# Interdependence between urinary cobalt concentrations and hemoglobin levels in pregnant women

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**ABSTRACT**

Cobalt is an essential trace element but may cause toxic effects upon occupational or environmental exposure. Women accumulate more cobalt than men at similar exposure levels which may be related to higher metabolic iron loss. During pregnancy these losses are much stronger but their influence on cobalt intake has not been studied. We have studied the associations between changes in hemoglobin and cobalt urinary excretion during pregnancy. 391 pairs of urine and blood samples from pregnant women were collected during the $12^{th}$ and $32^{nd}$ weeks of pregnancy and were analyzed for cobalt and hemoglobin. Mean concentrations of urinary cobalt were 0.73 and 1.6 µg/g creatinine during the first and third trimesters, respectively ($p < 0.001$). 84% of pregnant women had higher levels of cobalt in the third than in the first trimester. Cobalt concentrations were negatively associated to hemoglobin levels in the third trimester ($p<0.05$). Women with higher iron decreases between both trimesters had significant cobalt increases between these two periods. This correspondence involved a statistically significant difference in third trimester mean cobalt concentrations of anemic and non-anemic women, 1.8 and 1.5 µg/g creatinine, respectively ($p<0.05$). No significant differences between these two groups were found during the first trimester. These results were used to construct generalized additive models both in normal and anemic women. The strong association between the changes of both iron status and cobalt urine levels found in pregnant women may be related to higher intestinal absorption of cobalt at iron depletion such as in the last pregnancy period when iron body demands are high. Possible toxicity effects of these cobalt increases along pregnancy should be considered in cases of populations occupationally or environmentally exposed to this metal.

## 1. Introduction

Cobalt is a transition metal of widespread environmental occurrence. It is a minor component in a huge amount of minerals (Kim et al., 2006). It has been used for different applications such as pigments, catalysts in oil and gas production, battery electrodes, orthopedic prostheses and others (NHANES, 2009). It is then present in an important amount of manufactures, though human exposure to this metal depends mainly on diet. Its main sources are fish, green vegetables and fresh cereals (Unice et al., 2012). Cobalt is an essential trace metal used in the formation of vitamin B12 (also named cobalamin). 85% of the human body content of cobalt is this form, although only a small fraction of human cobalt intake is used for this purpose and most of the ingested cobalt is in inorganic form (Kim et al., 2006). This inorganic form has not an essential function and is not required in human diets. Cobalt deficiency has never been described in human metabolism (Simonsen et al., 2012). Remarkably, cobalt supplements are available and the manufacturers claim that this metal is useful for fat and carbohydrate metabolism, protein synthesis, red blood cell production and myelin sheath repair in the central nervous system (Finley et al., 2012). Cobalt has also been used as a homeopathic element to correct for eventual excessive excretion of estrogen during female hormone replacement therapy (Pausterbach et al., 2013). It is also suspected to have been used as doping agent due to its erythropoietic and angiogenetic properties (Lippi et al., 2006).

Occupational and accidental exposures to cobalt have been reported to originate asthma and respiratory problems (Nemery et al., 1992; Swennen et al., 1993), alterations of thyroid hormones (Prescott et al., 1992) and other effects. An oral reference dose of 0.03 mg/kg-day has been recently proposed as the maximum cobalt intake for non-cancer health effects in general population over lifetime exposure (Filey et al., 2012). This dose corresponds to 2.1 mg/day for a 70 kg adult, which is 50–400 fold higher than the average daily dietary cobalt intake of the US population (5–40 μg/day) (Finley et al., 2012). However, toxicological effects have been attributed to inorganic cobalt in its free ionic state, not bound to albumin, at lower concentrations than usual in subjects with

albumin alterations such as anephric patients, sepsis patients or sickle cell children (Pausterbach et al., 2013).

The maternal concentrations of metals, including cobalt may change along pregnancy which may also be related to variations in fetal exposure. Measurements of trace metal changes along pregnancy have been considered in some cases but these studies did not include cobalt. Iron depletion is one of the most relevant changes during pregnancy (Goonewardene et al., 2012). Barany et al. (2005) demonstrated that iron status has an influence in the concentration in blood of several metals such as cobalt. Moreover, animal studies have shown that iron depletion is associated with an increase of the intestinal absorption of divalent metals such as cobalt (Flanagan et al., 1980).

Gastrointestinal absorption of dietary cobalt can typically range from 10 to 35% (Unice et al., 2012). Intakes of 20% and 45% in males and females, respectively, have been considered as standard reference values in human biokinetic models (Unice et al., 2014). These gender differences are due to iron status. Menstrual losses in women may lead to lower iron which has been associated to higher levels of cobalt intake (Meltzer et al., 2010).

Toxicokinetic modeling and cobalt intake studies have long demonstrated that urinary cobalt is a good measure for cobalt concentrations in the human body. $CoCl_2$ intake and absorption is reflected in the urine cobalt concentrations (Christensen et al. 1993). Furthermore, urinary cobalt excretion was found to represent two thirds of daily intake in a group of women who self-measured their dietary intake (Harp and Scoular, 1952). Correspondences between decreases of iron and increases of cobalt have been observed when comparing differences in concentrations of this metal in subjects with abnormal and normal iron status (Barany et al., 2005). Hereditary hemochromatosis patients were found to accumulate both iron and cobalt (Nichols and Bacon, 1989).

Accordingly, urine is the preferred source of information for cobalt biomonitoring because it can be collected without invasive methods. It has been widely used in large environmental studies

with trace metals such as the German Environmental Survey for Children (GerES) and the National Health and Nutrition Examination (NHANES).

The present study is devoted to compare the levels of cobalt in urine of pregnant women in the first and third trimester of pregnancy and for assessment of the possible relationships of iron decrease occurring along pregnancy with the observed changes.

## 2. Materials and methods

### *2.1. Urine samples*

Between 2004 and 2006, in the context of the INMA research network (Childhood and environment) 657 pregnant women were recruited in their 12th week of pregnancy on occasion of a medical visit in the Primary Care Center II of Sant Fèlix Hospital (Sabadell, Catalonia). Recruitment conditions involved residence in Sabadell, age higher than 16 years, single pregnancy, voluntary incorporation to the program and scheduled birth at the Hospitals of Sabadell or Terrassa (a nearby city). Women suffering from chronic diseases, with communication impairment or assisted-reproduction pregnancy were excluded. After obtaining the consent from the admitted women, questionnaires were administered by trained interviewers in the 12th and 32th weeks of pregnancy.

Mean age of the mothers at the time of their last menstrual period was 31 years, ranging between 18 and 42 years. Their mean BMI before pregnancy was 23.62 kg/m$^2$, ranging between 17.35 and 54.82 kg/m$^2$, with 17.3 and 7.4% of overweight and obese women, respectively. 54.3% of the mothers were primiparous, 37.4% had another infant and 8.2% had more than two infants.

80 mL urine samples were drawn in both the 12th and 32nd week of pregnancy from 500 pregnant women of this cohort. The samples were stored at -20ºC in polyethylene tubes until further

processing. This study was approved by the Research Ethics Committee of the CREAL and all participant information was coded to maintain confidentiality. Participants gave written consent before start of the research described in the present paper.

### *2.2. Analysis of urine samples*

391 pairs of urine samples from the 12th and 32nd week of pregnancy from the Sabadell cohort were analyzed for cobalt by Q-ICP-MS (Quadrupole Inductive Coupled Plasma Mass Spetrometry). Prior to Q-ICP-MS analysis, digestion and dilution of the samples was performed to oxidize and remove organic matter and to keep the concentrations of inorganic solids to a minimum (Castillo et al., 2008; Krachler et al., 1998). The digestion protocol was validated by processing a Bio-Rad Level 1 urine reference sample (Lyphochek Urine Metals Control 1-69131; Marnes-la-Coquette, France) that contains metal concentrations close to those of urine in the studied population.

3 mL of each urine sample were introduced in Teflon vessels, together with 3 mL of Instra-Analysed 65% $HNO_3$ (J.T. Baker, Germany) and 1.5 mL of Instra-Analysed 30% $H_2O_2$ (Baker). They were then left in an oven at 90ºC overnight. After cooling, vessels were opened and placed on a heating plate at 250°C to evaporate the nitric acid. Once evaporated, the resulting solid samples were dissolved with 3 mL of 4% $HNO_3$ dilution, placed in 7 mL glass bottles and subsequently stored in a refrigerator until instrumental analysis. Before analysis, an internal standard of 10 ppb of In was introduced and depending on sample density samples were diluted with MilliQ water to 30 mL or 60 mL in order to avoid spectral interference. ICP-MS analysis was performed by a X-SERIES II device from Thermo Fisher SCIENTIFIC located in IDAEA-CSIC (Barcelona). One MilliQ water blank was processed in each batch of samples for control of possible contamination. Instrumental limit of detection referred to the urine sample was 0.2 ng/mL. Reagent blank levels were analyzed separately and the mean concentrations corresponded to 0.05 ng/ml. The method was validated by repeated analysis of Bio-Rad Level 1 reference urine samples (Lyphochek Urine

Metals Control 1–69131) which contains 6.8 ng/ml of cobalt. These concentrations are slightly higher than those found in our samples but they constitute the calibration set of lowest concentrations available and have been referred in several publications on urine metal analysis by ICP-MS (Heitland and Köster 2004). The resulting inter-assay relative standard deviation coefficient was 12 %.

All glassware and polypropilene material was thoroughly cleaned by soaking in 10% nitric acid for 24 h, followed by rinsing three times with MilliQ water. Teflon vessels were cleaned after every use by rinsing with 10% nitric acid (three times), then soaking with it in the oven at 90ºC overnight, and finally rinsing with abundant MilliQ water.

Creatinine was determined at Laboratories Echevarne (Barcelona) by the Jaffé method (kinetic with target measurement, compensated method) with Beckman Coulter© reactive in AU5400 (IZASA®).

### *2.3. Iron status measurements*

Hemoglobin in blood during the 12$^{th}$ and 32$^{nd}$ weeks of pregnancy was analyzed as a marker of iron status. Analysis was performed using a Sysmex XE-2100 system, where hemoglobin is determined by the sodium lauryl sulfate (SLS)-hemoglobin method.

### *2.4 Statistical analysis*

Mean, standard deviation (SD), median (IQR), and p90 values were calculated for the potential continuous variables, such as cobalt concentrations, hemoglobin and iron supplementation. Normality was tested by the Kolmogorov-Smirnov test. Absolute and relative frequencies were calculated for the potential categorical variables.

Cobalt 3rd-1st trimester individual ratios were calculated by division of the 3rd trimester by the 1st trimester concentrations. Furthermore, individual cobalt concentrations differences between both trimesters were also calculated.

Cobalt concentrations between the first and the third trimesters were compared using chi-squared tests and Spearman correlations. Spearman correlation rates were calculated to identify possible associations between different variables in non-parametric distributions. Mann-Whitney and Kruskall-Wallis testing was used to compare between groups of categorical variables. Univariate and multivariate linear regression models were performed to investigate which maternal factors were associated to cobalt concentrations.

Generalized additive models (GAM) were built in order to obtain graphics in which the associations could be observed excluding possible interferences.

All statistical analyses were performed using Stata 12.0 software.

## 3. Results

Cobalt levels, either in ng/mL or µg/g creatinine in the first and third trimesters are shown in Table 1. The distributions of individual concentrations were not gaussian but skewed to the left. Descriptive statistics in ng/mL or in µg/g creatinine were not significantly different. Accordingly, µg/g creatinine concentrations were generally used in the study of the associations. Median concentrations were 0.45 and 1.3 µg/g creatinine during the first and third trimesters, respectively. P90 was 1.4 and 2.9 µg/g creatinine during the first and third trimesters, respectively. The cobalt concentrations during the third and first trimesters were significantly correlated (Spearman coefficient 0.39; $p<0.001$).

The concentrations during both trimesters were significantly different ($p<0.001$). Calculation of the individual ratios between third and first trimesters provided an arithmetic mean

and median of 4.5 (SD 7) and 2.2 µg/g creatinine, respectively (Table 1). Most pregnant women from the studied population (84%) had higher cobalt levels during the third than the first trimesters.

Vitamin B12 intake showed no statistically significant association with cobalt levels in both trimesters involving Spearman's correlation rates of 0.0471 ($p = 0.36$) and -0.0690 ($p = 0.19$) during the first and third trimesters, respectively. Moreover, women taking supplementation either during the first or third trimesters did not show significant differences in cobalt concentrations when comparing with those who did not. Thus, in the first trimester, the mean urine concentrations of the two groups were 0.60 µg/g creatinine, standard deviation -SD- 0.58 µg/g creatinine, vs. 0.84 µg/g creatinine, SD 1.8 µg/g creatinine, ($p = 0.107$) and in the third trimester they were 1.7 µg/g creatinine, SD 2.8 µg/g creatinine, vs. 1.5 µg/g creatinine, SD 1.2 µg/g creatinine ($p = 0.624$).

The mean hemoglobin concentrations during the first and third trimesters were 12.63 g/dL and 11.55 g/dL, respectively. These differences were statistically significant ($p<0.001$). Women with higher hemoglobin decrease along pregnancy had significantly higher cobalt increase between both trimesters ($p<0.05$). This correlation was statistically significant (Figure 1). No statistically significant association was found for cobalt and hemoglobin during the first trimester but the association was statistically significant in the third trimester (-0.12; $p<0.05$).

A multivariate linear regression model was built taking into account different dietary and social maternal factors (Table 4). This model confirmed the negative significant association between cobalt and hemoglobin levels ($p<0.05$). Compilation of the same multivariate linear regression model on anemic (< 11 g/dL hemoglobin in blood) and non-anemic (> 11 g/dL) (Goonewardene et al., 2012) women showed that the former had significantly higher concentrations of cobalt. Consumption of coffee or tea also showed significant negative association with cobalt but with a lower beta coefficient ($p<0.05$). Therefore, during third trimester cobalt concentrations were negatively associated to hemoglobin levels and the trend was stronger among anemic women.

During the first trimester only 4.2% of pregnant women were anemic and this proportion rose up to 28% in the third trimester (Table 3). Anemic women had higher concentrations of cobalt

than women with normal levels in both trimesters, but the differences were only significant during the third trimester ($p<0.05$; Table 3).

Iron supplementation was taken by 8.1% (mean intake 19 mg/day) and 35% (mean intake 30 mg/day) in the first and third trimesters, respectively. A significant negative association between cobalt levels and iron supplementation was observed during the third trimester ($p<0.01$; Figure 2), but not during the first. When supplementation was high, this trend was not so well defined. Nevertheless, women taking supplementation had lower levels of cobalt during third trimester than those who were not taking it ($p<0.05$; Figure 2).

## 4. Discussion

The urine concentrations of cobalt in the studied cohort are similar to those found in general population from Europe (Minoia et al., 1990), USA (Komaromy-Hiller et al., 2000; NHANES, 2009), Japan (Ohashi et al., 2006) and Australia (Callan et al., 2013). In contrast, they are lower than levels reported in a mining area (Banza et al., 2009) or in occupational studies (Yokota et al., 2007). The levels during the third trimester were similar to those reported for pregnant women in Australia (Callan et al., 2013) (Table 2). According to the biokinetic model of Unice et al., (2014), assuming an inorganic cobalt intake of 10-20 µg/day during 30 years and 10% of gastrointestinal absorption, a urine concentration range between 0.6 and 1.2 ng/mL should be observed, which is in the same range as our data (Table 2). Cobalt intakes may change according to dietary differences between countries, e.g. 0.12 mg/day (United Kingdom), 29 µg/day (France) and 5-40 µg/day (USA) (Kim et al., 2006). Analysis of cobalt in 10-day representative diet items of an industrialized area of Spain led to estimate a mean intake of 9.8 µg/day (Domingo et al. 2012), which is consistent with these previous ranges. Thus, the observed cobalt concentrations of the present study are consistent with toxicokinetic data from general population of western countries in which the main exposure

and intake is due to the diet (Gál et al., 2008) and not to specific sources. The correlation between the concentrations in the first and third trimester of pregnancy is consistent with a constant exposure scenario along pregnancy for the whole studied population.

Cobalt is part of the vitamin B12 molecule. The absorption mechanisms of this vitamin are different from those of inorganic cobalt (Kim et al., 2006). Various studies have described mean daily losses of 0.1% of the B12 vitamin pool which may be enhanced when intake is high (IOM, 1998). No significant associations between intake of B12 supplements and urine cobalt concentrations either during the first or third trimesters of pregnancy have been observed in the present study. Accordingly, most of the ingested cobalt is in inorganic form and vitamin B12 intake has not an influence on the observed cobalt urinary levels (IOM, 1998; Kim et al., 2006).

Few previous studies have considered the changes in trace metal concentrations along pregnancy (Kilinc et al., 2010; Liu et al., 2010) and none of them included cobalt. Callan et al. (2013) analyzed cobalt concentrations in urine of pregnant women during the third trimester and the results were similar to those corresponding to the third trimester in the Sabadell cohort, but they did not describe results at other stages of pregnancy. Considering other metals concentration decreases of Cd, Se or Cs have been reported (Kilinc et al., 2010;) and have been attributed to the 40-50% increase in plasma volume along pregnancy (Hytten, 1985). In contrast, urinary Zn excretion was reported to increase along pregnancy due to the higher glomerular filtration rate that occurs along this process (Swanson and King, 1987). In the case of cobalt, the two-fold higher median increase between the first and third trimesters (Table 1) reflects the individual increase of this metal in 84% of women from this population. Although glomerular filtration rate increase could explain this change, we consider that a metabolic process may be responsible for an enhancement of cobalt absorption

Basal metabolic rate rises up to 60% during the third trimester of pregnancy (Hytten, 1985), increasing iron demand to transport sufficient oxygen for aerobic processes. Moreover, there is an

increased need for transport to the fetus for hematopoiesis. Actually, 80% of the iron present in the newborn term infant is accreted during the third trimester of pregnancy (Baker et al., 2010). Accordingly, requirements of absorbed dietary iron increase from 0.8 mg/day during the first trimester to 7.5 mg/day during the third (Milman, 2006). This increase in iron consumption leads to iron decrease along pregnancy, e.g. a mean hemoglobin decrease of 1.10 g/dL in the present study, including 28% of anemic women (less than 11 g/dL hemoglobin) in the third trimesters.

The observed urine cobalt increase may respond to a compensatory mechanism of iron decrease (Table 1, Figures 1 and 2). Accordingly, a statistically significant negative correlation is observed between cobalt and hemoglobin concentrations in the samples collected in the third trimester. The difference is defined even better when considering anemic women as they have significantly higher cobalt concentrations than women with normal hemoglobin levels (Figure 3). Hemoglobin decrease during pregnancy reflects iron depletion and leads to cobalt increase.

This relationship is consistent with previous studies. A correspondence between decrease of iron and increase of cobalt has been reported in adolescent girls and boys, particularly in girls, either by comparing serum ferritin or transferrin transporter and serum cobalt or from the differences of cobalt concentrations between subjects with abnormal and normal iron status (Bárány et al., 2005). Hereditary hemochromatosis patients were found to accumulate not only iron but also cobalt (Nichols and Bacon, 1989). Associations between cobalt blood concentrations and ferritin or hemoglobin status were reported in Norwegian women (Meltzer et al., 2010). Women with normal and high iron concentrations have been observed to retain less cobalt than women with iron depletion (Christensen, 1995; Christensen et al., 1993) Women are also known to retain more cobalt than men which may be related to iron losses by menstruation (Christensen, 1995; Christensen et al., 1993; Unice et al., 2012). Indeed, women have been observed to increase three times more cobalt in urine than men at equal intake of this compound in a short term study of gastrointestinal uptake of different inorganic cobalt compounds (Christensen, 1995; Christensen et al., 1993).

Increased intestinal cobalt absorption as consequence of iron deficiency by bleeding or diet was reported in mice experiments (Flanagan et al., 1980). Cobalt absorption has been demonstrated to be enhanced not only in iron depletion but also during adolescence or during the last stage of pregnancy when there are higher iron body demands (Barany et al., 2005). The increase of cobalt absorption may be mediated by the divalent metal transporter 1 (DMT1), an intestinal active transporter for inorganic Fe in its oxidized from Fe(II). This transporter has been demonstrated to be associated with the intestinal transport of other divalent cations such as Mn(II), Cu(II), Zn(II) or Co(II) (Gunshin et al., 1997). In vitro experiments with DMT1 animal transfected cells showed that this enzyme is able to transport cobalt into the cell (Garrick et al., 2006, Forbes and Gros, 2003), together with other divalent ions. This protein is up-regulated by iron status, either in iron depletion or when iron body demands are enhanced (Garrick et al., 2003; Mackenzie and Garrick, 2005). Hence, iron decrease together with higher demand occurring during the third trimester of pregnancy may enhance the DMT1 expression leading to a higher absorption of cobalt and higher concentrations of this metal in urine. This mechanism has already been proposed to justify observed associations between iron status and metal concentrations in human populations (Meltzer et al., 2010; Barany et al., 2005) and further studies are needed for a more comprehensive understanding of the processes involved. The present study provides evidence of the correspondence between iron status and cobalt in pregnant women.

Cobalt induces erythropoietin transcription, stimulating red blood cell production (Unice et al., 2012). In fact, it has been hypothesized that this metal may eventually be used by some athletes to increase erythropoietin activity (Lippi et al., 2006). The higher levels of cobalt during the third trimester may stimulate the production of red cells during late pregnancy to fulfill the $O_2$ requirements for the high metabolic rate occurring at this stage, as well as to provide sufficient iron for red cell production for the fetal growth.

Women taking iron supplementation had significantly lower levels of cobalt during the third trimester (Figure 2). As their iron requirements were fulfilled and hemoglobin levels were generally higher, lower cobalt absorption and lower cobalt levels in urine were observed.

The observed results may also be significant in populations occupationally or environmentally exposed to cobalt. Hence, in populations with daily cobalt intake close or higher to 0.03 mg/kg·day in women with albumin alterations (Pausterbach et al., 2013) or during pregnancy, the proposed chronic oral reference dose for maximum cohort intake (Field et al., 2012) could have health effects in the mothers and, most importantly, in their children. Dietary and control measures for avoiding iron depletion should be emphasized in these cases.

One of the main limitations of our study is the use of hemoglobin as the only measure of iron status. Hemoglobin reflects mass of circulating red blood cells. It should be complemented with other measurements such as serum ferritin or serum ferritin transporter in order to diagnose and iron-defficiency anemia status, although during pregnancy iron deficiency is difficult to confirm (Goonewardene et al., 2012). However, hemoglobin is a rapid and cheap parameter which do not require costly laboratory equipment and testing, thus it can be useful to have a first approach (Cameron and Neufeld, 2011).

Cobalt levels in maternal urine have been observed to rise significantly from the first to the third trimesters, probably due to the iron decrease along pregnancy. A significant negative correlation has been found between hemoglobin and urine cobalt concentrations in the third trimester, as well as between the differences between hemoglobin levels and urine cobalt concentrations between these two trimesters. This association has been previously reported in adolescents, women and hemochromatosis patients, but the present study is the first in which this trend is observed during pregnancy. Cobalt enhances transcription of erythropoietin, leading to higher red cell production. Higher absorption of this metal may tend to counterbalance iron depletion during last stages of pregnancy, when basal metabolic rate is high and 90% of fetal growth occurs and iron requirements are increased. This mechanism may be useful to contribute to

fulfill the oxygen demand of these processes that are crucial for proper fetus development. However, the present results recommend the implementation of monitoring programs of cobalt concentrations in pregnant women from populations occupationally or environmentally exposed to this metal. This strategy could allow to anticipate possible deleterious effects for the mother or fetus as consequence of enhanced cobalt accumulation.

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**TABLES**

Table 1. Metal urine concentrations of cobalt in the first and third trimesters of pregnancy, in ng/mL and µg/g creatinine. Correlation rate between concentrations in both trimesters and p-value of the difference.

| | % Detection | Arithmetic mean (SD) | Median (IQR) | P90 | p-Value | Spearman's correlation rho |
|---|---|---|---|---|---|---|
| **ng/mL** | | | | | | |
| 1st trimester | 73.6 | 0.57 (0.56) | 0.42 (0.74) | 1.2 | p<0.001 | 0.41 |
| 3rd trimester | 84.3 | 1.4 (1.4) | 1.2 (1.2) | 2.8 | | (p<0.001) |
| **3rd/1st ratio** | | **4.6(6.3)** | **2.1(4.3)** | **12** | | |
| **3rd – 1st difference** | | **0.88 (1.2)** | **0.70 (1.3)** | **2.1** | | |
| **µg/g creatinine** | | | | | | |
| 1st trimester | 73.6 | 0.73 (1.4) | 0.45 (0.70) | 1.4 | p<0.001 | 0.39 |
| 3rd trimester | 84.3 | 1.6 (2.5) | 1.3 (1.3) | 2.9 | | (p<0.001) |
| **3rd/1st ratio** | | **4.5 (7.0)** | **2.2 (3.8)** | **10** | | |
| **3rd – 1st difference** | | **0.90 (2.5)** | **0.72 (1.2)** | **2.2** | | |

475Table 2. Urine cobalt concentrations in the present cohort and in other populations, in ng/mL (µg/g

476creatinine in brackets)

| Reference | Origin | N | Co |
|---|---|---|---|
| Our study (1st trim) | Sabadell | 345 | 0.42(0.45)[b] |
| Our study (3rd trim) | Sabadell | 345 | 1.2(1.3) [b] |
| Callan et al., 2013 | Australia | 173 | (1.17) [b d] |
| Ohashi et al, 2006 | Japan | 1000 | 0.68 [a] |
| Banza et al 2009 | DR Congo (mining area) | 179 | (15.7) [a] |
| Yokota et al 2007 | Japan (battery plant) | 16 | 28 [c e] |
| Paschal et al 1998 | USA | 496 | (0.78) [a] |
| Minoia et al 1990 | Italy | 11-900 | 0.57 [a] |
| NHANES 03-04 | USA | 2500 | (0.314) [b] |
| Komaromy et al., 2000 | USA | 1000-16000 | (1) [a d] |

477[a] Geometric mean. [b] Median. [c] Arithmetic mean. [d] Pregnant women [e] Only men, just after leaving the
478plant
479

480Table 3. Cobalt concentrations (µg/g creatinine) in anemic and non-anemic pregnant women (under
481and over 11 g/dL hemoglobin in blood) during the first and third trimesters of pregnancy.

| | % women | Mean (SD) | Median (IQR) | P90 | p-Value |
|---|---|---|---|---|---|
| **1st trimester** | | | | | |
| >11 g/dL Hb | 96 | 0.85(0.71) | 0.44 (0.69) | 1.3 | 0.16 |
| <11 g/dL Hb | 4.2 | 0.72 (1.4) | 0.69 (0.71) | 2.1 | |
| | | | | | |
| **3rd trimester** | | | | | |
| >11 g/dL Hb | 72 | 1.5 (2.9) | 0.93 (1.2) | 2.6 | 0.016 |
| <11 g/dL Hb | 28 | 1.8 (1.4) | 1.2 (1.5) | 3.4 | |

482

Table 4. Multivariate regression model for maternal urine cobalt concentrations during the third trimester of pregnancy and its possible associated factors.

| | β (IC) | p-Value |
|---|---|---|
| Last measurement of hemoglobine in g/dL blood | -0.11 (-0.22, -0.00093) | 0.048 |
| Maternal origin | | |
| Spain | 1 | |
| Latin America | -0.081 (-0.54, 0.38) | 0.731 |
| Europe | 0.13 (-0.58, -0.85) | 0.710 |
| Rest of the world | -2.5 (-4.6, -0.49) | 0.020 |
| Exposure to heavy truck traffic | | |
| Practically never | 1 | |
| A few | 0.11 (-0.18, 0.41) | 0.450 |
| Quite often | 0.36 (-0.010, 0.72) | 0.057 |
| Continuous | 0.22 (-0.18, 0.51) | 0.146 |
| Smoking during 3rd trimester | | |
| No | 1 | |
| Yes | 0.33 (-0.0029, 0.67) | 0.052 |
| White meat consumption [a] | -0.0063 (-0.013, 0.00066) | 0.076 |
| Blue fish consumption [a] | 0.0056 (-0.00077,0.012) | 0.084 |
| Vegetables consumption [a] | 0.00098 (-0.00012, 0.0021) | 0.081 |
| Legumes consumption [a] | -0.0050 (-0.010, 0.00054) | 0.077 |
| Coffee/Tea comsumption [a] | -0.00070 (-0.0013, -0.00093) | 0.048 |
| $R^2$ | 0.094 | |

[a]gr/week of consumption

**Figure captions**

**Figure 1.** Graphic representation of generalized additive models (GAM) showing association (and 95 % confidence levels) between maternal blood hemoglobin levels and the logarithm of urine cobalt concentrations in the first and third trimesters of pregnancy and the increases of cobalt at decreasing hemoglobin. The symbols (+) on the X-axis show the hemoglobin levels of each subject (g/dL).

**Figure 2.** Association between iron supplementation intake and cobalt concentrations during the third trimester of pregnancy.