small for gestational age (birthweight below the 10th percentile).

- AGA: Appropriate for gestational age (birthweight between 10th percentile and 90th percentile)
- LGA: Large for gestational age (birthweight above the 90th percentile)

Statistical Analysis

Continuous variables that presented normal distribution were described using mean and standard deviation (SD), while continuous variables with non-normal distribution, namely urinary metals concentrations (UMC) were described as absolute frequencies (n), median, and interquartile range [25th percentile (P25); 75th percentile (P75)]. The normality of Continuous variables´ was tested by evaluating the symmetry of their histograms.

Furthermore, categorical variables were described using absolute frequencies (n) and relative frequencies (%).

To perform the statistical analysis, the UMC values below the limit of detection (LOD) were imputed as $LOD/\sqrt{2}$. Additionally, UMC were adjusted for urinary creatinine concentration and adjusted values were used for the analysis.

To test for differences in the median of UMC, the variables were properly categorized. Subsequently, the non-parametric Kruskal-Wallis or Mann-Whitney tests were applied, depending on the number of categories.

Statistical significance was set at 5%. Therefore, when $p<0.05$, the differences were considered statistically significant differences were considered statistically significant.

Analysis of all data used in this study was performed using IBM SPSS version 27™ software.

Results

15

Sociodemographic characteristics

Table 1 presents the sociodemographic characterization of the study sample.

Most of the population resided in Valongo (28%, n=93), Maia (26%, n=88) and Porto (18%, n=60). These are considered the principal municipalities covered by the CHUSJoão area.

The mean (SD) age of the participating women was 32 (5) years.

About 40% of the women were primiparous and 56% (n=175) had a BMI prior to pregnancy in the normal range (18.5 - 25 kg/m^2). Of the remaining women, 40% (n=125) had a pre-pregnancy BMI above the normal range, with 24% (n=76) being overweight and 16% (n=49) being obese; and only a small minority (5%, n=15) were underweight.

The mean (SD) variation in the participants´ gestational weight (the difference between weight at term and weight before pregnancy) was 14 (6) kg.

Regarding education, 52% (n=175) of the sample had higher degrees of education (≥13 years), 33% (n=112) had a medium level (10-12 years), and only 14% (n=48) had a low level of education (≤ 9 years).

In addition, about 96% (n=307) of the participants were employed and 18% (n=57) were health professionals (doctors, nurses, dentists, psychologists, health technicians, nutritionists, pharmacists, and healthcare assistants). Also, 34% (n=107) of the participants had a high-exposure occupation.

Only 17% (n=59) of the participants were "pregnancy smokers", i.e., they were current smokers at the time the questionnaire was administered or had stopped smoking within the first trimester of their pregnancy, at the time of the enrollment. About 5% (n=17) of the smoking participants reported smoking more than 5 cigarettes per day.

Referring to the intake of cow's milk per week, 58% (n=197) reported consuming 4 or more times. Furthermore, 69% (n=224) reported eating fish between 1 to 3 times per week.

As a result of their pregnancies, 52% (n=174) were females and 48% (n=161) were male babies. Analyzing the anthropometric parameters length, weight, and head circumference, the percentage of newborns with appropriate values for gestational age was 91% (n=307), 90% (n=300), and 85% (n=276), respectively.

Looking at the hospital's clinical records, it was possible to see that 50% (n=169) of deliveries were eutocic, 22% (n=73) vacuum-assisted, and 28% (n=94) were by cesarean section.

Besides that, 16% of pregnant women had complications in pregnancy, including: gestational diabetes mellitus (GDM) (8%, n=24), pre-eclampsia (2%, n=6), fetal growth restriction (4%, n=11), infection (0.3%, n=1), pre-term delivery (6%, n=17), and/or spontaneous fetal loss (0.3%, n=1). It was also found that only a small number had a foetus with malformations (2%, n=5) and complications at birth (1% n=4).

Finally, we could note that the urinary evaluation presented the following median urinary concentrations (P25; P75) (in µg/L): bismuth 0.02 (0.02; 0.02); thallium 0.02 (0.02; 0.018); nickel 4.13 (2.29; 12.09); antimony 0.04 (0.04; 0.04).

Table 1. Sociodemographic characteristics of the study sample.

Residence area, n (%)			
	Maia	88	(26)
	Porto	60	(18)
	Valongo	93	(28)
	Outros	95	(28)
Age, n (mean ± SD), years		348	(32±5)
Maternal education level, n (%)			
	Low (≤9 years)	48	(14)
	Medium (10-12 years)	112	(33)
	High (≥13 years)	175	(52)
BMI prior to pregnancy categories, n (%)			
	Underweight	15	(5)
	Normal	175	(56)
	Overweight	76	(24)
	Obesity	49	(16)
Gestational weight variation, n (mean ± SD), kgs		294	(14±6)
Employed			
	Yes	307	(97)
	No	11	(3)
Health professional, n (%)			
	Yes	57	(18)
	No	261	(82)

Table 1. (cont.)

High-exposure occupation, n (%)

Yes	107	(34)
No	211	(66)

Primiparity, n (%)

Yes	134	(40)
No	198	(60)

Smoking habits, n (%)

Non smoker	241	(70)
Pregnancy smoker (current + former ≤ 2 months)	59	(17)
Former smoker (>2months)	45	(13)

Number of cigarettes per day, n (%)

0 cigarettes per day	286	(87)
1 to 5 cigarettes per day	24	(7)
> 5 cigarettes per day	17	(5)

Frequency of ingestion of cow milk, n (%)

< 3 times per month	82	(24)
1 – 3 times per week	62	(18)
4 ou > times per week	197	(58)

Frequency of ingestion of fish, n (%)

< 3 times per month	52	(16)
1-3 times per week	224	(69)
4 ou > times per week	50	(15)

Anthropometric parameters of the newborns

Newborn sex n (%)

Male	161	(48)
Female	174	(52)

Birth length

SGA (small for gestational age)	25	(7)
AGA (adequate for gestational age)	307	(91)
LGA (large for gestational age)	4	(1)

Birth weight

SGA (small for gestational age)	26	(8)
AGA (adequate for gestational age)	300	(90)
LGA (large for gestational age)	9	(3)

Birth head circumference

SGA (small for gestational age)	28	(9)
AGA (adequate for gestational age)	276	(85)
LGA (large for gestational age)	21	(6)

Table 1. (cont.)

Pregnancy outcomes

Type of delivery, n (%)

Eutocic	169	(50)
Vacuum	73	(22)
Cesarian	94	(28)

Pregnancy complications, n (%)

No	251	(84)
Yes	49	(16)

Delivery complications, n (%)

No	290	(99)
Yes	4	(1)

Urinary metal concentration

	n	median (P25; P75)
Bismuth (n, median (P25; P75), in µg/L)	349	0.02 (0.02; 0.02)
Thallium (n, median (P25; P75), in µg/L)	349	0.02 (0.02; 0.18)
Nickel (n, median (P25; P75), in µg/L)	349	4.13 (2.29; 12.09)
Antimony (n, median (P25; P75), in µg/L)	349	0.04 (0.04; 0.04)

Missing data interval, n (%): 0-55 (0-16)

Toxic metals urinary concentrations

Table 2 presents a summary of the results obtained for the 4 metals that were the object of this study, indicating the minimum, maximum and median value, and the 5th (P5), 25th (P25), 75th (P75) and 95th (P95) percentiles. Urinary metal concentrations (µg/L) were adjusted for urinary creatinine concentration (g/L), so that results are expressed as µg of metal / g of creatinine.

Table 2 also presents the distribution of pregnant women by the three levels of urinary concentration considered: <LOD, between LOD and P95; above P95.

LOD (the lowest urinary concentration detectable in the ICP-MS analytical procedure used) were (in µg/L): 0.02 for Bi, 0.02 for Tl, 0.46 for Ni and 0.05 for Sb.

The detection rates (defined as the percentage of samples with an urinary metal concentration ≥LOD) were: 10.9% for Bi, 47.6% for Tl, 98.3% for Ni and 16.1% for Sb.

Table 2. Summary of the results obtained in the determination of the urinary metals concentration normalized for urinary creatinine concentration (µg of metal / g of creatinine), and distribution of pregnant women by three levels of urinary concentration (<LOD, between LOD and P95; above P95).

	n	%	Min	5th	25th	Median	75th	95th	Max
						Percentiles (µg/g)			
Bi			0.00	0.01	0.01	0.02	0.04	1.14	29.28
< LOD	311	(89)							
LOD to P95	21	(6)							
> P95	17	(5)							
Detection rate	38	(11)							
Tl			0.01	0.01	0.03	0.06	0.17	0.70	3.20
< LOD	183	(52)							
LOD to P95	149	(43)							
> P95	17	(5)							
Detection rate	166	(48)							
Ni			0.24	1.29	2.42	4.37	14.36	86.33	969.22
< LOD	6	(2)							
LOD to P95	326	(93)							
> LOD	17	(5)							
Detection rate	343	(98)							
Sb			0.02	0.02	0.03	0.05	0.09	0.18	0.75
< LOD	293	(84)							
LOD to P95	39	(11)							
> LOD	17	(5)							
Detection rate	56	(16)							

Charcacteristics of the study sample and exposure to toxic metals

Table 3 shows the association between urinary metal levels adjusted for urinary creatinine (µg metal / g creatinine) and sociodemographic characteristics of the study sample.

Median urinary Tl excretion was associated with residence area, with higher values for pregnant women residing in Valongo, compared to other municipalities. Also, exposure to Bi tended to be higher in Valongo compared to Porto or Maia, but this association was not statistically significant. No other metal showed association with residence area.

Increasing maternal age was associated with higher urinary excretions. This association is statistically significant for Bi and Sb, and marginally significant for Tl (p=0.052).

Regarding BMI, Bi, Ni, and Sb urinary excretion consistently decreased from normal weight to obese women. This association was statistically significant for Bi (p=0.015) and marginally significant for Sb (p=0.061).

Being unemployed was not associated with urinary metal excretion.

Occupation as a health professional was associated with higher urinary excretion of Bi and Ni, but the latter association was only marginally significant (p=0.055). Occupations categorized as "high exposure" were also associated with higher excretions, but this association was marginally significant for Tl, Ni and Sb (p values of 0.050, 0.069 and 0.055, respectively).

Regarding maternal education level, urinary Ni excretion appeared to decrease consistently with higher levels of education. However, the differences were not statistically significant.

Maternal urinary Ni excretion tended to increase consistently with smoking habits, but the association was not statistically significant. No other consistent associations were found between metal excretion and smoking habits, and there was no association between the number of cigarettes smoked and metal excretion.

Urinary Tl excretion appeared to increase consistently with frequency of fish consumption, with participants consuming fish 4 or more times a week having a higher median excretion (0.12 µg/g) than those consuming fish less than 3 times a month (0.04 µg/g). No other metal was associated with fish consumption, and none was associated with milk consumption.

Table 3. Sociodemographic characteristics of the study population sample and exposure to toxic metals, expressed as urinary metal concentration adjusted for urinary creatinine.

	n	(%)	Bi (µg/g) P25	Median	P75	p-value	Tl (µg/g) P25	Median	P75	p-value	Ni (µg/g) P25	Median	P75	p-value	Sb (µg/g) P25	Median	P75	p-value
Residence Area (n, %)						0.097[a]				0.032[a]				0.242[a]				0.692[a]
Maia	88	(26)	0.0127	0.0174	0.0363		0.03	0.066	0.19		2.48	3.95	8.28		0.033	0.049	0.083	
Porto	60	(18)	0.0098	0.0143	0.0304		0.03	0.072	0.25		2.74	6.91	23.7		0.031	0.053	0.108	
Valongo	93	(28)	0.0141	0.0209	0.0334		0.03	0.074	0.22		2.21	4.47	14.53		0.036	0.059	0.084	
Other	95	(28)	0.0128	0.0212	0.0495		0.02	0.047	0.11		2.38	4.07	15.07		0.033	0.053	0.101	
Age categories						**0.012[a]**				**0.052[a]**				0.387[a]				**0.011[a]**
<30 years	126	(36)	0.0112	0.0171	0.0286		0.02	0.046	0.14		2.43	4.32	14.19		0.031	0.047	0.075	
30-35 years	129	(37)	0.0131	0.0204	0.0389		0.03	0.061	0.20		2.38	4.01	10.99		0.034	0.054	0.089	
>35 years	93	(27)	0.0132	0.0237	0.0472		0.03	0.081	0.19		2.46	4.88	25.15		0.036	0.065	0.110	
Employed						0.104[b]				0.895[b]				0.730[b]				0.306[b]
No	11	(3)	0.0086	0.0119	0.0245		0.03	0.065	0.19		2.17	6.4	22.56		0.022	0.044	0.070	
Yes	307	(97)	0.0123	0.0193	0.0384		0.02	0.061	0.17		2.40	4.27	13.92		0.033	0.052	0.089	
Healthcare professional						**0.014[b]**				0.773[b]				0.055[b]				0.120[b]
no	261	(82)	0.0119	0.0180	0.0346		0.02	0.061	0.17		2.31	4.25	11.79		0.033	0.049	0.087	
yes	57	(18)	0.0145	0.0241	0.0495		0.03	0.061	0.18		2.79	5.53	25.96		0.033	0.061	0.101	

23

Table 3 (cont.)

	n	(%)				p				p				p				p
High-exposure occupation						**0.009**[b]				0.050[b]				0.069[b]				**0.055**[b]
no	211	(66)	0.0112	0.0175	0.0334		0.02	0.055	0.15		2.21	4.06	14.22		0.032	0.049	0.084	
yes	107	(34)	0.0138	0.0221	0.0456		0.03	0.08	0.27		2.95	5.31	14.19		0.035	0.056	0.106	
Maternal education level (n, %)						0.082[a]				0.504[a]				0.787[a]				0.219[a]
Low (≤ 9 years)	48	(14)	0.0124	0.0182	0.0368		0.02	0.049	0.18		2.72	5.36	12.99		0.035	0.05	0.087	
Medium (10 - 12 years)	112	(33)	0.0115	0.0172	0.0273		0.02	0.057	0.21		2.37	4.37	15.55		0.032	0.049	0.078	
High (≥ 13 years)	175	(52)	0.0125	0.0212	0.0402		0.03	0.061	0.17		2.31	4.07	14.75		0.033	0.056	0.092	
Pre-pregnancy BMI categories (n, %)						**0.015**[a]				0.772[a]				0.350[a]				0.061[a]
Underweight	15	(5)	0.013	0.024	0.221		0.03	0.074	0.17		2.4	3.1	9.59		0.032	0.061	0.071	
Normal	175	(56)	0.013	0.021	0.039		0.02	0.061	0.16		2.58	4.71	14.53		0.036	0.056	0.101	
Overweight	76	(24)	0.011	0.02	0.039		0.03	0.072	0.23		2.25	4.3	19.41		0.03	0.049	0.082	
Obesity	49	(16)	0.009	0.014	0.025		0.02	0.06	0.18		2.11	3.87	10.79		0.025	0.041	0.07	

Table 3 (cont)

	n	(%)				0.385[a]				0.878[a]				0.598[a]				0.946[a]
Smoking Habits (n, %)																		
Non smoker	241	(70)	0.013	0.021	0.039		0.027	0.061	0.171		2.31	4.26	14.75		0.033	0.053	0.093	
Former smoker (> 2 months)	45	(13)	0.012	0.017	0.03		0.026	0.057	0.169		2.21	4.36	14.35		0.03	0.049	0.103	
Pregnancy smoker	59	(17)	0.012	0.018	0.028		0.02	0.054	0.178		2.78	5.74	13.22		0.038	0.054	0.07	
Number of cigarettes per day (n, %)						0.630[a]				0.655[a]				0.943[a]				0.308[a]
0 cigarettes	286	(87)	0.012	0.02	0.038		0.026	0.061	0.171		2.31	4.27	14.75		0.033	0.052	0.097	
1-5 cigarettes	24	(7)	0.012	0.018	0.023		0.018	0.056	0.174		2.73	4.65	13.2		0.038	0.048	0.062	
> 5 cigarettes	17	(5)	0.014	0.021	0.025		0.032	0.094	0.165		3.07	3.63	8.56		0.052	0.064	0.075	
Frequency of ingestion of fish (n, %)						0.548[a]				**0.030**[a]				0.098[a]				0.767[a]
< 3 times per month	52	(16)	0.013	0.019	0.028		0.024	0.04	0.108		2.97	6.37	16.51		0.032	0.053	0.073	
1 - 3 times per week	224	(69)	0.012	0.019	0.041		0.026	0.065	0.186		2.29	3.94	10.89		0.034	0.051	0.088	
≥ 4 times per week	50	(15)	0.01	0.02	0.033		0.036	0.118	0.343		2.31	5.38	25.44		0.028	0.065	0.101	

Table 3 (cont.)

Frequency of ingestion of cow milk (n, %)			0.065[a]			0.184[a]			0.132[a]			0.818[a]		
< 3 times per month	82	(24)	0,014	0.023	0.047	0.026	0.051	0.133	2.65	5.23	18.82	0.034	0.053	0.086
1 - 3 times per week	62	(18)	0,012	0.017	0.035	0.026	0.051	0.146	2.2	3.62	9.24	0.034	0.062	0.088
≥ 4 times per week	197	(58)	0,012	0.018	0.033	0.027	0.076	0.225	2.55	4.71	14.8	0.033	0.051	0.089

p-values were obtained through the non-parametric tests aKruskal-Wallis and bMan-Whitney. Bold values indicate statistical significance.

Association of Toxic metal excretion with newborn and pregnancy outcomes

Association analyses for newborn anthropometry and pregnancy complications were only performed for Ni and Tl, as they were the only metals that presented a significant percentage of urine samples with detectable levels (above 45%).

Newborn outcomes and exposure to toxic metals

Table 4 summarizes the association between urinary metal levels adjusted for urinary creatinine (µg metal / g creatinine) and newborn outcomes.

Female newborns tended to have a lower median urinary metal excretion for Ni (4.27 µg/g) when compared to male newborns (5.18 µg/g).

Urinary Tl excretion consistently increased with increasing anthropometric categories of birth weight, birth head circumference and birth length, with marginal statistical significance for birth weight adequacy (p=0.074). Regarding Ni, it tended to show higher levels of excretion for adequate categories of birth head circumference and birth length, but none of these associations was statistically significant.

Table 4. Newborn outcomes and exposure to toxic metals, expressed as **maternal urinary metal concentration** adjusted for urinary creatinine.

	n	(%)	P25	Median	P75	p-value	P25	Median	P75	p-value
				Tl (µg/g)				**Ni (µg/g)**		
New-born sex (n, %)						0.769 [b]				0.254 [b]
Male	161	(48)	0.03	0.06	0.17		2.68	5.18	16.1	
Female	174	(52)	0.03	0.06	0.18		2.31	4.27	14.19	
Birth weight						**0.074** [a]				0.238 [a]
SGA (small for gestational age)	26	(8)	0.02	0.04	0.07		2.1	3.55	5.74	
AGA (adequate for gestational age)	300	(90)	0.03	0.07	0.18		2.5	4.70	14.99	
LGA (large for gestational age)	9	(3)	0.06	0.09	0.19		3.26	4.72	25.15	
Birth head circumference						0.574 [a]				0.134 [a]
SGA (small for gestational age)	28	(9)	0.02	0.06	0.16		2.16	3.55	6.06	
AGA (adequate for gestational age)	276	(85)	0.03	0.06	0.17		2.61	4.99	15.14	
LGA (large for gestational age)	21	(6)	0.04	0.09	0.19		2.4	3.50	13.05	
Birth length						0.199 [a]				0.198 [a]
SGA (small for gestational age)	25	(7)	0.02	0.03	0.15		2.57	3.47	6.40	
AGA (adequate for gestational age)	307	(91)	0.03	0.06	0.17		2.45	4.71	15.11	
LGA (large for gestational age)	4	(1)	0.03	0.12	0.22		1.15	1.98	25.3	

p-values were obtained through the non-parametric tests [a]Kruskal-Wallis and [b]Mann-Whitney

Pregnancy outcomes and exposure to toxic metals

Table 5 summarizes the association between urinary metal levels adjusted for urinary creatinine (µg metal / g creatinine) and pregnancy outcomes.

Interestingly, the median for urinary Ni excretion was much higher in women who had delivery complications (11.3 µg/g). It is also found that urinary Ni excretion was higher in women who have had a baby with malformations (16.9 µg/g). However, these differences were not statistically significant.

There were no relationships between urinary Tl excretion and delivery complications or pregnancy complications.

Table 5. Pregnancy outcomes and exposure to toxic metals, expressed as maternal urinary metal concentration adjusted for urinary creatinine.

	n	(%)	P25	Median	P75	p-value	P25	Median	P75	p-value
				Tl (µg/g)				**Ni** (µg/g)		
Delivery complications (n, %)						0.071[b]				0.179[b]
no	290	(99)	0.03	0.06	0.17		2.43	4.42	14.35	
yes	4	(1)	0.02	0.02	0.04		5.91	11.25	28.33	
Pregnancy complications (n, %)						0.165[b]				**0.053**[b]
no	251	(84)	0.02	0.07	0.19		2.54	4.71	14.97	
yes	49	(16)	0.03	0.04	0.09		2.13	3.47	7.63	
Foetal malformations (n, %)						0.450[b]				0.536[b]
no	289	(98)	0.02	0.06	0.17		2.45	4.48	14.22	
yes	5	(2)	0.02	0.07	0.08		3.23	16.88	21.43	

p-values were obtained through the non-parametric tests [a]Kruskal-Wallis and [b]Mann-Whitney

Discussion

Exposure to heavy metals during pregnancy is harmful to humans, with possible negative consequences both in the short and in the long-term. Exposure to these metals can occur orally (mainly through contaminated water and food) [11], by inhalation [9] or absorption through the skin [21].

Physiological changes during pregnancy, with increased needs for specific nutrients, including trace elements, make it a unique period of vulnerability in a woman's life, with risks to the health of the pregnant woman and the fetus [1].

Heavy metals do not have a specific biochemical role in the human body and, on the contrary, systemic exposure to these elements, even at very low levels, can have a great toxicological impact [1]. This is particularly true in crucial period of fetal life, making it very relevant to study the possible impacts of those metals on pregnancy and the health of the newborn.

The present study aimed to evaluate the level of exposure to four heavy metals – Bi, Tl, Ni and Sb – in the IoMum cohort, by determining their respective urinary concentrations, and to correlate the level of exposure of pregnant women with sociodemographic characteristics, anthropometric parameters of the newborn and pregnancy outcomes.

Analyses of the association between urinary metal concentrations and newborn and pregnancy outcomes were performed only Ni and Tl, as these were the only metals detected in a significant percentage of the analyzed urine samples. There are no well-defined reference intervals for urinary concentrations of the metals studied, particularly in pregnant women. Anyway, using the reference ranges reported by LabCorp (a large international clinical laboratory), we could conclude that: the median Bi concentration (0.015 µg/L) was below the reference range for environmental exposure of 0.3−4.6 µg/L [34] and the median concentrations of Tl, Ni and Sb (0.020, 4.13 and 0.039 µg/L, respectively) were well within (and even at the very bottom, in the case of Tl and Sb) of the corresponding reference ranges for environmental exposure: <1.5 µg/L for Tl [35], <9.9 µg/L for Ni [36] and <10 µg/L for Sb [37].

Sociodemographic characteristics of the study sample and exposure to toxic metals

Exposure to Tl was associated with residence area, being higher in Valongo, and a similar trend was observed for Bi. Valongo is a mining area in Portugal and, although Tl is not the main metal extracted, it is often found in deposits of other elements such as Sb and As, which are present in the main minerals extracted in the region [38, 39]. Despite this, exposure levels proved to be very low, according to the reference ranges for environmental exposure mentioned above.

Particularly noteworthy is the finding that the median urinary Ni excretion in Porto (6.91 μg/g) was the highest of all residence areas studied, although the lack of statistical significance.

Regarding maternal age categories, statistically significant differences were found in the urinary excretion of Bi, Tl and Sb urinary concentrations: the median values increased from women younger than 30 years to women older than 35 years. This suggests that there may be a body burden of these metals over time, which has also been suggested for other heavy metals in non-pregnant women [40]. This increase in urinary excretion with age can be at least partially explained by a decrease in creatinine excretion in older age groups, but trend in urinary creatinine was not statistically significant in our study (data not shown). In any case, Bi, Tl and Sb urinary concentrations remain quite low in all age groups and would not be expected to pose toxicity concerns.

Sources of Tl include coal combustion, semiconductor manufacturing, exhaust emissions and it is also currently used in the semiconductor and optical industries [9]. To date, only a few studies have been published on the possible biological effects on human health and in cell cultures at Tl low-doses exposure (below 100 μg/L). However, a brief review on the literature on this subject was carried out and all the studies found highlight that exposure to this metal, even at very low concentrations, is a threat to human health, with an impact on the newborns outcomes, such as low birth weight [10].

It is noteworthy that this study showed that professional occupation could be a source of exposure to heavy metals. In this context, being a health professional was associated with increased levels of Bi and Ni. Other occupations, including housekeeping, hairdressing, cooking and working in factories, also appeared to increase exposure to all metals analyzed.

In fact, as previously mentioned, Ni is a metal widely used in various industrial processes (mining, refining, food processing, stainless steel, and electronic equipment production), putting factory workers and warehouse operators at a particularly increased risk of exposure. Individual systemic exposure to Ni occurs by inhalation, ingestion and skin contact [21].

Ni is also present in fossil fuels and, therefore, their burning contaminates the environment, which can be an important source of exposure for firefighters, taxi drivers and air traffic controllers. Health care workers may be occupationally exposed to Ni through medical equipment where this metal is used such as medical electric equipment and medical implants [21].

We could also observe that urinary Ni excretion tended to decrease with increasing education level. In 2004, a systematic review carried out to investigate whether low health literacy was related to worse use of health care and poorer health outcomes concluded that it was associated with poorer health outcomes [41]. Extrapolating the conclusions of this review to the results found in our study, there is a possibility that a higher level of education is associated with lower exposure to toxic metals and, therefore, better health outcomes.

Although no statistically significant differences were observed in the urinary excretion of metals between pregnant non-smokers, smokers, and ex-smokers, the presence of Ni [31] and Tl [32] in different tobacco products is confirmed. Interestingly, when looking at the results of this study, the median urinary excretion of Tl and Ni did not appear to be related to daily cigarette consumption. That is, the urinary excretion of Tl and Ni was not correlated with the number of cigarettes smoked per day. As these metals are present in tobacco, it would be expected that pregnant smokers would excrete more Tl and Ni in the urine, which was not observed in this study.

Regarding the specific exposure to Tl through diet, it was found that urinary excretion was consistently higher with the frequency of fish consumption (p=0.03). Some studies on marine fish from the central Pacific Ocean revealed that Tl levels in tissues were between 0.041-2.45 µg/g (Lin et al. 2001; Couture et al. 2011). Analysis of muscle tissue from alpine trout form a Canadian lake (Ellesmere Island, Nunavut, Kanada) showed a wide range of Tl levels, but much lower (14.3-124.7 ng/g) (Gantner et al. 2009). The highest levels of Tl were found in fish from aquatic environments close to contaminated areas, reaching 96 (Palermo et al. 1983) and 117.5 ug/g (Zitko et al. 1975) [12]. Our data corroborate the hypothesis of fish consumption as a source of Tl exposure.

Newborn outcomes and exposure to toxic metals

Regarding the anthropometric parameters of newborns, even though no statistically significant associations were found, urinary Ni excretion tended to increase consistently from SGA (small for gestational age) to AGA (appropriate for gestational age). That is, greater exposure to Ni, with higher levels of Ni excretion, seemed to result in the adequacy of anthropometry for gestational age.

A study carried out in China, with a final sample of 156 pregnant women, in which there was no association between exposure to heavy metals, namely Ni, and length at birth Li, Zhuang [42]. On the other hand, Howe et al. [43] showed a positive association between urinary Ni concentration in early pregnancy and neonatal birth weight, which has also been reported by others. Despite this, we cannot disregard the fact that Ni is not currently considered an essential trace element for humans, and EFSA does not considered it as a nutrient [44]. In fact, concerns about Ni toxicity are raising and a limit of exposure has been set for Ni [45].

A similar positive association, although not statistically significant, was found for Tl and all the neonatal anthropometric parameters studied. These results are contradictory to those of a nested case-control study with 816 participants residing in Hubei Province, China, which showed that higher maternal urinary Tl concentrations were associated with an increased risk of low birth

weight [18]. However, it should be noted that the urinary Tl concentrations found in that study in China, also measured by ICP-MS, were 10 times higher than ours. This suggests that Tl may not present fetal toxicity in the range of concentrations found in our study [18].

Pregnancy outcomes and exposure to toxic metals

The results of our study show that, although not reaching statistical significance, urinary Ni excretion was much higher in pregnant women who had childbirth complications or who had a baby with malformations. It is important to emphasize that urinary Ni excretion in these categories was close to the 75th percentile of the overall sample. A study conducted in 2090 pregnant women from a cohort in China, where urine samples were collected before the 20th week of gestation and an oral glucose tolerance test was performed, showed that five metals, including Ni, were significantly and positively associated with gestational diabetes mellitus (GDM) [23]. In that study, the Ni concentrations associated with GDM (2.84 µg/g) were lower than those found in our study for the group of delivery complications or fetal malformations.

On the contrary, we could observe that Tl was not associated with delivery complications. To the best of our knowledge, only one prospective study has been conducted so far to investigate the association between urinary Tl levels during pregnancy and women's risk of developing GDM. This cohort study used a sample of 1798 pregnant women and suggested the possible existence of a positive association between exposure to Tl during pregnancy and an increased risk of developing GDM [46].

Thus, given the small amount of reported but still existing studies and results, the effect of these metals on pregnancy outcomes should be further explored.

Limitations and strengths

This study has some limitations and strengths that must be considered during the analysis and interpretation of its results.

One of the limitations, and despite the use of the most sensitive analytical technique currently available, is the low detection rate, due to the low concentrations of metals that the samples actually showed. Thus, we did not perform association analyzes of Bi and Sb with neonatal or pregnancy outcomes.

Another limitation of this study is the lack of detailed information on the specific exposure risk associated with some professional occupations and on specific sources of exposure (food, water, etc.).

Another limitation is that urinary concentrations may not truly reflect actual exposure. Although urine is an appropriate specimen to evaluate the excretion of the studied metals, the samples collected for this study are spot samples (random urine) and therefore only provide information about a narrow time window of exposure. That is to say, the result of a random urine sample may be due to an occasional, exceptional intake of water or food rich in one of the metals, for example. In addition, we assumed that the point concentrations found were a good estimate of the longer exposure that pregnant women had over time.

In terms of strengths of this study, urinary concentrations of Bi, Tl, Ni and Sb were used as biomarkers of prenatal exposure simultaneously. In few studies available in the literature, such a comprehensive approach was performed, with these metals simultaneously tested in pregnant women.

In addition, the participants of this study were pregnant women who had attended the routine prenatal surveillance consultations held at CHUSJoão. In other words, recruitment was not exclusively carried out only among pregnant women who were being followed at CHUSJoão consultations for a specific health problem. Thus, the results are representative of women with a normal course of pregnancy and not just women suffering from known or emerging pathologies during pregnancy.

Finally, after recruiting the pregnant women who were invited to participate in this study, some exclusion criteria were applied. And yet the population used for the final analysis was considerably large, resulting in 349 spot urine samples.

Conclusion

In conclusion, exposure to metals Bi, Tl, Ni and Sb was evaluated in a cohort of Portuguese pregnant women (the IoMum cohort) by determining the respective urinary levels, and it was found that no sociodemographic factor was a significantly associated with environmental exposure.

In addition, the statistically significant differences found in this study and described above are not of concern regarding possible impact on maternal or newborn health.

Thus, while it is certain that pregnant women are inevitably exposed to toxic metals, the levels of exposure found do not appear to pose a significant health risk.

Finally, the evidence in the literature on these and other toxic metals, with some uncertainty in several cases, highlights the importance of carrying out more studies in the future in Portugal that analyze the possible relationships between metals and pregnancy and newborn outcomes.

Statements & Declarations

Competing Interests

The authors declare that there are no conflicts of interest.

Author Contributions

Manuscript writing: A.D., E.K., V.F. A.A., and, B.M.; Study conception and design: E.K., C.M.C., L.F.R.A., C.C., J.C.L., and C.R.; Population recruitment: J.G., C.P., C.P., C.M., C.R., and D.P., Data collection: J.G., C.P., C.R., and D.P.; Biochemical analyses: E.P., C.M., C.D.M., A.A., and V.C.F.; Statistical analyses: A.M.R. and C.C.D.

Ethics approval

The data used for the IoMum study were obtained according to the research protocol (ClinicalTrials.gov #NCT04010708) that was subsequently approved by the Ethics Committee of Centro Hospitalar Universitário de São João (CHUSJoão) / Faculty of Medicine of the University of Porto (FMUP) (ClinicalTrials.gov #NCT04010708).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

References

1. Wang, X., et al., *Urinary concentrations of environmental metals and associating factors in pregnant women.* Environ Sci Pollut Res Int, 2019. **26**(13): p. 13464-13475.

2. Poddalgoda, D., S.M. Hays, and A. Nong, *Derivation of biomonitoring equivalents (BE values) for bismuth.* Regul Toxicol Pharmacol, 2020. **114**: p. 104672.

3. Badrigilan, S., et al., *A Review on the Biodistribution, Pharmacokinetics and Toxicity of Bismuth-Based Nanomaterials.* Int J Nanomedicine, 2020. **15**: p. 7079-7096.

4. Zagari, R.M., et al., *Treatment of Helicobacter pylori infection: a clinical practice update.* Minerva Med, 2021. **112**(2): p. 281-287.

5. Wang, R., et al., *Elevated non-essential metals and the disordered metabolism of essential metals are associated to abnormal pregnancy with spontaneous abortion.* Environ Int, 2020. **144**: p. 106061.

6. Cabrera-Rodríguez, R., et al., *Occurrence of 44 elements in human cord blood and their association with growth indicators in newborns.* Environ Int, 2018. **116**: p. 43-51.

7. Mahadevan, U., *Gastrointestinal medications in pregnancy.* Best Pract Res Clin Gastroenterol, 2007. **21**(5): p. 849-77.

8. Body, C. and J.A. Christie, *Gastrointestinal Diseases in Pregnancy: Nausea, Vomiting, Hyperemesis Gravidarum, Gastroesophageal Reflux Disease, Constipation, and Diarrhea.* Gastroenterol Clin North Am, 2016. **45**(2): p. 267-83.

9. Kemnic, T.R. and M. Coleman, *Thallium Toxicity*, in *StatPearls*. 2022, StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC.: Treasure Island (FL).

10. Campanella, B., et al., *Toxicity of Thallium at Low Doses: A Review.* Int J Environ Res Public Health, 2019. **16**(23).

11. Anaya-Ramos, L., et al., *The acute systemic toxicity of thallium in rats produces oxidative stress: attenuation by metallothionein and Prussian blue.* Biometals, 2021. **34**(6): p. 1295-1311.

12. Karbowska, B., *Presence of thallium in the environment: sources of contaminations, distribution and monitoring methods.* Environ Monit Assess, 2016. **188**(11): p. 640.

13.   Fisher, R.M. and V. Gupta, *Heavy Metals*, in *StatPearls*. 2022, StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC.: Treasure Island (FL).

14.   Poudyal, B., P. Shrestha, and Y.S. Chowdhury, *Thallium-201*, in *StatPearls*. 2022, StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC.: Treasure Island (FL).

15.   Zou, H. and S. Zou, *Advanced thallium toxicity.* Pract Neurol, 2022.

16.   Chen, X., et al., *Effect of maternal thallium exposure in early pregnancy on the risk of preterm birth.* Environ Sci Pollut Res Int, 2022. **29**(33): p. 49966-49975.

17.   Zhu, B., et al., *Association between serum thallium in early pregnancy and risk of gestational diabetes mellitus: The Ma'anshan birth cohort study.* J Trace Elem Med Biol, 2019. **52**: p. 151-156.

18.   Xia, W., et al., *A Case-Control Study of Prenatal Thallium Exposure and Low Birth Weight in China.* Environ Health Perspect, 2016. **124**(1): p. 164-9.

19.   Genchi, G., et al., *Nickel: Human Health and Environmental Toxicology.* Int J Environ Res Public Health, 2020. **17**(3).

20.   Nielsen, F., *Nickel.* Adv Nutr, 2021. **12**(1): p. 281-282.

21.   Lee, H.W., C.C. Jose, and S. Cuddapah, *Epithelial-mesenchymal transition: Insights into nickel-induced lung diseases.* Semin Cancer Biol, 2021. **76**: p. 99-109.

22.   Fu, Z. and S. Xi, *The effects of heavy metals on human metabolism.* Toxicol Mech Methods, 2020. **30**(3): p. 167-176.

23.   Wang, X., et al., *Exposure to multiple metals in early pregnancy and gestational diabetes mellitus: A prospective cohort study.* Environ Int, 2020. **135**: p. 105370.

24.   Ding, A.L., et al., *Pregnancy complications effect on the nickel content in maternal blood, placenta blood and umbilical cord blood during pregnancy.* World J Clin Cases, 2021. **9**(28): p. 8340-8348.

25.   Boreiko, C.J. and T.G. Rossman, *Antimony and its compounds: Health impacts related to pulmonary toxicity, cancer, and genotoxicity.* Toxicol Appl Pharmacol, 2020. **403**: p. 115156.

26.   Nishad, P.A. and A. Bhaskarapillai, *Antimony, a pollutant of emerging concern: A review on industrial sources and remediation technologies.* Chemosphere, 2021. **277**: p. 130252.

27.   Gutierrez Guarnizo, S.A., et al., *Metabolite Biomarkers of Leishmania Antimony Resistance.* Cells, 2021. **10**(5).

28.   Zhang, G., et al., *Antimony in urine during early pregnancy correlates with increased risk of gestational diabetes mellitus: A prospective cohort study.* Environ Int, 2019. **123**: p. 164-170.

29.   Howe, C.G., et al., *Prenatal metal mixtures and fetal size in mid-pregnancy in the MADRES study.* Environ Res, 2021. **196**: p. 110388.

30.   Weir, C.B. and A. Jan, *BMI Classification Percentile And Cut Off Points*, in *StatPearls*. 2022, StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC.: Treasure Island (FL).

31.   Steuerwald, A.J., et al., *Trace element analysis of human urine collected after administration of Gd-based MRI contrast agents: characterizing spectral interferences using inorganic mass spectrometry.* J Anal At Spectrom, 2013. **28**(6): p. 821-830.

32.   Brodzka, R., M. Trzcinka-Ochocka, and B. Janasik, *Multi-element analysis of urine using dynamic reaction cell inductively coupled plasma mass spectrometry (ICP-DRC-MS) - a practical application.* Int J Occup Med Environ Health, 2013. **26**(2): p. 302-12.

33.   Chou, J.H., S. Roumiantsev, and R. Singh, *PediTools Electronic Growth Chart Calculators: Applications in Clinical Care, Research, and Quality Improvement.* J Med Internet Res, 2020. **22**(1): p. e16204.

34.   *Bismuth, Urine.* 2021; Available from: https://www.labcorp.com/tests/706507/bismuth-urine.

35.   *Thallium, Urine.* 24/09/2022]; Available from: https://www.labcorp.com/tests/706580/thallium-urine.

36.   *Nickel, Urine.* Available from: https://www.labcorp.com/tests/071563/nickel-urine.

37.   *Antimony, Urine.* Available from: https://www.labcorp.com/tests/071605/antimony-urine.

38.   Barneo-Caragol, C., et al., *Strontium and its role in preeclampsia.* J Trace Elem Med Biol, 2018. **47**: p. 37-44.

39.   *Mindat.org.* Available from: https://www.mindat.org/min-52512.htmlhttps://miningwatch.pt/mapadominerio/index.html.

40.   Berglund, M., et al., *Gender and age differences in mixed metal exposure and urinary excretion.* Environ Res, 2011. **111**(8): p. 1271-9.

41.   Berkman, N.D., et al., *Low health literacy and health outcomes: an updated systematic review.* Ann Intern Med, 2011. **155**(2): p. 97-107.

42.   Li, A., et al., *Heavy metals in maternal and cord blood in Beijing and their efficiency of placental transfer.* J Environ Sci (China), 2019. **80**: p. 99-106.

43.   Howe, C.G., et al., *Prenatal metal(loid) mixtures and birth weight for gestational age: A pooled analysis of three cohorts participating in the ECHO program.* Environ Int, 2022. **161**: p. 107102.

44.   6/01/2022]; DIRECTIVA 2002/46/CE DO PARLAMENTO EUROPEU E DO CONSELHO de 10 de Junho de 2002 relativa à aproximação das legislações dos Estados-Membros respeitantes aos suplementos alimentares]. Available from: https://eur-lex.europa.eu/legal-content/PT/TXT/HTML/?uri=CELEX:02002L0046-20150402&from=PT.

45.   6/01/2023

]; Air quality standards]. Available from: https://www.eea.europa.eu/themes/air/air-quality-concentrations/air-quality-standards.

46.   Zhang, Q.Q., et al., *Association between maternal thallium exposure and risk of gestational diabetes mellitus: Evidence from a birth cohort study.* Chemosphere, 2021. **270**: p. 128637.

Annex

Annex 1 – Categorization of variables

Continuous Variables

- Age
- Gestational weight variation
- Urinary Bi concentration at T1 in µg/L obtained from urine sample analysis (values below LOD were imputed as LOD/√2)
- Urinary Tl concentration at T1 in µg/L obtained from urine sample analysis (values below LOD were imputed as LOD/√2)
- Urinary Ni concentration at T1 in µg/L obtained from urine sample analysi (values below LOD were imputed as LOD/√2)
- Urinary Sb concentration at T1 in µg/L obtained from urine sample analysis (values below LOD were imputed as LOD/√2)
- Urinary Bi concentration at T1 obtained from urine sample analysis (values below LOD imputed as LOD/√2) adjusted for urinary creatinine (results as µg Bi / g creatine)
- Urinary Tl concentration at T1 obtained from urine sample analysis (values below LOD imputed as LOD/√2) adjusted for urinary creatinine (results as µg Tl / g creatine)
- Urinary Ni concentration at T1 obtained from urine sample analysis (values below LOD imputed as LOD/√2) adjusted for urinary creatinine (results as µg Ni / g creatine)
- Urinary Sb concentration at T1 obtained from urine sample analysis (values below LOD imputed as LOD/√2) adjusted for urinary creatinine (results as µg Sb / g creatine)

Ordinal Variables

- Maternal education level
- Pre-pregnancy BMI categories
- Employed
- Smoking habits
- Number of cigarretes per day
- Frequency of cow's milk intake
- Frequency of fish intake
- Urinary Bi concentration categories
- Urinary Tl concentration categories
- Urinary Ni concentration categories
- Urinary Sb concentration categories
- Length at birth categories according to WHO standards

- Weight at birth according to WHO standards
- Head circunference at birth according to WHO standards
- Birth weight according to WHO categories
- Birth head circunference according to WHO categories

Nominal Variables

- Health professional
- High-exposure occupation
- Primiparity
- Residence area
- Smoking habits
- Newborn sex
- Type of delivery
- Delivery complications
- Pregnancy complications
- Foetal malformations

Comissão de Ética

De:	Elisa Keating [keating@med.up.pt]
Enviado:	quinta-feira, 15 de Fevereiro de 2018 15:47
Para:	'Comissão de Ética'
Assunto:	Projeto iomum - questões adicionais a considerar
Anexos:	iomum_anexo 1_colheita amostra sangue seco.docx
Importância:	Alta

Exm.o Senhor
Presidente da Comissão de Ética para a Saúde do CHS João/FMUP
Professor Doutor Filipe Almeida

Venho por este meio apresentar duas questões a considerar no contexto do projeto iomum, recentemente aprovado por esta mesma Comissão, e que só se colocaram posteriormente a esta aprovação (27 Novembro 2017). Em reflexão de equipa consideramos que estas questões não alteram a aceitabilidade ética do projeto iomum. No entanto, compete-nos deixá-las para reflexão superior pela Comissão de Ética e solicitar autorização para as incluir no projeto iomum.

1. **Introdução de colheita de gota de sangue seco por picada de dedo, no protocolo do estudo.**
   Justificação: Embora o *outcome* primário do projeto iomum se mantenha a quantificação de iodo na urina, a equipa de investigação considera importante avaliar também os níveis de tiroglobulina em gota de sangue seco, no tempo 2 (T2) – consulta do termo da gravidez. Este doseamento tem 2 objetivos específicos:
   a) Validar a tiroglobulina em gota de sangue seco como um método fácil e pouco invasivo para avaliação do estado de iodo em mulheres grávidas
   b) Investigar de que forma a toma de iodo em suplemento afeta os níveis de tiroglobulina

   A colheita será feita por enfermeiros devidamente treinados, segundo protocolo previamente estabelecido (ver anexo). Uma vez autorizado, este procedimento será incluído na informação ao participante.

2. **Alteração ao modelo de consentimento informado, nomeadamente:**
   a) **Remoção da frase: "Declaro não ter sido incluída em nenhum outro projeto de investigação nos últimos três meses".**
      Justificação: O iomum é um projeto de natureza observacional, que não envolve qualquer intervenção ao participante, e que envolve apenas a colheita de urina em 2 pontos de tempo e de uma picada no dedo única, sendo assim minimamente exigente para as participantes. Por este motivo, consideramos que no contexto deste estudo observacional não se coloca o problema ético de a participante estar envolvida também noutros estudos de investigação, desde que o faça voluntariamente, premissa para a assinatura do consentimento informado. Consideramos ainda que a presença desta frase poderá inibir a adesão ao estudo.
   b) **Introdução de um campo para inserção de contactos da participante.**
      Justificação: Esta informação possibilitará à equipa iomum lembrar a participante do segundo contacto com o projeto, na data de consulta de termo da gravidez.

Sem mais assunto, peço deferimento e envio os meus cumprimentos cordiais
Elisa Keating

Investigadora Responsável
Projeto iomum

Elisa Keating
Professora Auxiliar
Dept. de Biomedicina – Unidade de Bioquímica
Faculdade de Medicina da Universidade do Porto
Centro de Investigação Médica (CIM), 2º piso
R. Dr. Plácido da Costa
4200-450 Porto, Portugal
Tel: +351220426650

1

Comissão de Ética

De: Elisa Keating [keating@med.up.pt]
Enviado: segunda-feira, 4 de Fevereiro de 2019 14:08
Para: 'Comissão de Ética'
Assunto: Projeto iomum - questões adicionais a considerar
Anexos: iomum_adenda_CES_04Fev_anexos.docx

Importância: Alta

Exm.o Senhor
Presidente da Comissão de Ética para a Saúde do CHUS João/FMUP
Professor Doutor Filipe Almeida

Venho por este meio apresentar duas questões adicionais a considerar no contexto do projeto iomum, aprovado por esta mesma Comissão de Ética em 27 de Novembro de 2017, e que se colocaram posteriormente a esta aprovação, especificamente no âmbito do desenvolvimento do trabalho de Mestrado em Nutrição Clínica pela estudante Juliana Cristina Diniz Guimarães, subordinada o tema "State of iodine in Portuguese pregnant women and the anthropometric profile of offspring at birth: results from the IoMum cohort". Em reflexão de equipa consideramos que as questões que ora apresentamos não alteram a aceitabilidade ética do projeto iomum. Importa referir também que estas questões estão incluídas na sua totalidade no âmbito da proposta anteriormente aprovada pela CES, pelo que os detalhes de financiamento se mantêm os mesmos.

Compete-nos ainda assim deixar estas questões para reflexão superior pela Comissão de Ética e solicitar autorização para as incluir no projeto iomum.

1. Biomonitorização na cohort Iomum

Embora o *outcome* primário do projeto iomum se mantenha a quantificação de iodo na urina, a equipa de investigação considera importante avaliar adicionalmente, como outcome secundário, o grau de exposição das grávidas da cohort iomum a poluentes ambientais pela sua quantificação na urina, tais como oligoelementos (cádmio, arsénico, chumbo, selénio, alumínio, entre outros), e outros poluentes (pesticidas e metabolitos de pesticidas, retardantes de chama, entre outros). Esta avaliação tem como objetivo a biomonitorização humana em grávidas para gerar informação relevante em termos de saúde pública e de avaliação e gestão de riscos neste grupo populacional particularmente suscetível, importando realçar que os dados científicos sobre estas exposições em mulheres grávidas portuguesas são à data inexistentes.

Adicionalmente, a quantificação desta exposição será cruzada com os dados antropométricos do recém-nascido, bem como com outros desfechos da gravidez, de forma a encontrar associações com os níveis de exposição ambiental.

Esta utilização adicional da cohort iomum não acarreta nenhuma ação adicional, incómodo ou desconforto para as participantes, uma vez que os referidos doseamentos serão feitos nas amostras de urina recolhidas e previstas inicialmente no protocolo do iomum. Este pedido representa pois um esforço de rentabilização das amostras recolhidas que nos parece recomendável.

Importa referir que a equipa de investigação do iomum dispõe do conhecimento e dos meios humanos e técnicos que garantem a qualidade na metodologia (anexo 1) de análise química necessária, bem como no tratamento dos respetivos dados (anexo 2).

2. Acesso aos dados do processo clínico do recém-nascido

Embora tenha sido inicialmente previsto o acesso ao processo clínico das grávidas participantes no iomum, vimos solicitar o acesso ao processo clínico e aos dados do recém-nascido. Desta forma ser-nos-á possível

Título do Projeto: IoMum - Monitorização do estado do iodo em grávidas Portuguesas: impacto da suplementação

Nome da Investigadora Principal: Prof Doutora Elisa Keating

Onde decorre o Estudo: No Serviço de Ginecologia e Obstetrícia do CHSJ. Apresentou declaração do Prof. Doutor Nuno Montenegro, que será também o profissional de ligação.

Objectivos do Estudo:

1) Monitorizar o estado de iodo em mulheres grávidas portuguesas,

2) Avaliar a eficácia da orientação da DGS através da avaliação da adesão dos médicos de família e obstetras a esta orientação e 3) Avaliar o impacto da suplementação no estado de iodo das grávidas.

Concepção e Pertinência do estudo:

Em Portugal, um estudo realizado entre 2005 e 2007 evidenciou uma prevalência elevada de deficiência de iodo em mulheres grávidas, com apenas 16.8% a apresentar valores de iodúria adequados. Este estudo motivou a implementação, em 2013, de uma orientação (nº 011/2013) de suplementação com iodo durante a gravidez pela Direção Geral de Saúde (DGS). Atualmente, 10 anos após a última avaliação do estado de iodo em mulheres grávidas, e 4 anos após a publicação da orientação da DGS, nenhum outro estudo se debruçou sobre este tema ou sobre a caraterização da adesão dos médicos à recomendação da DGS. Este cenário torna-se particularmente crítico considerando que a própria orientação obriga à monitorização do seu impacto 2 anos após a publicação, prática que é reforçada pela Organização Mundial de Saúde (OMS). O IoMum surge neste contexto, para colmatar esta ausência de conhecimento, e permitirá a caraterização do estado de iodo atual em mulheres grávidas portuguesas e a aferição da eficácia da suplementação na redução da insuficiência de iodo nesta população, contribuindo para o esclarecimento da comunidade médica e das entidades de saúde pública competentes.

O IoMum tem **duas populações alvo**:

• Grávidas saudáveis para avaliação das iodúrias e recolha de informação sobre estilos de vida, saúde e alimentação (questionário em anexo).

Estão definidos critérios de inclusão e exclusão.

• Médicos de Medicina Geral e Familiar e de Obstetrícia cuja participação será unicamente a resposta a um questionário sobre a sua prática clínica de suplementação com iodo durante a gravidez (questionário em anexo). Este questionário inclui uma secção inicial de informação sobre o Projeto que ajudará cada Médico na decisão de preenchimento e por isso de participação no projeto.

O desenho de estudo do IoMum tira partido do facto de a guideline da Direção Geral de Saúde (DGS) para o aporte de iodo em mulheres grávidas não ter carácter obrigatório, dando assim a possibilidade de encontrar na população de mulheres grávidas, um grupo suplementado e um grupo não suplementado, com base nas indicações clínicas de seguimento rotineiro da gravidez.

O Recrutamento será efetuado por investigadores experientes.

Benefício/risco:

A participação neste estudo permitirá às grávidas participantes um esclarecimento e sensibilização sobre a importância do iodo na gravidez e sobre o risco de deficiência deste micronutriente podendo, desta forma, contribuir ativamente para a alteração de comportamentos em prole de uma maior ingestão deste micronutriente.

Não haverá riscos para os participantes. As grávidas participantes apenas terão que disponibilizar-se para colher duas amostras pontuais de urina em dois tempos distintos da gravidez, bem como ceder cerca alguns minutos (máximo 10 minutos) do seu tempo para leitura da informação ao participante, assinatura de consentimento e preenchimento dos questionários que serão feitas no período dedicado às consultas de rotina, no Hospital. A participação das grávidas não obrigará a visitas ao Hospital/centro de saúde além das que estão previstas na vigilância de rotina da gravidez. As avaliações clínicas serão totalmente não-invasivas.

Os participantes, médicos, terão que ceder breves minutos para o preenchimento de apenas um questionário, que se estima que não exceda os 10 minutos.

O IoMum é um estudo observacional, que não prevê nenhuma espécie de intervenção pelo que não implica riscos para os participantes.

Confidencialidade dos dados:

Toda a informação recolhida permanecerá anónima e confidencial estando assegurada a privacidade de todos os participantes. A cada participante será atribuído um código único para que os nomes não sejam associados aos resultados. Os resultados deste estudo serão publicados em meios científicos, mas os participantes não serão identificados.

Respeito pela liberdade e autonomia do sujeito de ensaio:

A participação das grávidas será sujeita a assinatura de consentimento informado livre e esclarecido (Modelo da CES) e adequada informação ao participante (em anexo).

A participação dos médicos será considerada consentida sempre que de livre vontade acederem responder ao questionário online que lhes será disponibilizado.

Curriculum **da investigadora**: Adequado à investigação.

Data previsível da conclusão do estudo: novembro de 2020

Conclusão: Proponho um parecer favorável à realização deste projecto de investigação.

Porto, 27 de Novembro de 2017

O Relator da CES,

n.º 292/17

SÃO JOÃO

PEDIDO DE AUTORIZAÇÃO

Realização de Investigação

Exmo. Senhor Presidente do Conselho de Administração
do Centro Hospitalar de São João

AUTORIZADO

CONSELHO DE ADMINISTRAÇÃO ● REUNIÃO DE 18 JAN 2018

Presidente do Conselho de Administração

(Dr. António Oliveira e Silva)

Diretor Clínico / Enfermeira Diretora / Vogal Executivo / Vogal Executivo

(Prof. Dr. José Artur Paiva) (Enf.ª Filomena Cardosa) (Dr. Luís Porto Gomes) (Dr. Renato O. Matos)

Nome do Investigador Principal:

Elisa Oliveira Braga Keating

Título da Investigação:

IoMum - Monitorização do estado de iodo em grávidas Portuguesas: impacto da suplementação.

Pretendendo realizar no(s) Serviço(s) de:

Ginecologia e Obstetrícia

a investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efetivação.

Para o efeito, anexo toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de São João/Faculdade de Medicina da Universidade do Porto respeitante à investigação, à qual endereçei pedido de apreciação e parecer.

Com os melhores cumprimentos.

O Investigador/Promotor

Porto, 8 de Novembro de 2017.

assinatura

CES-IM005-0

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

SÃO JOÃO

Questionário para submissão de Investigação

*Exmo. Sr. Presidente da Comissão de Ética do Centro Hospitalar de São João/
Faculdade de Medicina da Universidade do Porto,*

Pretendendo realizar a investigação infracitada, solicito a V. Exa., na qualidade de Investigador, a sua apreciação e a elaboração do respetivo parecer. Para o efeito, anexo toda a documentação requerida.

IDENTIFICAÇÃO DO ESTUDO

Título da investigação: IoMum - Monitorização do estado do iodo em grávidas Portuguesas: impacto da suplementação

Nome do investigador: Elisa Oliveira Braga Keating

Endereço eletrónico: keating@med.up.pt   **Contacto telefónico:** 914696021

Caracterização da investigação:

☐ Estudo retrospetivo   ☒ Estudo observacional   ☐ Estudo prospetivo

☐ Inquérito   ☐ Outro. Qual?_____

Tipo de investigação:

☐ Com intervenção   ☒ Sem intervenção

Formação do investigador em boas práticas clínicas *(GCP)*: ☐ Sim   ☒ Não

Promotor *(se aplicável):* _____

Nome do orientador de dissertação/tese *(se aplicável):* Elisa Oliveira Braga Keating

Endereço eletrónico: keating@med.up.pt

Local/locais onde se realiza a investigação: Centro Hospitalar de S. João, Hospital de Braga, Hospital de Cascais

Data prevista para início: 01 / 12 / 2017   Data prevista para o término: 31 / 11 / 2020

PROTOCOLO DO ESTUDO

Síntese dos objetivos:

1) Monitorizar o estado de iodo em mulheres grávidas portuguesas, 2) Avaliar a eficácia da orientação da DGS através da avaliação da adesão dos médicos de família e obstetras a esta orientação e 3) Avaliar o impacto da suplementação no estado de iodo das grávidas.

Fundamentação ética (ganhos em conhecimento/inovação; ponderação benefícios/riscos):

Em Portugal, um estudo realizado entre 2005 e 2007 evidenciou uma prevalência elevada de deficiência de iodo em mulheres grávidas, com apenas 16.8% a apresentar valores de iodúria adequados. Este estudo motivou a implementação, em 2013, de uma orientação (nº 011/2013) de suplementação com iodo durante a gravidez pela Direção Geral de Saúde (DGS). Atualmente, 10 anos após a última avaliação do estado de iodo em mulheres grávidas, e 4 anos após a publicação da orientação da DGS, nenhum outro estudo se debruçou sobre este tema ou sobre a caraterização da adesão dos médicos à recomendação da DGS. Este cenário torna-se particularmente crítico considerando que a própria orientação obriga à monitorização do seu impacto 2 anos após a publicação, prática que é reforçada pela Organização Mundial de Saúde (OMS). O IoMum surge neste contexto, para colmatar esta ausência de conhecimento, e permitirá a caraterização do estado de iodo atual em mulheres grávidas portuguesas e a aferição da eficácia da suplementação na redução da insuficiência de iodo nesta população, contribuindo para o esclarecimento da comunidade médica e das entidades de saúde pública competentes.
O IoMum tem duas populações alvo:
• Grávidas saudáveis para avaliação das iodúrias e recolha de informação sobre estilos de vida, saúde e alimentação (questionário em anexo)

CES-IM007-0

LISTA DE DOCUMENTOS ANEXOS

☒ Pedido de autorização ao Presidente do Conselho de Administração do Centro Hospitalar de São João *(se aplicável)*

☐ Pedido de autorização à Diretora da Faculdade de Medicina da Universidade do Porto *(se aplicável)*

☒ Protocolo do estudo

☒ Declaração do Diretor de Serviço onde decorre o estudo

(sendo um estudo na área de enfermagem deve anexar também a concordância da chefia de enfermagem)

☒ Profissional de ligação

☐ Informação dos orientadores

☒ Informação ao participante

☒ Modelo de consentimento

☒ Instrumentos a utilizar *(inquéritos, questionários, escalas, p.ex.):* questionário às grávidas, questionário aos médicos

☒ Curriculum Vitae abreviado *(máx. 3 páginas)*

☐ Protocolo financeiro

☐ Outros:

COMPROMISSO DE HONRA E DECLARAÇÃO DE INTERESSES

Declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (1960 e respetivas emendas), e da Organização Mundial da Saúde, Convenção de Oviedo e das "Boas Práticas Clínicas" (GCP/ICH) no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo, nos últimos três meses. Comprometo-me a entregar à CES o relatório final da investigação, assim que concluído.
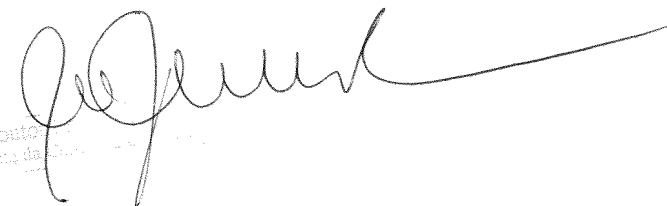
Porto, 9 *de* Novembro *de* 2017

Nome legível: Elisa Oliveira Braga Keating

assinatura

Parecer da Comissão de Ética do Centro Hospitalar de São João/FMUP   Emitido na reunião plenária da CE de 2̶7̶ / 11 / 17

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1, 2

Urinary toxic levels and pregnancy and newborn outcomes: results of the IoMum cohort biomonitoring

Abstract

This study aimed to evaluate the association between exposure of pregnant women to bismuth (Bi), thallium (Tl), nickel (Ni), and antimony (Sb) and sociodemographic characteristics of the study sample, pregnancy outcomes, and anthropometric parameters of the newborn.

This was a prospective study based on the IoMum cohort. Pregnant women undergoing routine first-trimester ultrasounds at Centro Hospitalar São João, from April 2018 to April 2019, were invited to participate. Inclusion criteria were gestational age ≥ 10 and < 14 weeks, confirmed foetal vitality and informed consent signature. Spot urine samples (n=349) were analyzed using ICP-MS to quantify metals concentrations.

Median urinary metal concentrations were, in µg/L: Bi 0.02, Tl 0.02, Ni 4.3 and Sb 0.04. Tl and Bi excretion were associated with residence area, with higher values in Valongo. Maternal age was positively associated with excretions of Bi, Sb, and Tl. Bi, Ni, and Sb excretion decreased from normal weight to obese women (pre-pregnancy BMI). Occupation as a health professional was associated with higher excretion of Bi and Ni. Occupations categorized as "high exposure" (including industry workers, cooks and housekeeping and hairdressing professionals) were associated with higher excretions of all the metals. Tl excretion increased with the frequency of fish consumption and with increasing anthropometric categories of weight, head circumference, and length at birth.

The present study showed that some professions may increase the exposure of pregnant women to the studied toxic metals. Despite this, the levels of exposure are not of concern regarding possible impact on maternal or newborn health.

Keywords: Pregnancy, Newborns, Anthropometry, Heavy metals, Occupational exposure;

Introduction

Exposure to metals during pregnancy

Individuals are naturally subject to exposure to different metals[1] through daily intake of food and water, as well as inhalation of airborne particulate matter. Moreover, different occupational activities may represent an additional and specific source of exposure. An overload of these metals in the human body can have important adverse health effects [1].

There are several metals that are biochemically important and must be ingested continuously to maintain health. Some examples of these nutritionally essential metals are iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu). On the other hand, several metals, such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), have no known biological function and are toxic even when present in the human body in very low concentrations[1].

The physiological adaptations that occur during pregnancy increase the demand for essential trace elements, as well as increase the susceptibility to the harmful effects of toxic metals[2]. Pregnancy and fetal development are seen as a specific time window of vulnerability, during which the toxic effects of heavy metals can result in disturbed fetal development and poor pregnancy outcomes, such as reduced fetal growth, impaired neurological development, congenital malformation, spontaneous abortion, stillbirth, preterm birth and low birth weight [1].

Most studies on the effects of natural exposure to metals are epidemiological studies in which the degree of exposure of the general population is evaluated, together with the associated effects [1]. Studies on specific populations (e.g., pregnant women), however, are quite scarce – and particularly in the case of Portugal, there are almost no studies on the exposure to toxic metals of pregnant women, as well as the consequences on fetal health and pregnancy outcomes.

Below we review the main sources and possible known consequences of exposure regarding the four metals that were the focus of our study: bismuth, thallium, antimony, and nickel.

---

[1] Strictly speaking, some of the elements referred to throughout this text (e.g., arsenic), including one of the elements that was the subject of this study (antimony), are not metals but "metalloids" (since they present some characteristics of metals but others of non-metals). However, for the purposes of simplifying writing, the generic designation of "metals" will always be used.

[2] Often collectively referred to as "heavy metals", since physiochemically most of them are in fact heavy metals.

| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 9 |

Aims

There is a lack of relevant studies on exposure to toxic metals and their consequences on fetal health and pregnancy outcomes, particularly in Portugal.

Thus, the main objectives of this study were:

(1) to evaluate the level of exposure to the four toxic metals Bi, Tl, Ni and Sb in pregnant women form the IoMum cohort, based on their urinary concentration;

(2) to investigate the correlation between exposure levels and sociodemographic characteristics of the study sample;

(3) to investigate the correlation between exposure levels and pregnancy outcomes and anthropometric parameters of newborns.

Methods

| Study design | 4 | Present key elements of study design early in the paper | 10 |

Pregnant women who underwent routine first-trimester ultrasound at CHUSJoão during the period from April 2018 to April 2019 were invited to participate in the IoMum cohort study. Based on this cohort, a prospective observational study was subsequently performed.

| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 10 |

Pregnant women who underwent routine first-trimester ultrasound at CHUSJoão during the period from April 2018 to April 2019 were invited to participate in the IoMum cohort study. Based on this cohort, a prospective observational study was subsequently performed.

Pregnant women with confirmed gestational age between 10 weeks and 13 weeks plus 6 days, with confirmed foetal vitality on the day of recruitment, and who agreed to sign the informed consent form were included in the study. The gestational age of each pregnant woman was assessed by measuring the crown-rump length and fetal rump length. Thus, 548 pregnant women were enrolled at baseline.

Exclusion criteria were gestational age at recruitment ≤10 and >14 weeks, unsigned standard informed consent, unsigned newborn informed consent,

and twin pregnancy. After the exclusion criteria were applied, the final study sample became 352 participants, and 349 urine samples were analyzed for use in the study.

At the time of enrollment (timepoint 1, T1), participants received a questionnaire to assess various demographic and lifestyle factors. This questionnaire included questions such as age, area of residence, education level, pre-pregnancy weight, height, gestational age, primiparity, smoking habits (and the number of cigarettes smoked per day, if applicable), and frequency of some types of food (including fish and dairy) consumption.

Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	10
		Pregnant women who underwent routine first-trimester ultrasound at CHUSJoão during the period from April 2018 to April 2019 were invited to participate in the IoMum cohort study. Based on this cohort, a prospective observational study was subsequently performed. Pregnant women with confirmed gestational age between 10 weeks and 13 weeks plus 6 days, with confirmed foetal vitality on the day of recruitment, and who agreed to sign the informed consent form were included in the study.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Clinical information about the newborns and pregnancy of the women who participated in the study was taken from clinical records, and included: maternal occupation, pregnancy outcomes and complications, type of delivery, gestational age at delivery, and newborn´s anthropometry.	11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group "This questionnaire included questions such as age, area of residence, education level, pre-pregnancy weight, height, gestational age, primiparity, smoking habits (and the number of cigarettes smoked per day, if applicable), and frequency of some types of food (including fish and dairy) consumption."	10, 11

"Clinical information about the newborns and pregnancy of the women who participated in the study was taken from clinical records, and included: maternal occupation, pregnancy outcomes and complications, type of delivery, gestational age at delivery, and newborn´s anthropometry."

Bias	9	Describe any efforts to address potential sources of bias	15

To perform the statistical analysis, the UMC values below the limit of detection (LOD) were imputed as $LOD/\sqrt{2}$. Additionally, UMC were adjusted for urinary creatinine concentration and adjusted values were used for the analysis.
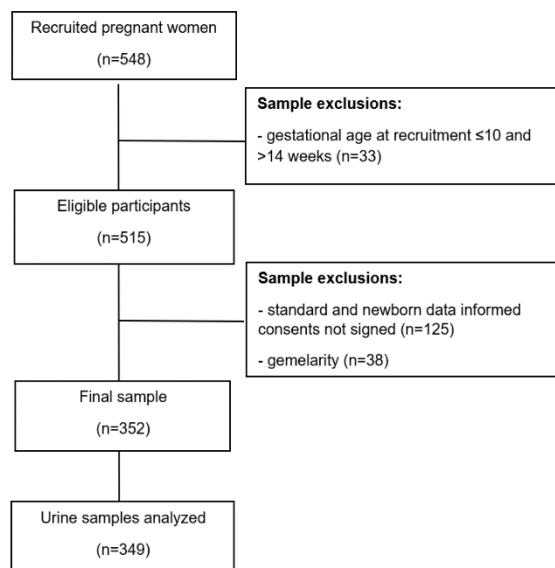
Study size	10	Explain how the study size was arrived at	10

Pregnant women who underwent routine first-trimester ultrasound at CHUSJoão during the period from April 2018 to April 2019 were invited to participate in the IoMum cohort study. Based on this cohort, a prospective observational study was subsequently performed.

Pregnant women with confirmed gestational age between 10 weeks and 13 weeks plus 6 days, with confirmed foetal vitality on the day of recruitment, and who agreed to sign the informed consent form were included in the study. The gestational age of each pregnant woman was assessed by measuring the crown-rump length and fetal rump length. Thus, 548 pregnant women were enrolled at baseline.

Exclusion criteria were gestational age at recruitment ≤10 and >14 weeks, unsigned standard informed consent, unsigned newborn informed consent, and twin pregnancy. After the exclusion criteria were applied, the final study sample became 352 participants, and 349 urine samples were analyzed for use in the study.

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	13, 14,

"Urinary levels of toxic metals

There is extensive evidence that urine is a suitable specimen for assessing metal exposure [31]. So, the urinary excretion of each metal was measured using inductively coupled mass spectrometry (ICP-MS), according to the

method developed by the CDC´s Urine Multi-element ICP-DRC-MS: Method 3018.3 [32]."

"BMI calculation and categorization

The calculation of body mass index (BMI) calculation was based on pre-pregnancy weight and height which were self-reported by pregnant women in the questionnaires and was calculated by dividing weight, in kilograms, by the square of height, in meters. Subsequently, participants were divided into 4 different BMI categories according to the WHO classification [30]:

Underweight: BMI <18.5;
Normal: BMI [18.5 – 24,9];
Overweight: BMI [25.0 – 29,9];
Obese: BMI >30.0."

"Occupational exposure categorization

To categorize occupational exposure, the Portuguese Classification of Professions 2010 (CPP/2010) was used, based on the International Classification of Occupations 2008 (ISCO/2008) by the Instituto Nacional de Estatistica, I.P. (INE, I.P.). This defines the most relevant new professions, tasks, and functions as part of CIPT/2008."

"Anthropometric data categorization

The Categorization of newborns´ anthropometric variables (weight, length and head circumference) was based on percentiles stipulated in the WHO child growth standards."

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	15
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e̲*) Describe any sensitivity analyses	

Statistical Analysis

Continuous variables that presented normal distribution were described using mean and standard deviation (SD), while continuous variables with non-normal distribution, namely urinary metals concentrations (UMC) were described as absolute frequencies (n), median, and interquartile range [25th

6

percentile (P25); 75th percentile (P75)]. The normality of Continuous variables´ was tested by evaluating the symmetry of their histograms.

Furthermore, categorical variables were described using absolute frequencies (n) and relative frequencies (%).

To perform the statistical analysis, the UMC values below the limit of detection (LOD) were imputed as $LOD/\sqrt{2}$. Additionally, UMC were adjusted for urinary creatinine concentration and adjusted values were used for the analysis.

To test for differences in the median of UMC, the variables were properly categorized. Subsequently, the non-parametric Kruskal-Wallis or Mann-Whitney tests were applied, depending on the number of categories.

Statistical significance was set at 5%. Therefore, when $p<0.05$, the differences were considered statistically significant differences were considered statistically significant.

Analysis of all data used in this study was performed using IBM SPSS version 27™ software.

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage	12

(c) Consider use of a flow diagram

Recruited pregnant women
(n=548)

Sample exclusions:
- gestational age at recruitment ≤10 and >14 weeks (n=33)

Eligible participants
(n=515)

Sample exclusions:
- standard and newborn data informed consents not signed (n=125)
- gemelarity (n=38)

Final sample
(n=352)

Urine samples analyzed
(n=349)

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	16, 17

(c) Summarise follow-up time (eg, average and total amount)

Sociodemographic characteristics

Most of the population resided in Valongo (28%, n=93), Maia (26%, n=88) and Porto (18%, n=60). These are considered the principal municipalities covered by the CHUSJoão area.

The mean (SD) age of the participating women was 32 (5) years.

About 40% of the women were primiparous and 56% (n=175) had a BMI prior to pregnancy in the normal range (18.5 - 25 kg/m^2). Of the remaining women, 40% (n=125) had a pre-pregnancy BMI above the normal range, with 24% (n=76) being overweight and 16% (n=49) being obese; and only a small minority (5%, n=15) were underweight.

The mean (SD) variation in the participants´ gestational weight (the difference between weight at term and weight before pregnancy) was 14 (6) kg.

Regarding education, 52% (n=175) of the sample had higher degrees of education (≥13 years), 33% (n=112) had a medium level (10-12 years), and only 14% (n=48) had a low level of education (≤ 9 years).

In addition, about 96% (n=307) of the participants were employed and 18% (n=57) were health professionals (doctors, nurses, dentists, psychologists, health technicians, nutritionists, pharmacists, and healthcare assistants). Also, 34% (n=107) of the participants had a high-exposure occupation.

Only 17% (n=59) of the participants were "pregnancy smokers", i.e., they were current smokers at the time the questionnaire was administered or had stopped smoking within the first trimester of their pregnancy, at the time of the enrollment. About 5% (n=17) of the smoking participants reported smoking more than 5 cigarettes per day.

Referring to the intake of cow's milk per week, 58% (n=197) reported consuming 4 or more times. Furthermore, 69% (n=224) reported eating fish between 1 to 3 times per week.

As a result of their pregnancies, 52% (n=174) were females and 48% (n=161) were male babies. Analyzing the anthropometric parameters length, weight, and head circumference, the percentage of newborns with appropriate values for gestational age was 91% (n=307), 90% (n=300), and 85% (n=276), respectively.

Looking at the hospital's clinical records, it was possible to see that 50% (n=169) of deliveries were eutocic, 22% (n=73) vacuum-assisted, and 28% (n=94) were by cesarean section.

Besides that, 16% of pregnant women had complications in pregnancy, including: gestational diabetes mellitus (GDM) (8%, n=24), pre-eclampsia (2%, n=6), fetal growth restriction (4%, n=11), infection (0.3%, n=1), pre-term delivery (6%, n=17), and/or spontaneous fetal loss (0.3%, n=1). It was also found that only a small number had a foetus with malformations (2%, n=5) and complications at birth (1% n=4).

Finally, we could note that the urinary evaluation presented the following median urinary concentrations (P25; P75) (in µg/L): bismuth 0.02 (0.02; 0.02); thallium 0.02 (0.02; 0.018); nickel 4.13 (2.29; 12.09); antimony 0.04 (0.04; 0.04).

Outcome data	15*	Report numbers of outcome events or summary measures over time	20

Table 2 presents a summary of the results obtained for the 4 metals that were the object of this study, indicating the minimum, maximum and median value, and the 5th (P5), 25th (P25), 75th (P75) and 95th (P95) percentiles. Urinary metal concentrations (µg/L) were adjusted for urinary creatinine concentration (g/L), so that results are expressed as µg of metal / g of creatinine.

Table 2 also presents the distribution of pregnant women by the three levels of urinary concentration considered: <LOD, between LOD and P95; above P95.

LOD (the lowest urinary concentration detectable in the ICP-MS analytical procedure used) were (in µg/L): 0.02 for Bi, 0.02 for Tl, 0.46 for Ni and 0.05 for Sb.

The detection rates (defined as the percentage of samples with an urinary metal concentration ≥LOD) were: 10.9% for Bi, 47.6% for Tl, 98.3% for Ni and 16.1% for Sb.

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	22, 27, 29

"Charcacteristics of the study sample and exposure to toxic metals

Table 3 shows the association between urinary metal levels adjusted for urinary creatinine (μg metal / g creatinine) and sociodemographic characteristics of the study sample.

Median urinary Tl excretion was associated with residence area, with higher values for pregnant women residing in Valongo, compared to other municipalities. Also, exposure to Bi tended to be higher in Valongo compared to Porto or Maia, but this association was not statistically significant. No other metal showed association with residence area.

Increasing maternal age was associated with higher urinary excretions. This association is statistically significant for Bi and Sb, and marginally significant for Tl (p=0.052).

Regarding BMI, Bi, Ni, and Sb urinary excretion consistently decreased from normal weight to obese women. This association was statistically significant for Bi (p=0.015) and marginally significant for Sb (p=0.061).

Being unemployed was not associated with urinary metal excretion.

Occupation as a health professional was associated with higher urinary excretion of Bi and Ni, but the latter association was only marginally significant (p=0.055). Occupations categorized as "high exposure" were also associated with higher excretions, but this association was marginally significant for Tl, Ni and Sb (p values of 0.050, 0.069 and 0.055, respectively).

Regarding maternal education level, urinary Ni excretion appeared to decrease consistently with higher levels of education. However, the differences were not statistically significant.

Maternal urinary Ni excretion tended to increase consistently with smoking habits, but the association was not statistically significant. No other consistent associations were found between metal excretion and smoking habits, and there was no association between the number of cigarettes smoked and metal excretion.

Urinary Tl excretion appeared to increase consistently with frequency of fish consumption, with participants consuming fish 4 or more times a week having a higher median excretion (0.12 µg/g) than those consuming fish less than 3 times a month (0.04 µg/g). No other metal was associated with fish consumption, and none was associated with milk consumption."

"Newborn outcomes and exposure to toxic metals

Table 4 summarizes the association between urinary metal levels adjusted for urinary creatinine (µg metal / g creatinine) and newborn outcomes.

Female newborns tended to have a lower median urinary metal excretion for Ni (4.27 µg/g) when compared to male newborns (5.18 µg/g).

Urinary Tl excretion consistently increased with increasing anthropometric categories of birth weight, birth head circumference and birth length, with marginal statistical significance for birth weight adequacy (p=0.074). Regarding Ni, it tended to show higher levels of excretion for adequate categories of birth head circumference and birth length, but none of these associations was statistically significant."

"Pregnancy outcomes and exposure to toxic metals

Table 5 summarizes the association between urinary metal levels adjusted for urinary creatinine (µg metal / g creatinine) and pregnancy outcomes.

Interestingly, the median for urinary Ni excretion was much higher in women who had delivery complications (11.3 µg/g). It is also found that urinary Ni excretion was higher in women who have had a baby with malformations (16.9 µg/g). However, these differences were not statistically significant.

There were no relationships between urinary Tl excretion and delivery complications or pregnancy complications."

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	20

Toxic metals urinary concentrations

Table 2 presents a summary of the results obtained for the 4 metals that were the object of this study, indicating the minimum, maximum and median value, and the 5th (P5), 25th (P25), 75th (P75) and 95th (P95) percentiles. Urinary metal concentrations

(µg/L) were adjusted for urinary creatinine concentration (g/L), so that results are expressed as µg of metal / g of creatinine.

Table 2 also presents the distribution of pregnant women by the three levels of urinary concentration considered: <LOD, between LOD and P95; above P95.

LOD (the lowest urinary concentration detectable in the ICP-MS analytical procedure used) were (in µg/L): 0.02 for Bi, 0.02 for Tl, 0.46 for Ni and 0.05 for Sb.

The detection rates (defined as the percentage of samples with an urinary metal concentration ≥LOD) were: 10.9% for Bi, 47.6% for Tl, 98.3% for Ni and 16.1% for Sb.

Discussion

Key results	18	Summarise key results with reference to study objectives	31, 35

"Exposure to heavy metals during pregnancy is harmful to humans, with possible negative consequences both in the short and in the long-term. Exposure to these metals can occur orally (mainly through contaminated water and food) [11], by inhalation [9] or absorption through the skin [21].

Physiological changes during pregnancy, with increased needs for specific nutrients, including trace elements, make it a unique period of vulnerability in a woman's life, with risks to the health of the pregnant woman and the fetus [1].

Heavy metals do not have a specific biochemical role in the human body and, on the contrary, systemic exposure to these elements, even at very low levels, can have a great toxicological impact [1]. This is particularly true in crucial period of fetal life, making it very relevant to study the possible impacts of those metals on pregnancy and the health of the newborn.

The present study aimed to evaluate the level of exposure to four heavy metals – Bi, Tl, Ni and Sb – in the IoMum cohort, by determining their respective urinary concentrations, and to correlate the level of exposure of pregnant women with sociodemographic characteristics, anthropometric parameters of the newborn and pregnancy outcomes."

"In conclusion, exposure to metals Bi, Tl, Ni and Sb was evaluated in a cohort of Portuguese pregnant women (the IoMum cohort) by determining the respective

urinary levels, and it was found that no sociodemographic factor was a significantly associated with environmental exposure.

In addition, the statistically significant differences found in this study and described above are not of concern regarding possible impact on maternal or newborn health.

Thus, while it is certain that pregnant women are inevitably exposed to toxic metals, the levels of exposure found do not appear to pose a significant health risk.

Finally, the evidence in the literature on these and other toxic metals, with some uncertainty in several cases, highlights the importance of carrying out more studies in the future in Portugal that analyze the possible relationships between metals and pregnancy and newborn outcomes."

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	34, 35

Limitations and strengths

This study has some limitations and strengths that must be considered during the analysis and interpretation of its results.

One of the limitations, and despite the use of the most sensitive analytical technique currently available, is the low detection rate, due to the low concentrations of metals that the samples actually showed. Thus, we did not perform association analyzes of Bi and Sb with neonatal or pregnancy outcomes.

Another limitation of this study is the lack of detailed information on the specific exposure risk associated with some professional occupations and on specific sources of exposure (food, water, etc.).

Another limitation is that urinary concentrations may not truly reflect actual exposure. Although urine is an appropriate specimen to evaluate the excretion of the studied metals, the samples collected for this study are spot samples (random urine) and therefore only provide information about a narrow time window of exposure. That is to say, the result of a random urine sample may be due to an occasional, exceptional intake of water or food rich in one of the metals, for example. In addition, we assumed that the point concentrations found were a good estimate of the longer exposure that pregnant women had over time.

In terms of strengths of this study, urinary concentrations of Bi, Tl, Ni and Sb were used as biomarkers of prenatal exposure simultaneously. In few studies available in

the literature, such a comprehensive approach was performed, with these metals simultaneously tested in pregnant women.

In addition, the participants of this study were pregnant women who had attended the routine prenatal surveillance consultations held at CHUSJoão. In other words, recruitment was not exclusively carried out only among pregnant women who were being followed at CHUSJoão consultations for a specific health problem. Thus, the results are representative of women with a normal course of pregnancy and not just women suffering from known or emerging pathologies during pregnancy.

Finally, after recruiting the pregnant women who were invited to participate in this study, some exclusion criteria were applied. And yet the population used for the final analysis was considerably large, resulting in 349 spot urine samples.

| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 31, 32, 33, 34 |

Sociodemographic characteristics of the study sample and exposure to toxic metals

Exposure to Tl was associated with residence area, being higher in Valongo, and a similar trend was observed for Bi. Valongo is a mining area in Portugal and, although Tl is not the main metal extracted, it is often found in deposits of other elements such as Sb and As, which are present in the main minerals extracted in the region [38, 39]. Despite this, exposure levels proved to be very low, according to the reference ranges for environmental exposure mentioned above.

Particularly noteworthy is the finding that the median urinary Ni excretion in Porto (6.91 µg/g) was the highest of all residence areas studied, although the lack of statistical significance.

Regarding maternal age categories, statistically significant differences were found in the urinary excretion of Bi, Tl and Sb urinary concentrations: the median values increased from women younger than 30 years to women older than 35 years. This suggests that there may be a body burden of these metals over time, which has also been suggested for other heavy metals in non-pregnant women [40]. This increase in urinary excretion with age can be at least partially explained by a decrease in creatinine excretion in older age groups, but trend in urinary creatinine was not statistically significant in our study (data not shown). In any case, Bi, Tl and Sb urinary concentrations remain quite low in all age groups and would not be expected to pose toxicity concerns.

Sources of Tl include coal combustion, semiconductor manufacturing, exhaust emissions and it is also currently used in the semiconductor and optical industries [9].

14

To date, only a few studies have been published on the possible biological effects on human health and in cell cultures at Tl low-doses exposure (below 100 µg/L). However, a brief review on the literature on this subject was carried out and all the studies found highlight that exposure to this metal, even at very low concentrations, is a threat to human health, with an impact on the newborns outcomes, such as low birth weight [10].

It is noteworthy that this study showed that professional occupation could be a source of exposure to heavy metals. In this context, being a health professional was associated with increased levels of Bi and Ni. Other occupations, including housekeeping, hairdressing, cooking and working in factories, also appeared to increase exposure to all metals analyzed.

In fact, as previously mentioned, Ni is a metal widely used in various industrial processes (mining, refining, food processing, stainless steel, and electronic equipment production), putting factory workers and warehouse operators at a particularly increased risk of exposure. Individual systemic exposure to Ni occurs by inhalation, ingestion and skin contact [21].

Ni is also present in fossil fuels and, therefore, their burning contaminates the environment, which can be an important source of exposure for firefighters, taxi drivers and air traffic controllers. Health care workers may be occupationally exposed to Ni through medical equipment where this metal is used such as medical electric equipment and medical implants [21].

We could also observe that urinary Ni excretion tended to decrease with increasing education level. In 2004, a systematic review carried out to investigate whether low health literacy was related to worse use of health care and poorer health outcomes concluded that it was associated with poorer health outcomes [41]. Extrapolating the conclusions of this review to the results found in our study, there is a possibility that a higher level of education is associated with lower exposure to toxic metals and, therefore, better health outcomes.

Although no statistically significant differences were observed in the urinary excretion of metals between pregnant non-smokers, smokers, and ex-smokers, the presence of Ni [31] and Tl [32] in different tobacco products is confirmed. Interestingly, when looking at the results of this study, the median urinary excretion of Tl and Ni did not appear to be related to daily cigarette consumption. That is, the urinary excretion of Tl and Ni was not correlated with the number of cigarettes smoked per day. As these metals are present in tobacco, it would be expected that pregnant smokers would excrete more Tl and Ni in the urine, which was not observed in this study.

Regarding the specific exposure to Tl through diet, it was found that urinary excretion was consistently higher with the frequency of fish consumption (p=0.03). Some

studies on marine fish from the central Pacific Ocean revealed that Tl levels in tissues were between 0.041-2.45 µg/g (Lin et al. 2001; Couture et al. 2011). Analysis of muscle tissue from alpine trout form a Canadian lake (Ellesmere Island, Nunavut, Kanada) showed a wide range of Tl levels, but much lower (14.3-124.7 ng/g) (Gantner et al. 2009). The highest levels of Tl were found in fish from aquatic environments close to contaminated areas, reaching 96 (Palermo et al. 1983) and 117.5 ug/g (Zitko et al. 1975) [12]. Our data corroborate the hypothesis of fish consumption as a source of Tl exposure.

Newborn outcomes and exposure to toxic metals

Regarding the anthropometric parameters of newborns, even though no statistically significant associations were found, urinary Ni excretion tended to increase consistently from SGA (small for gestational age) to AGA (appropriate for gestational age). That is, greater exposure to Ni, with higher levels of Ni excretion, seemed to result in the adequacy of anthropometry for gestational age.

A study carried out in China, with a final sample of 156 pregnant women, in which there was no association between exposure to heavy metals, namely Ni, and length at birth Li, Zhuang [42]. On the other hand, Howe et al. [43] showed a positive association between urinary Ni concentration in early pregnancy and neonatal birth weight, which has also been reported by others. Despite this, we cannot disregard the fact that Ni is not currently considered an essential trace element for humans, and EFSA does not considered it as a nutrient [44]. In fact, concerns about Ni toxicity are raising and a limit of exposure has been set for Ni [45].

A similar positive association, although not statistically significant, was found for Tl and all the neonatal anthropometric parameters studied. These results are contradictory to those of a nested case-control study with 816 participants residing in Hubei Province, China, which showed that higher maternal urinary Tl concentrations were associated with an increased risk of low birth weight [18]. However, it should be noted that the urinary Tl concentrations found in that study in China, also measured by ICP-MS, were 10 times higher than ours. This suggests that Tl may not present fetal toxicity in the range of concentrations found in our study [18].

Pregnancy outcomes and exposure to toxic metals

The results of our study show that, although not reaching statistical significance, urinary Ni excretion was much higher in pregnant women who had childbirth complications or who had a baby with malformations. It is important to emphasize that

urinary Ni excretion in these categories was close to the 75th percentile of the overall sample. A study conducted in 2090 pregnant women from a cohort in China, where urine samples were collected before the 20th week of gestation and an oral glucose tolerance test was performed, showed that five metals, including Ni, were significantly and positively associated with gestational diabetes mellitus (GDM) [23]. In that study, the Ni concentrations associated with GDM (2.84 µg/g) were lower than those found in our study for the group of delivery complications or fetal malformations.

On the contrary, we could observe that Tl was not associated with delivery complications. To the best of our knowledge, only one prospective study has been conducted so far to investigate the association between urinary Tl levels during pregnancy and women's risk of developing GDM. This cohort study used a sample of 1798 pregnant women and suggested the possible existence of a positive association between exposure to Tl during pregnancy and an increased risk of developing GDM [46].

Thus, given the small amount of reported but still existing studies and results, the effect of these metals on pregnancy outcomes should be further explored.

				31
Generalisability	21	Discuss the generalisability (external validity) of the study results		

"Exposure to heavy metals during pregnancy is harmful to humans, with possible negative consequences both in the short and in the long-term. Exposure to these metals can occur orally (mainly through contaminated water and food) [11], by inhalation [9] or absorption through the skin [21].

Physiological changes during pregnancy, with increased needs for specific nutrients, including trace elements, make it a unique period of vulnerability in a woman's life, with risks to the health of the pregnant woman and the fetus [1].

Heavy metals do not have a specific biochemical role in the human body and, on the contrary, systemic exposure to these elements, even at very low levels, can have a great toxicological impact [1]. This is particularly true in crucial period of fetal life, making it very relevant to study the possible impacts of those metals on pregnancy and the health of the newborn."

Other information

				37
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based		

Author Contributions

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Springer

Search Q   Authors & Editors   Log in

Biological Trace Element Research

Submission guidelines

Contents

Instructions for Authors

Types of papers

Original article, Brief communication, Short communication

Back to top ↑

General

Biological Trace Element Research is an international journal dedicated to publishing high-quality articles that advance knowledge of the importance and roles of trace elements for the health and well-being of humans and animals. This knowledge may be conveyed by original research articles, brief communications, and reviews, including those describing negative results from well-preformed experiments giving unexpected or unusual findings.

Classical experiments using animal and human models to study deficiency, supra-nutritional, pharmacological, and toxicological aspects of trace elements are especially desired. In vitro, cell culture and microbial models that help in defining the biochemical and physiological roles of trace elements are also welcomed. Studies involving plant models are invited if they are related to the provision of trace elements to animals and humans. Articles involving the importance of trace elements for plant growth, composition, and reproduction will be considered more suitable for other journals focusing on that area of science.

Starting in 2015, Biological Trace Element Research will be published monthly on-line to provide timely and useful information to nutritionists, physiologists, biochemists, chemists, and toxicologists about the biological importance of trace elements.

Dr. Forrest H. Nielsen, Co-Editor-in-Chief

Dr. John B. Vincent, Co-Editor-in-Chief

Back to top ↑

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

Back to top ↑

Title Page

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

- Trial registration number and date of registration for prospectively registered trials
- Trial registration number and date of registration, followed by "retrospectively registered", for retrospectively registered trials

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Back to top ↑

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using [Springer Nature's LaTeX template](#).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Back to top ↑

Scientific style

- Please always use internationally accepted signs and symbols for units (SI units).
- Nomenclature: Insofar as possible, authors should use systematic names similar to those used by Chemical Abstract Service or IUPAC.
- Genus and species names should be in italics.
- Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.
- Please use the standard mathematical notation for formulae, symbols, etc.:Italic for single letters that denote mathematical constants, variables, and unknown quantities Roman/upright for numerals, operators, and punctuation, and commonly defined functions or abbreviations, e.g., cos, det, e or exp, lim, log, max, min, sin, tan, d (for derivative) Bold for vectors, tensors, and matrices.

Back to top ↑

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The entries in the list should be numbered consecutively.

If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

- Journal article
  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
- Article by DOI
  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086
- Book
  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter
  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document
  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
- Dissertation
  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

[ISSN.org LTWA](#)

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibliography style file sn-basic.bst which is included in the [Springer Nature Article Template](#).

Back to top ↑

Statements & Declarations

The following statements must be included in your submitted manuscript under the heading 'Statements and Declarations'. This should be placed after the References section. Please note that submissions that do not include required statements will be returned as incomplete.

Funding

Please describe any sources of funding that have supported the work. The statement should include details of any grants received (please give the name of the funding agency and grant number).

Example statements:

"This work was supported by […] (Grant numbers […] and […]). Author A.B. has received research support from Company A."

"The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

Competing Interests

Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work.

Example statements:

"Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M. Dr. C has received speaker honorarium and research funding from Company M and Company N. Author D has received travel support from Company O. Non-financial interests: Author D has served on advisory boards for Company M and Company N."

"The authors have no relevant financial or non-financial interests to disclose."

Please refer to the "Competing Interests" section below for more information on how to complete these sections.

Author Contributions

Authors are encouraged to include a statement that specifies the contribution of every author to the research and preparation of the manuscript.

Example statement:

"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Please refer to the "Authorship Principles " section below for more information on how to complete this section.

Data Availability

All original research must include a Data Availability Statement. Data Availability Statements should provide information on where data supporting the results reported in the article can be found. Statements should include, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. When it is not possible to share research data publicly, for instance when individual privacy could be compromised, data availability should still be stated in the manuscript along with any conditions for access.

Example statements:

"The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT LINK TO DATASETS]"

"The datasets generated during and/or analysed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.]."

Please refer to the "Research Data Policy and Data Availability" section below for more information on how to complete this section.

In addition to the above, manuscripts that report the results of studies involving humans and/or animals should include the following declarations:

Ethics approval

Authors of research involving human or animal subjects should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee and reference number, if available). For research involving animals, their data or biological material, authors should supply detailed information on the ethical treatment of their animals in their submission. If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

"This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No....)."

"This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required."

For detailed information on relevant ethical standards and criteria, please refer to the sections on "Research involving human participants, their data or biological material", "Research involving animals, their data or biological material".

Consent to participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript.

Example statement:

"Informed consent was obtained from all individual participants included in the study."

"Written informed consent was obtained from the parents."

Please refer to the section on "Informed Consent" for additional help with completing this information.

Consent to publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. This is in particular applicable to case studies. A statement confirming that consent to publish has been received from all participants should appear in the manuscript.

Example statement:

"The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c."

Please refer to the section on "Informed Consent" for additional help with completing this information.

Back to top ↑

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Back to top ↑

Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art
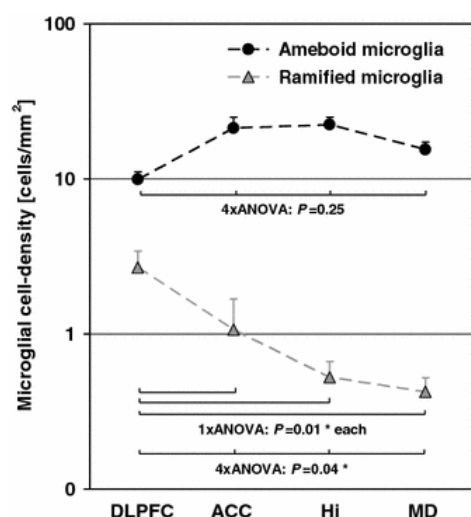
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art
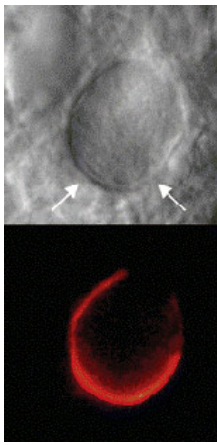
- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art
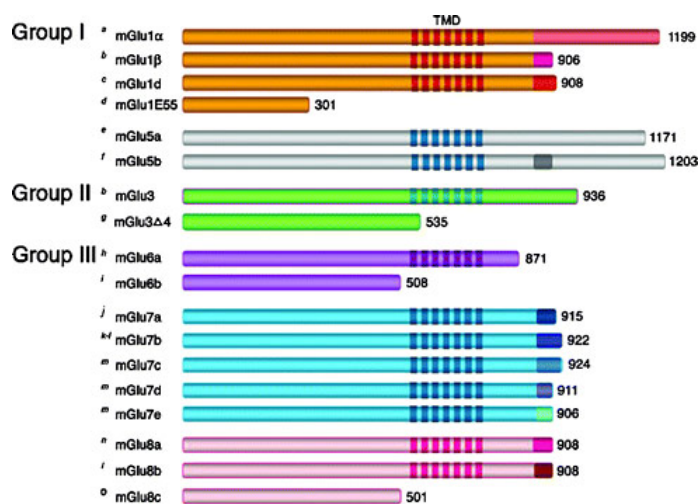
- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.

- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
- For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Back to top ↑

Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

Audio, Video, and Animations

- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as "Online Resource", e.g., "... as shown in the animation (Online Resource 3)", "... additional data are given in Online Resource 4".
- Name the files consecutively, e.g. "ESM_3.mpg", "ESM_4.pdf".

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Back to top ↑

Editing Services

English

How can you help improve your manuscript for publication?

Presenting your work in a well-structured manuscript and in well-written English gives it its best chance for editors and reviewers to understand it and evaluate it fairly. Many researchers find that getting some independent support helps them present their results in the best possible light. The experts at Springer Nature Author Services can help you with manuscript preparation—including **English language editing, developmental comments, manuscript formatting, figure preparation, translation**, and more.

[Get started and save 15%](#)

You can also use our free [Grammar Check](#) tool for an evaluation of your work.

Please note that using these tools, or any other service, is not a requirement for publication, nor does it imply or guarantee that editors will accept the article, or even select it for peer review.

Chinese (中文)

您怎么做才有助于改进您的稿件以便顺利发表？

如果在结构精巧的稿件中用精心组织的英语展示您的作品，就能最大限度地让编辑和审稿人理解并公正评估您的作品。许多研究人员发现，获得一些独立支持有助于他们以尽可能美好的方式展示他们的成果。Springer Nature Author Services 的专家可帮助您准备稿件，具体包括**润色英语表述、添加有见地的注释、为稿件排版、设计图表、翻译**等。

[开始使用即可节省 15% 的费用](#)

您还可以使用我们的[免费语法检查工具](#)来评估您的作品。

请注意，使用这些工具或任何其他服务不是发表前必须满足的要求，也不暗示或保证相关文章定会被编辑接受（甚至未必会被选送同行评审）。

Japanese (日本語)

発表に備えて、論文を改善するにはどうすればよいでしょうか？

内容が適切に組み立てられ、質の高い英語で書かれた論文を投稿すれば、編集者や査読者が論文を理解し、公正に評価するための最善の機会となります。多くの研究者は、個別のサポートを受けることで、研究結果を可能な限り最高の形で発表できると思っています。Springer Nature Author Servicesのエキスパートが、**英文の編集、建設的な提言、論文の書式、図の調整、翻訳**など、論文の作成をサポートいたします。

[今なら15%割引でご利用いただけます](#)

原稿の評価に、無料[の文法チェック](#)ツールもご利用いただけます。

これらのツールや他のサービスをご利用いただくことは、論文を掲載するための要件ではありません。また、編集者が論文を受理したり、査読に選定したりすることを示唆または保証するものではないことにご注意ください。

Korean (한국어)

게재를 위해 원고를 개선하려면 어떻게 해야 할까요?

여러분의 작품을 체계적인 원고로 발표하는 것은 편집자와 심사자가 여러분의 연구를 이해하고 공정하게 평가할 수 있는 최선의 기회를 제공합니다. 많은 연구자들은 어느 정도 독립적인 지원을 받는 것이 가능한 한 최선의 방법으로 자신의 결과를 발표하는 데 도움이 된다고 합니다. Springer Nature Author Services 전문가들은 **영어 편집, 발전적인 논평, 원고 서식 지정, 그림 준비, 번역** 등과 같은 원고 준비를 도와드릴 수 있습니다.

[지금 시작하면 15% 할인됩니다.](#)

또한 당사의 무료 [문법 검사](#)도구를 사용하여 여러분의 연구를 평가할 수 있습니다.

이러한 도구 또는 기타 서비스를 사용하는 것은 게재를 위한 필수 요구사항이 아니며, 편집자가 해당 논문을 수락하거나 피어 리뷰에 해당 논문을 선택한다는 것을 암시하거나 보장하지는 않습니다.

Back to top ↑

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

- The manuscript should not be submitted to more than one journal for simultaneous consideration.

- The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').

- A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').

- Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

- Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

- No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

- Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).

- Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.

- Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.

- Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).

- Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.

*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

- If the manuscript is still under consideration, it may be rejected and returned to the author.

- If the article has already been published online, depending on the nature and severity of the infraction:
  - an erratum/correction may be placed with the article

  - an expression of concern may be placed with the article

  - or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is **maintained on the platform**, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

- The author's institution may be informed

- A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Back to top ↑

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

- Disclosure of potential conflicts of interest

- Research involving Human Participants and/or Animals

- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Back to top ↑

Competing Interests

Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Editorial Board Members and Editors are required to declare any competing interests and may be excluded from the peer review process if a competing interest exists. In addition, they should exclude themselves from handling manuscripts in cases where there is a competing interest. This may include – but is not limited to – having previously published with one or more of the authors, and sharing the same institution as one or more of the authors. Where an Editor or Editorial Board Member is on the author list they must declare this in the competing interests section on the submitted manuscript. If they are an author or have any other competing interest regarding a specific manuscript, another Editor or member of the Editorial Board will be assigned to assume responsibility for overseeing peer review. These submissions are subject to the exact same review process as any other manuscript. Editorial Board Members are welcome to submit papers to the journal. These submissions are not given any priority over other manuscripts, and Editorial Board Member status has no bearing on editorial consideration.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement.Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under 'summary of requirements' (see below) funding information should be included in the '**Declarations**' section.

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

- Partial financial support was received from [...]
- The research leading to these results received funding from [...] under Grant Agreement No[...].
- This study was funded by [...]
- This work was supported by [...] (Grant numbers [...] and [...])

Examples of statements to be used when there is no funding:

- The authors did not receive support from any organization for the submitted work.
- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this study.
- No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

- **Financial interests:** Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.
  Non-financial interests: Author C is an unpaid member of committee Z.
- **Financial interests:** The authors declare they have no financial interests.
  Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.
- **Financial interests:** Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.
  Non-financial interests: none.
- **Financial interests:** Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.
  Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no competing interests to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Back to top ↑

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki

Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the [NCBI database](#) for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the [International Cell Line Authentication Committee](#) (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: *Filip1$^{tm1a(KOMP)Wtsi}$* **RRID:MMRRC_055641-UCD**

Cell Line: RST307 cell line **RRID:CVCL_C321**

Antibody: Luciferase antibody DSHB Cat# LUC-3, **RRID:AB_2722109**

Plasmid: mRuby3 plasmid **RRID:Addgene_104005**

Software: ImageJ Version 1.2.4 **RRID:SCR_003070**

RRIDs are provided by the [Resource Identification Portal](). Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly [register a new resource]() and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as "A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions" and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example [www.clinicaltrials.gov]() or any of the primary registries that participate in the [WHO International Clinical Trials Registry Platform]().

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the [EQUATOR Network]() when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials [(CONSORT)]() and Study protocols [(SPIRIT)]()

Observational studies [(STROBE)]()

Systematic reviews and meta-analyses [(PRISMA)]() and protocols [(Prisma-P)]()

Diagnostic/prognostic studies [(STARD)]() and [(TRIPOD)]()

Case reports [(CARE)]()

Clinical practice guidelines [(AGREE)]() and [(RIGHT)]()

Qualitative research [(SRQR)]() and [(COREQ)]()

Animal pre-clinical studies [(ARRIVE)]()

Quality improvement studies [(SQUIRE)]()

Economic evaluations [(CHEERS)](CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. …).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date…/No. …).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: …).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Back to top ↑

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for

coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

[here. (Download docx, 36 kB)](#) ⬇

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

Back to top ↑

Research involving animals, their data or biological material

The welfare of animals (vertebrate and higher invertebrate) used for research, education and testing must be respected. Authors should supply detailed information on the ethical treatment of their animals in their submission. For that purpose they may use the ARRIVE checklist which is designed to be used when submitting manuscripts describing animal research.

For studies involving client-owned animals, authors must also document informed consent from the client or owner and adherence to a high standard (best practice) of veterinary care.

Authors are recommended to comply with:

• The International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and consult the IUCN red list index of threatened species.

• Convention on the Trade in Endangered Species of Wild Fauna and Flora

When reporting results authors should indicate:

• … that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted. Please provide the name of ethics committee and relevant permit number;

• … whether the legal requirements or guidelines in the country and/or state or province for the care and use of animals have been followed.

Researchers from countries without any legal requirements or guidelines voluntarily should refer to the following sites for guidance:

– The Basel Declaration describes fundamental principles of using animals in biomedical research

– The International Council for Laboratory Animal Science (ICLAS) provides ethical guidelines for researchers as well as editors and reviewers

– The Association for the study of Animal Behaviour describes ethical guidelines for the treatment of animals in research and teaching

– The International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics provide guidelines for authors on animal ethics and welfare

Researchers may wish to consult the most recent (ethical) guidelines available from relevant taxon-oriented professional societies.

If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Examples of statements to be used when ethics approval has been obtained:

• All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (No. 29 12 2014, Kocaeli, Turkey).

• All procedures performed in the study were in accordance with the ARVO Statement for Use of Animals in Ophthalmic Vision and Research. The ethical principles established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011) were followed. The research protocol was approved by the Ethics Committee on Animal Use (Protocol No. 06174/14) of FCAV/Unesp, Jaboticabal.

• This study involved a questionnaire-based survey of farmers as well as blood sampling from their animals. The study protocol was assessed and approved by Haramaya University, research and extension office. Participants provided their verbal informed consent for animal blood sampling as well as for the related survey questions. Collection of blood samples was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare.

• All brown bear captures and handling were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Application C18/15) and the Swedish Environmental Protection Agency in compliance with Swedish laws and regulations.

• The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Maiduguri Senate committee on Medical Research ethics. Proper permit and consent were obtained from the Maiduguri abattoir management, before the faecal samples of the cattle and camels slaughtered in this abattoir were used for this experiment.

Examples of statements to be used when no ethical approval is required/exemption granted:

• No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

• As the trappings of small mammals were conducted as part of regular pest control measures in accordance with the NATO Standardized Agreement 2048 "Deployment Pest and Vector Surveillance and Control ", no approval by an ethics committee was required.

• All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Department of Zoology, Utkal University, Bhubaneswar, Odisha, India. However, the insect species used in this study is reared for commercial production of raw silk materials, as a part of agro-based industry. Therefore, use of this animal in research does not require ethical clearance. We have obtained permission from the office of Research officer sericulture, Baripada, Orissa, India for the provision of infrastructure and support for rearing of silkworm both in indoor and outdoor conditions related to our study to promote sericulture practices.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Back to top ↑

After acceptance

Upon acceptance, your article will be exported to Production to undergo typesetting. Once typesetting is complete, you will receive a link asking you to confirm your affiliation, choose the publishing model for your article as well as arrange rights and payment of any associated publication cost.

Once you have completed this, your article will be processed and you will receive the proofs.

Article publishing agreement

Depending on the ownership of the journal and its policies, you will either grant the Publisher an exclusive licence to publish the article or will be asked to transfer copyright of the article to the Publisher.

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Online publication of color illustrations is free of charge. For color in the print version, authors will be expected to make a contribution towards the extra costs.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Back to top ↑

Open Choice

Open Choice allows you to publish open access in more than 1850 Springer Nature journals, making your research more visible and accessible immediately on publication.

Article processing charges (APCs) vary by journal – view the full list

Benefits:

- Increased researcher engagement: Open Choice enables access by anyone with an internet connection, immediately on publication.

- Higher visibility and impact: In Springer hybrid journals, OA articles are accessed 4 times more often on average, and cited 1.7 more times on average*.

- Easy compliance with funder and institutional mandates: Many funders require open access publishing, and some take compliance into account when assessing future grant applications.

It is easy to find funding to support open access – please see our funding and support pages for more information.

*) Within the first three years of publication. Springer Nature hybrid journal OA impact analysis, 2018.

Open Choice

Funding and Support pages

Copyright and license term – CC BY

Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License.

Find more about the license agreement

Back to top ↑

Research Data Policy and Data Availability Statements

This journal operates a type 3 research data policy. A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Data availability

All original research must include a data availability statement. Data availability statements should include information on where data supporting the results reported in the article can be found, if applicable. Statements should include, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. For the purposes of the data availability statement, "data" is defined as the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. When it is not possible to share research data publicly, for instance when individual privacy could be compromised, data availability should still be stated in the manuscript along with any conditions for access. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

2. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

3. All data generated or analysed during this study are included in this published article [and its supplementary information files].

4. The datasets generated during and/or analysed during the current study are not publicly available due [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.].

5. Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

6. The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

More templates for data availability statements, including examples of openly available and restricted access datasets, are available here:

Data availability statements

Data repositories

This journal strongly encourages that all datasets on which the conclusions of the paper rely are available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and

appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories.

List of Repositories

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Data citation

The journal also requires that authors cite any publicly available data on which the conclusions of the paper rely. Data citations should include a persistent identifier (such as a DOI), should be included in the reference list using the minimum information recommended by DataCite, and follow journal style. Dataset identifiers including DOIs should be expressed as full URLs.

Research data and peer review

Peer reviewers are encouraged to check the manuscript's Data availability statement, where applicable. They should consider if the authors have complied with the journal's policy on the availability of research data, and whether reasonable effort has been made to make the data that support the findings of the study available for replication or reuse by other researchers. Peer reviewers are entitled to request access to underlying data (and code) when needed for them to perform their evaluation of a manuscript.

If the journal that you're submitting to uses double-blind peer review and you are providing reviewers with access to your data (for example via a repository link, supplementary information or data on request), it is strongly suggested that the authorship in the data is also blinded. There are data repositories that can assist with this and/or will create a link to mask the authorship of your data.

Authors who need help understanding our data sharing policies, help finding a suitable data repository, or help organising and sharing research data can access our Author Support portal for additional guidance.

For more information:

http://www.springernature.com/gp/group/data-policy/faq

Back to top ↑

Open access publishing

To find out more about publishing your work Open Access in *Biological Trace Element Research*, including information on fees, funding and licenses, visit our Open access publishing page.

Back to top ↑

For authors

Submission guidelines

Manuscript editing services

Ethics & disclosures

Open Access fees and funding

Contact the journal

Submit manuscript

Working on a manuscript?

Avoid the most common mistakes and prepare your manuscript for journal editors.

Learn more →

Explore

Online first articles

Volumes and issues

Sign up for alerts

Springer

Publish with us

Authors & Editors

Journal authors

Publishing ethics

Open Access & Springer

Discover content

SpringerLink

Books A-Z

Journals A-Z

Video

Other services

Instructors

Librarians (Springer Nature)

Societies and Publishing Partners

Advertisers

Shop on Springer.com

About Springer

About us

Help & Support

Contact us

Press releases

Impressum

Legal

General term & conditions

California Privacy Statement

Rights & permissions

Privacy

How we use cookies

Manage cookies/Do not sell my data

Accessibility

SPRINGER NATURE

© 2023 Springer Nature Switzerland AG. Part of Springer Nature.