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Selenium, selenoproteins and selenometabolites in mother and baby at time of birth.

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ABSTRACT

Deficiency of selenium (Se), an essential micronutrient, has been implicated in adverse pregnancy outcomes. Our study was designed to determine total serum Se, selenoproteins [extracellular glutathione peroxidase (GPX3), selenoprotein P (SELENOP), selenoalbumine (SeAlb) and selenometabolites (SeMetab)] in healthy women and their newborns at delivery. A cross-sectional study included 83 healthy mother-baby couples. Total Se and Se-species concentrations were measured in maternal and umbilical-cord serum by an in-series coupling of two-dimensional size-exclusion and affinity high-performance liquid chromatography (2D/SE-AF-HPLC). Additional measurements were made of SELENOP concentration by ELISA and of GPX3 enzyme activity. Total Se concentration was significantly higher in maternal serum than in cord serum (68.9±15.2 and 56.1±14.6 µg/L respectively; $p<0.01$). There were significant correlations between selenoprotein and SeAlb concentrations in mothers and newborns, although they also showed significant differences: GPX3 (11.2±3.7 vs 10.5±3.5 µg/L) SELENOP (42.5±9.5 vs 28.1±7.7 µg/L) and SeAlb (11.6±3.6 vs 14.1±4.3 µg/L) in maternal and cord serum respectively. GPX3 activity and concentration were correlated positively in mothers ($r=0.33$; $p=0.038$) but not in newborns. GPX activity in cord serum was significantly correlated with gestational age ($r=0.44$; $p=0.009$). Se Alb concentration was significantly higher in babies while SELENOP and GPX3 were significantly higher in mothers. The differences cannot be explained by simple diffusion; specific transfer mechanisms are probably involved. GPX3 concentrations in mothers at delivery are related to maternal Se status, while the GPX activity in cord serum depends on gestational age.

INTRODUCTION

Selenium (Se) is an essential micronutrient with a wide range of protective functions, which are exerted through its incorporation into selenoproteins [1]. Inadequate dietary Se intake can compromise human reproduction since Se deficiency has been associated with obstetric and perinatal complications such as male and female infertility, miscarriage, pre-eclampsia, intrauterine growth restriction, preterm delivery and neural defects in the offspring [2-4].

The maternal-fetal transfer mechanisms for Se and selenoproteins have been studied in mice [5] and in placental models *in vitro* [6], leading to the identification of some specific placental transporters [6,7]. Though a number of studies have measured Se concentrations in mothers and newborns at the time of birth, they have had contradictory results [8-10]. To some extent, these contradictions might be explained by differences in Se status between geographic regions [11], distribution patterns between Se species and the reliability of the method of determination of Se [12]. Whatever the case, those studies were limited by measurement only of total serum/plasma Se which does not allow distinction between different species of Se that may be transferred from mother to baby by different mechanisms.

In investigating the relative concentrations of Se in mother and newborn it is important to take account of the different Se components in serum/plasma[13]: two selenoproteins [selenoprotein P1 (SELENOP)[14] and the extracellular GPX3], Se incorporated non-specifically as selenomethionine (SeMet) in lieu of methionine in albumin (SeAlb), and other plasma proteins, and a small amount of non-protein-bound low molecular weight Se metabolites (SeMetab). SELENOP is the most abundant selenoprotein in plasma and is a good indicator of Se status in non-Se-replete humans [15]. The activity of GPX3 in human serum is a complementary marker of Se status. Importantly,GPX3 has been identified as a key enzyme in the defence against oxidative stress during decidualization (the post-ovulatory process of endometrial remodeling in preparation for implantation) by reducing H_2O_2 in the endometrium [16].

We have been able to determine these species in serum from mother and baby pairs at time of birth owing to our previous development of a three dimensional chromatographic method for simultaneous speciation of serum GPX3, SELENOP, SeAlb and SeMetab [17]. Although the accuracy of this method has been assessed by analyzing a commercial human serum (BCR-637), it has not previously been applied in a clinical setting.

The aims of the current study were: (i) to determine simultaneously Se, selenoproteins, SeAlb and SeMetab concentrations by this new method in both maternal and cord sera from uncomplicated pregnancies at the time of birth; (ii) to compare maternal and neonatal concentrations in order to

elucidate potential maternal-fetal transfer mechanisms; (iii) to assess whether SELENOP measured by 3D/SE-AF-AEC-HPLC corresponded to that measured by ELISA and to assess the coherence between GPX3 measured as a concentration and GPX3 enzyme activity. Our study may also help to assess Se requirements in pregnancy, as recommended by the European Food Safety Authority (EFSA) Panel [18].

METHODS

A cross-sectional study was performed on 83 healthy mother-baby couples. Participants were recruited at random from pregnant women without any maternal or neonatal risk factors, who gave birth at term, at the Department of Obstetrics and Gynecology, Hospital de Riotinto, during the years 2014-2015. Women with diseases, multiple gestation and perinatal complications such as obstructed labor, low Apgar score (below 5) or where there was suspicion of infant pathology were excluded.

A full history was taken from the enrolled mothers which included whether or not they took a multivitamin/mineral supplement [a standard formula approved by the Department of Health, containing 200 µg iodide (I^-), 55µg Se, 10 mg zinc and 200 mg docosahexaenoic acid (DHA)] during pregnancy. Medical data from clinical examination of mothers and babies were also recorded at birth.

This study was approved by the local Ethics Committee and written informed consent was obtained from all the participants. Since we also collected samples from cord blood, the parents provided separate, signed, informed consent for this specific purpose.

Analytical procedures

Blood samples were collected from mothers during the 24 hours before delivery. At birth, a sample of cord blood was obtained and its pH was measured. Serum was separated and frozen at -80ºC until further analysis.

Selenium and selenoprotein concentrations were measured by a three-dimensional chromatographic method that used in-series size-exclusion, affinity and anion-exchange high performance liquid chromatography (3D/SE-AF-AEC-HPLC), as previously described, that overcomes common spectral interferences from chloride and bromide [17]. SELENOP concentration was also analyzed by enzyme immunoassay (ELISA) (USCN Business Co., Ltd., Houston, TX), and GPX activity by a commercial kit (Cayman Chemical, Ann Arbor, MI).

Statistical analysis

Power: With 38 subjects per mother-baby group, and a standard deviation as 15 µg/L of selenium in mean serum Se concentration [obtained from a recent study of Spanish pregnant women (71.2± 14.9 µg/L)][19], our study had 80% power to detect a difference of at least 10 µg/L in total serum Se between mother and baby groups at a two-sided, 5% significance level.

As our sample included women who took a mutivitamin/mineral supplement and women who did not, a comparison of means between these two groups was made to see whetheher this supplementation had a significant effect. Assuming a two- sided hypothesis, the conventional alpha and beta levels, and a proportion of 3:1 for the studied groups (the use of multivitamin/mineral supplements in our population does not reach a quarter of pregnant women), the sample size calculated was 19 women for the supplemented group and 57 pregnant women for the non-supplemented group.

Data are presented as medians and standard deviations for continuous variables and as percentages for categorical variables. The contrast hypothesis for two samples was evaluated using Fisher´s exact test for categorized variables and Student´s t-test for continuous variables. Wilcoxon signed-rank test was used to compare matched samples. The correlation between variables was determined using the Spearman test, designing multiple linear regression models in those cases where it was desired to predict the variance adjusted for other variables, besides the main variable. The contrast hypothesis for more than two samples was determined with an analysis of variance (ANOVA). All *P*-values were two-sided, and statistical significance was declared at $P < 0.05$. All data were analyzed using SPSS 20.0 (IBM SPSS Statistics).

RESULTS

Maternal and neonatal characteristics

Table 1 shows maternal and neonatal characteristics. Since exclusively healthy pregnant women were recruited, the cesarean section rate was only 7.5% (compared to the usual cesarean section rate at our center of around 16%), and perinatal outcomes were good (there were no admissions to the Neonatal Intensive Care Unit).

No correlations were found between maternal age, parity, body mass index or level of education and maternal Se concentration, nor were any correlations found between neonatal weight at birth or gestational age with maternal or neonatal serum Se concentrations. However, there was a negative correlation between hours of fasting prior to blood draw and total Se concentration in maternal serum ($r = -0.30$; $p<0.05$).

Women who did not take multivitamins /mineral supplements (containing 55 µg Se) were significantly younger than women who did take them (29.42±5.35 vs 31.16±4.80 years, respectively; $p<0.05$). No significant differences in parity, body mass index or level of education were found between women who took multivitamin/mineral supplements and those who did not (data not shown).

Se and selenoprotein concentrations by 3D/SE-AF-AEC-HPLC

Total Se and selenoprotein concentrations in maternal and cord serum are summarized in Table 2. Total Se, GPX3 and SELENOP concentrations were significantly higher in maternal than in cord serum, while SeAlb was significantly more concentrated in cord serum. There was no significant difference in the SeMetab concentration between maternal and cord serum.

The correlations between maternal and cord Se species were significant in all cases, reaching the highest value for GPX3 and the lowest for SELENOP.

The proportion of Se species in maternal and cord serum was different; while SELENOP accounted for 65% of total amount Se in maternal serum, it only represents 50% of total Se in cord serum. By contrast, SeAlb constituted 15% of total Se in maternal serum but 28% in cord serum. The percentages for GPX3 and SeMetab were similar in maternal and cord sera: 14% for GPX3 in both, and 7% and 8% for SeMetab in maternal and cord sera, respectively (Figure 1a). When the Se species concentrations are represented graphically, the different pattern in maternal and cord serum is apparent (Figure 1b).

There were no significant differences in the concentrations of Se species in maternal or cord serum between women who took multivitamins/minerals containing Se and those who did not (Table 3).

Maternal total serum Se was strongly correlated with all the Se species in maternal serum, i.e. GPX3 ($r=0.71$; $p<0.01$), SELENOP ($r=0.89$; $p<0.01$), SeAlb ($r=0.80$; $p<0.01$) and SeMetab ($r=0.42$; $p<0.01$), and moderately correlated with the concentrations of the Se species in cord serum, i.e. total Se in cord serum ($r=0.56$; $p<0.01$), GPX3 ($r=0.47$; $p<0.01$), SELENOP ($r=0.43$; $p<0.01$), SeAlb ($r=0.45$; $p<0.01$) and SeMetab ($r=0.48$; $p<0.01$).

Total Se in cord serum was correlated with all the Se species studied in cord serum , i.e. GPX3 ($r=0.78$; $p<0.01$), SELENOP ($r=0.89$; $p<0.01$), SeAlb ($r=0.80$; $p<0.01$) and SeMetab ($r=0.60$; $p<0.01$), and moderately correlations with the concentration of the Se species in maternal serum, i.e. GPX3 ($r=0.64$; $p<0.01$), SELENOP ($r=0.32$; $p<0.01$), SeAlb ($r=0.61$; $p<0.01$) and SeMetab ($r=0.66$; $p<0.01$).

Negative correlations were found between umbilical-cord pH and maternal SeAlb (r= -0.33; p=0.01), cord total Se (r= -0.30; p=0.04) and cord SeAlb (r= -0.30; p=0.02).

GPX activity

GPx activity was significantly higher in maternal than in cord serum (112.87±68.91 and 38.67±25.03 nmol/min/ml, respectively; p<0.01), and there was no correlation between the activities (r=0.12; p=0.48). There was a significant correlation between GPX3 concentration and its activity in maternal serum (r=0.33; p=0.04), but this correlation was not apparent in cord serum (data not shown). Maternal GPX activity also correlated significantly with total Se concentration in maternal serum (r=0.36; p=0.03) and with SeMetab concentration in cord serum (r=0.50; p<0.01), but not with other Se species in either maternal or cord serum (data not shown). GPX activity in cord serum was significantly correlated with birth weight (r=0.39; p=0.02) and gestational age (r=0.43; p<0.01). This last correlation remained significant after adjusting by birth weight and height (R^2=0.23; p=0.04). However, GPX activity in cord serum did not correlate with total Se or Se species either in cord or maternal serum (data not shown).

SELENOP measured by ELISA.

SELENOP concentration determined by ELISA was significantly different in maternal and cord serum (38.27±12.34 µg/L and 1.93±0.98 µg/L respectively, p<0.01). No correlation was found between SELENOP in maternal and cord serum when measured by ELISA. There was no correlation between SELENOP concentrations measured by HPLC and ELISA in either maternal or cord serum (data not shown). Although SELENOP concentration in maternal serum measured by HPLC and ELISA were not significantly different (42.49 ± 9.49 µg/L and 38.27 ± 12.34 µg/L respectively, p=0.10), there was a significant difference between these two methods of determination in cord serum (28.06 ± 7.69 µg/L by HPLC and 1.93 ± 0.98 µg/L by ELISA, p<0.01). Maternal SELENOP measured by ELISA correlated significantly with GPX3 concentration in cord serum (r=0.33; p=0.04).

DISCUSSION

This is the first study to analyze simultaneously total Se and Se species in maternal and cord serum at the time of birth using a new optimized HPLC method. Although some of these factors have previously been measured at delivery, the interrelationships between the different Se species in maternal and cord sera are largely unknown, as is the biological significance of such relationships. Our findings indicate that, at birth, total Se, GPX3 and SELENOP concentrations are significantly

higher in maternal than in cord serum while the opposite is the case for SeAlb. In general, simple diffusion cannot explain the maternal-neonatal transfer of Se and Se species around the time of birth.

Oxidative stress is tightly related to perinatal morbidity and mortality, so the antioxidant selenoproteins may have a central role in protecting against adverse pregnancy outcomes[20]. Thus total Se concentration and GPX3 activity in cord serum were found to increase with advancing pregnancy[21] while oxidative stress during labor was associated with elevated fetal GPX3 activity[22]. A study in rats showed that a sufficient Se supply was required to ensure antioxidative protection to the fetus during the oxygen transformations that take place during delivery and early postnatal life[23]. By contrast, in a UK population of slightly higher Se status than ours, maternal blood Se concentrations fell from 12 to 35 weeks of gestation (believed to be partly due to the expansion of plasma volume and partly due to receptor-mediated transfer of SELENOP from mother to fetus) and maternal GPX activity did not change[24].

Our study found lower Se concentrations than those reported in other studies of Spanish pregnant women[19,25]. Using the most restrictive cut-off of 70 µg/L of total serum Se concentration required to optimize GPX3 activity[26], 57 of 83 (69%) pregnant women in our study had insufficient serum Se to fulfill this requirement. However, based on alternative estimates of the serum Se concentration required for optimal GPX3 activity range from 89-114 µg/L[11], 90% of the women in our sample would have not reached this optimal status.

It might have been expected that pregnant women who took multivitamins/multiminerals containing sodium selenite would have had significantly higher concentrations of at least some Se species than those who did not, as sodium selenite can be used for the synthesis of selenoproteins such as GPX3 or SELENOP (though it will not increase SeAlb)[12]. However, the absence of higher concentrations in the women on supplements is not surprising as the Se dose was low (55 µg/day) and only 25% of the women took such supplements; furthermore, we have no data on their compliance.

In recent years, Se status in maternal and cord blood at delivery has been determined in a number of studies, though with varied outcomes[27-29]. Al-Saleh et al[27] examined the role of Se in reducing oxidative stress induced by cadmium and its impact on birth measures. They concluded that the extent of benefit afforded by Se is not governed only by its concentration but also by the different chemical forms of Se that interact with various proteins. Chen et al[28] attempted to clarify concentrations for cadmium, mercury, lead and Se in mothers and newborns and their placental transfer. Finding a high degree of maternal-fetal Se transfer, they concluded that there was free

trans-placental passage of Se from mother to fetus. However, our results do not support that conclusion; the different pattern of transfer of SELENOP and SeAlb implies that different mechanisms are involved in the transfer of these different Se species from mother to fetus.

It appears likely that receptor-mediated uptake exists in humans as previously found in pregnant mice[5]. Burk and colleagues identified two mechanisms of Se transfer; from early to mid-gestation, plasma GPX and SELENOP were transported *via* the uterine fluid, probably by pinocytosis, whereas in the latter half of gestation, placental transfer of maternal SELENOP occurred through the apoE receptor 2 (apoER2)[(5)]. In fact, the higher levels of SeAlb in cord serum compared to maternal serum suggest the existence of a further maternal-fetal Se transfer mechanisms[(5,30)]. Studies in mice and rats suggest that this other pathway involves the transfer of Se as SeMet-containing proteins or SeMet itself (*via* methionine transporters) to the fetus[(5,30)]. This mechanism depends on the amount of SeMet in the diet of the mother, appears to be unregulated and is highly dependent on the mother´s Se status, thus it will be less effective under conditions of Se deficiency[(5)].

The similar concentrations for SeMetab and their strong correlation in maternal and cord serum can be explained by a simple diffusion mechanism of trans-placental passage that does not occur for other Se species.

GPX3 activity was measured to see whether there was a correlation between concentrations and activity. As expected, the GPX3 activity in maternal serum significantly correlated with maternal total Se and GPX3 concentrations, but GPX3 activity in cord serum only correlated with gestational age and birth weight. These results are in agreement with previous findings[(31)]; it seems that GPX activity in mothers at delivery is related to maternal Se status, while the GPX activity in cord serum depends on gestational age[(22)].

Finally, our study compared different methods of determining SELENOP: 3D/SE-AF-AEC-HPLC and ELISA. The growing replacement of traditionally immunoassay techniques by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has highlighted the advantages and limitations of each system as well as the disparity in results by the two methods[(32)]. In our sample, the concentration of SELENOP in maternal serum measured by both methods gave a similar result, but this was not the case in cord serum where SELENOP concentration measured by ELISA was significantly lower than that measured by HPLC, and did not correlate with any other parameters. While these results may suggest that ELISA is not a reliable method of quantifying SELENOP in cord serum, other explanations are possible. SELENOP has three transcripts in humans although all variants begin with the same non-coding exon[(33)]. The most abundant transcript in all tissues was

SELENOPa. However, all variants (SELENOPa, b and c) showed tissue and developmental stage-specific expression patterns in neonatal and adult tissues[33]. These transcripts can synthetize different isoforms, varying in size and Se content[33] as previously proposed[34]. Moreover, the silencing of each transcript results in a differential synthesis of SELENOP isoforms[33]. In this respect, the antibodies used in ELISA kits could have a different affinity for the SELENOP isoforms present in serum. This could affect the binding of the antibody to the SELENOP protein in the ELISA and therefore give different results than techniques that do not use antibodies. Alternatively, this discordance in the results of SELENOP concentration between 3D/SE-AF-AEC-HPLC and ELISA may suggest that in the newborn, the predominant SELENOP isoform is different from that found in the mothers, and perhaps, with different functions[33]. It is also likely that full-length SELENOP has a higher Se content that the shorter isoforms[15]. It has been suggested that while liver exports full-length SELENOP, other tissues secrete mostly shorter SELENOP isoforms, possibly for local use as a redox enzyme or as a signaling molecule[15]).

The current study has some limitations. Firstly, the absence of a food frequency questionnaire did not allow us to assess Se intake. Furthermore, the small proportion (25%) of women taking a supplement containing Se may have prevented our finding significantly higher markers of status in that group.

Our study has also certain strengths; our design made possible the collection of data and samples from healthy pregnant women and their newborns at the time of birth. The multiple biomarkers studied from the same patients and the availability of a robust and reliable method to determine simultaneously different Se species gave us an insight into the relationships between these Se species in mother and newborn.

In summary, we have investigated the ratio of Se species in mother and baby at the time of birth. Our main finding is that the different pattern of Se species in maternal and cord serum suggests the existence of different mechanisms of trans-placental passage of Se species from mother to fetus which are species-dependent. The lack of concordance in the SELENOP concentration measured by 3D/SE-AF-AEC-HPLC and ELISA suggests the possibility that the predominant SELENOP isoform in newborn may be different from that found in the mother; this possibility needs to be followed up in a later study.

CONCLUSION

There is a different pattern of Se species in maternal and cord serum at the time of birth which suggests the existence of different mechanisms of trans-placental passage of Se species from mother to fetus that are species-dependent.

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Conflict of Interest:

The authors declared that no competing interests exist.

Authorship:

*Santos and Velasco contributed equally to this study, designing the study, recruiting participants, collecting the samples and writing the manuscript. García-Fuentes performed the biochemical tests and the statistical analysis. García-Barrera and Gómez-Ariza designed the analytical experiments, the instrumental couplings and supervised the results. Santos and Callejón-Leblic performed the analytical experiments and the tandem mass spectrometry analysis. Rayman contributed intellectually to the study and to the writing of the manuscript.

REFERENCES

1. Duntas LH, Benvenga S. (2015) Selenium: an element for life. *Endocrine* **48**,756–75.
2. Pieczyńska J, Grajeta H. (2015) The role of selenium in human conception and pregnancy. *J Trace Elem Med Biol* **29**, 31–8.
3. Rayman MP, Bode P, Redman CW.(2003) Low selenium status is associated with the occurrence of the pregnancy disease preeclampsia in women from the United Kingdom. *Am J Obstet Gynecol* **189**, 1343–9.
4. Rayman MP, Wijnen H, Vader H, Kooistra *et al.* (2011) Maternal selenium status during early gestation and risk for preterm birth. *CMAJ* **183**, 549–55.
5. Burk RF, Olson GE, Hill KE, *et al*. (2013) Maternal-fetal transfer of selenium in the mouse. *FASEB J* **27**, 3249–56.
6. Nandakumaran M, Dashti HM, Al-Saleh E, *et al*. (2003) Transport kinetics of zinc, copper, selenium, and iron in perfused human placental lobule in vitro. *Mol Cell Biochem* **252**, 91–6.
7. Miyauchi S, Srinivas SR, Fei YJ, *et al*. (2006) Functional Characteristics of NaS2, a Placenta-specific Na+-coupled Transporter for Sulfate and Oxyanions of the Micronutrients Selenium and Chromium. *Placenta* **27**, 550–9.
8. Jariwala M, Suvarna S, Kiran Kumar G, *et al*. (2014) Study of the Concentration of Trace Elements Fe, Zn, Cu, Se and Their Correlation in Maternal Serum, Cord Serum and Colostrums. *Indian J Clin Biochem* **29**, 181–8.
9. Bermúdez L, García-Vicent C, López J, *et al*. (2015) Assessment of ten trace elements in umbilical cord blood and maternal blood: association with birth weight. *J Transl Med* 13, 291.
10. Özdemir HS, Karadas F, Pappas AC, *et al*. (2008) The Selenium Levels of Mothers and Their Neonates Using Hair, Breast Milk, Meconium, and Maternal and Umbilical Cord Blood in Van Basin. *Biol Trace Elem Res* **122**, 206–15.
11. Rayman MP. (2012) Selenium and human health. *Lancet* **379**, 1256–68.
12. Fairweather-Tait SJ, Collings R, Hurst R. (2010) Selenium bioavailability : current knowledge and future research. *Am J Clin Nutr* **91**, 1484S–91S.
13. Combs GF, Watts JC, Jackson MI, *et al.* (2011) Determinants of selenium status in healthy adults. *Nutr J* **10**, 75.
14. Gladyshev VN, Arnér ES, Berry MJ, *et al*. (2016) Selenoprotein Gene Nomenclature. *J Biol Chem* **291**, 24036-40.
15. Burk RF, Hill KE. (2009) Selenoprotein P- Expression, Funtions, and Roles in Mamals.

Biochim Biophys Acta **1790**, 1441–7.

16. Xu X, Leng JY, Gao F, *et al*. (2014) Differential expression and anti-oxidant function of glutathione peroxidase 3 in mouse uterus during decidualization. *FEBS Lett* **588**, 1580–9.
17. García-Sevillano MA, García-Barrera T, Gómez-Ariza JL. (2013) Development of a new column switching method for simultaneous speciation of selenometabolites and selenoproteins in human serum. *J Chromatogr A* **1318**, 171–9.
18. EFSA NDA Panel. (2014) Scientific Opinion on Dietary Reference Values for selenium. *EFSA J* **12**, 3846.
19. Bermúdez L, García-Vicent C, López J, *et al.* (2015) Assessment of ten trace elements in umbilical cord and maternal blood: association with birth weight. *J Transl Med* **13**, 291.
20. Mariath AB, Bergamaschi DP, Rondó PHC, *et al.* (2011) The possible role of selenium status in adverse pregnancy outcomes. *Br J Nutr* **105**, 1418–28.
21. Makhoul IR, Sammour RN, Diamond E, *et al*. (2004) Selenium concentrations in maternal and umbilical cord blood at 24–42 weeks of gestation: basis for optimization of selenium supplementation to premature infants. *Clin Nutr* **23**, 373–81.
22. Katzer D, Mueller A, Welzing L, *et al*. (2015) Antioxidative status and oxidative stress in the fetal circulation at birth: the effects of time of delivery and presence of labor. *Early Hum Dev* **91**,119–24.
23. Nogales F, Ojeda ML, Fenutría M, *et al*. (2013) Role of selenium and glutathione peroxidase on development, growth, and oxidative balance in rat offspring. *Reproduction* **146**, 659–67.
24. Rayman MP, Bath SC, Westaway J, *et al.* (2015) Selenium status in UK pregnant women and its relationship with hypertensive conditions of pregnancy. *Br J Nutr* **113**, 249–58.
25. Izquierdo Alvarez S, Castañón SG*, et al*. (2007) Updating of normal levels of copper, zinc and selenium in serum of pregnant women. *J Trace Elem Med Biol* **21**, 49–52.
26. Combs GF Jr. (2015) Biomarkers of Selenium Status. *Nutrients* **7**, 2209–36.
27. Al-saleh I, Al-rouqi R, Angela C, *et al*. (2015) Interaction between cadmium (Cd), selenium (Se) and oxidative stress biomarkers in healthy mothers and its impact on birth anthropometric measures. *Int J Hyg Environ Health* **218**, 66–90.
28. Chen Z, Myers R, Wei T,*et al*. (2014) Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. *J Expo Sci Env Epidemiol* **24**, 537–44.
29. Yang X, Bao Y, Fu H, *et* al. (2014) Selenium protects neonates against neurotoxicity from prenatal exposure to manganese. *PLoS One* **9**, e86611.
30. Anan Y, Ogra Y, Somekawa L, *et al*. (2009) Effects of chemical species of selenium on maternal transfer during pregnancy and lactation. *Life Sci* **84**, 88–93.
31. Darlow BA, Inder TE, Graham PJ, *et al*. (1995) The relationship of selenium status to respiratory outcome in the very low birth. *Pediatrics* **96**, 314-9.
32. Taylor AE, Keevil B, Huhtaniemi IT. (2015) Mass spectrometry and immunoassay: How to measure steroid hormones today and tomorrow. *Eur J Endocrinol***173**, D1–12.
33. Dewing AST, Rueli RH, Robles MJ, *et al*. (2012) Expression and regulation of mouse selenoprotein P transcript variants differing in non-coding RNA. *RNA Biol* **9**, 1361–9.
34. Akesson B, Bellew T BR. (1994) Purification of selenoprotein P from human plasma. *Biochim Biophys Acta* **1204**, 243–9.

Table 1: Main characteristics of participants.

	Mean	SD
Maternal characteristics		
Maternal age (Years)	29.7	5.7
Maternal weight (kg)	81.48	14.35
Body mass index (kg/m^2)	31.84	8.32
Parity		
- Primiparous	49.4 %	
- Multiparous	51.6 %	
Previous miscarriages		
- Yes	22.2 %	
- No	77.8%	
Use of supplements		
- None	11/83 (13.3%)	
- Potassium iodide (KI)	51/83 (61.4%)	
- Multivitamins	21/83 (25.3%)	
Level of education		
- Low	90%	
- Medium	10%	
- High	0%	
Working woman		
- No	80%	
- Yes	20%	
Consumption of iodized salt		
- No	69.5 %	
- Yes	30.5 %	
Weight gain during pregnancy (kg)	12.64	5.06
Type of delivery:		
- Normal vaginal delivery	75%	
- Vacuum/Forceps	17.5%	
- Cesarean section	7.5%	

Neonatal characteristics		
Gestational age at birth (days)	275.2	8.4
Birth weight (gr)	3392.84	408.91
Birth height (cm)	50.32	1.56
Head circumference (cm)	33.48	1.44
Abdominal circumference (cm)	33.40	1.67

Table 2: Comparisons between concentrations of total Se, selenoproteins, SeAlb and SeMetab in maternal and cord sera measured by 3D/SE-AF-AEC-HPLC.

	Maternal serum	Cord serum	Correlation (*r; p*)		Difference in means (*p)*
Total Se (µg/L)	68.95 ± 15.23	56.14 ± 14.61	0.56	<0.01	<0.01
GPX3 concentration (µg/L)	11.24 ± 3.68	10.46 ± 3.48	0.74	<0.01	<0.01
SELENOP concentration (µg/L)	42.49 ± 9.49	28.06 ± 7.69	0.25	<0.01	<0.01
SeAlb (µg/L)	11.35 ± 3.59	14.14 ± 4.26	0.60	<0.01	<0.01
SeMetab (µg/L)	4.32 ± 2.86	3.98 ± 2.38	0.60	<0.01	n.s

Table 3: Concentration of various Se species in maternal and cord serum in women who took multivitamin/mineral supplement containing Se during pregnancy and those who did not take such supplements.

	No supplements (N=62)	Multivitamins (N=21)	*p*
Maternal Total Se (µg/L)	67.40 ± 14.24	69.72 ± 15.32	0.54
Maternal GPX3 (µg/L)	10.84 ± 3.38	11.57 ± 3.92	0.43
Maternal SELENOP (µg/L)	41.44 ± 9.04	44.86 ± 10.25	0.16
Maternal SeAlb (µg/L)	11.16 ± 3.38	11.09 ± 3.31	0.41
Maternal SeMetab (µg/L)	4.35 ± 2.96	3.21 ± 1.45	0.18
Cord Total Se (µg/L)	55.24 ± 13.32	55.12 ± 11.75	0.97
Cord GPX3 (µg/L)	10.13 ± 3.24	10.84 ± 3.52	0.42
Cord SELENOP (µg/L)	28.12 ± 7.70	27.12 ± 6.36	0.62
Cord SeAlb (µg/L)	13.49 ± 3.46	14.51 ± 3.62	0.28
Cord SeMetab (µg/L)	3.82 ± 2.16	3.67 ± 2.26	0.83

FIGURE LEGEND

Figure 1: Comparison between (a) percentage of selenoproteins and SeMetab in maternal and cord sera (b) concentrations of total Se, selenoproteins and SeMetab in maternal and cord sera.

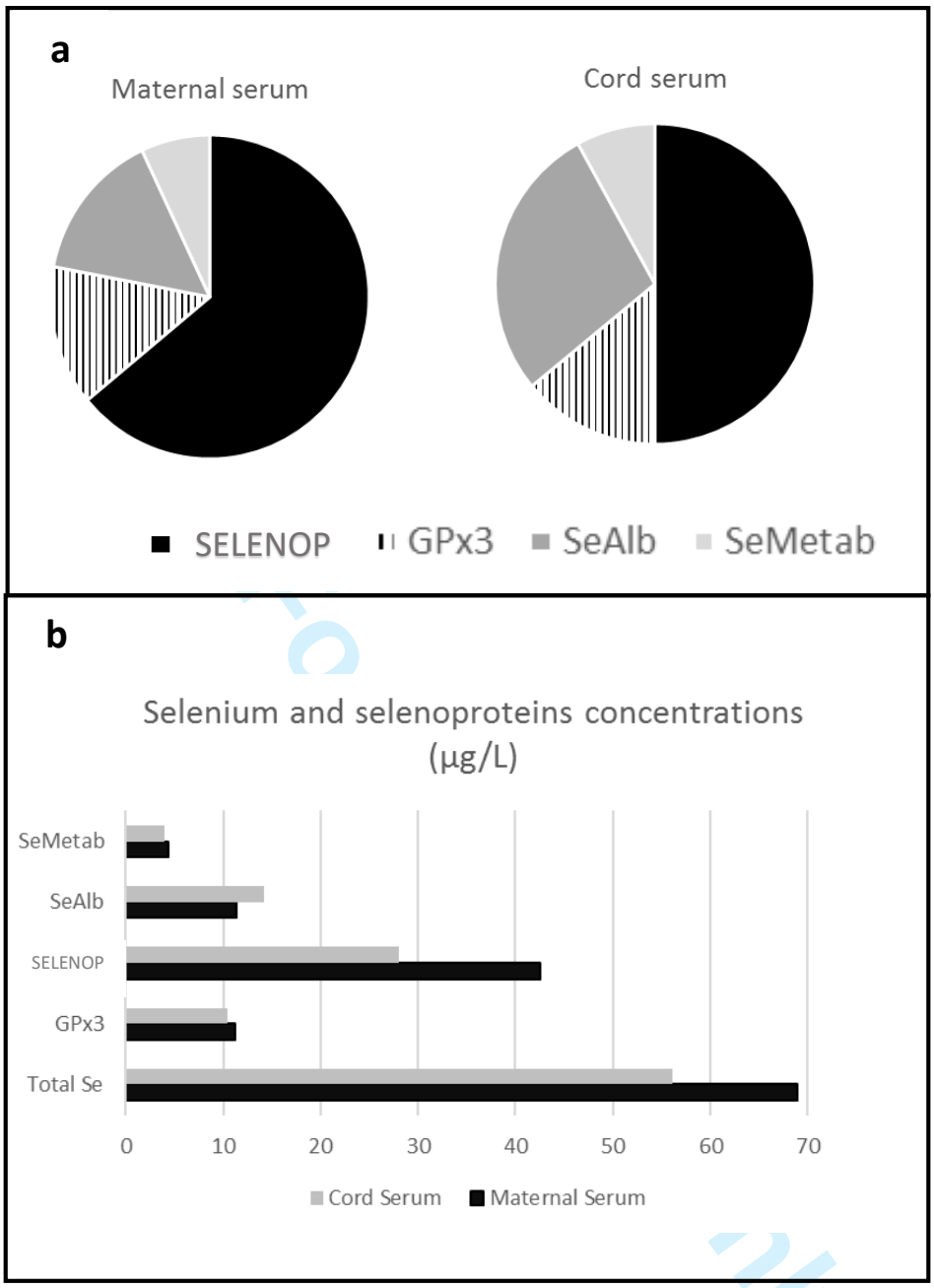

Figure 1: Comparison between (a) percentage of selenoproteins and selenometabolites in maternal and cord sera (b) concentrations of total selenium, selenoproteins and selenometabolites in maternal and cord sera.