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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 12th and 32nd 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 (Paustenbach 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

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cobalt intake for non-cancer health effects in general population over lifetime exposure (Finley 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 (Paustenbach 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). Bárány 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 (Bárány 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 −20 °C 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-Analyzed 65% HNO3 (J.T. Baker, Germany) and 1.5 mL of Instra-Analyzed 30% H2O2 (Baker). They were then left in an oven at 90 °C 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% HNO3 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 90 °C 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 Colter© reactive in AU5400 (IZASA®).