Role of selenium and glutathione peroxidase on development, growth, and oxidative balance in rat offspring

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Abstract

Selenium (Se), an essential trace metal, is important in both growth and reproduction and is the constituent of different selenoproteins. The glutathione peroxidase (GPx) family is the most studied as it prevents oxidative stress. Liver oxidation is considered as another mechanism involved in low birth weight. Therefore, in order to ascertain whether GPx is related to the effects of Se on growth during gestation and lactation, three groups of rat pups were used: control, Se deficient (SD), and Se supplemented (SS). Morphological parameters and reproductive indices were evaluated. Hepatic Se levels were measured by graphite furnace atomic absorption while spectrophotometry was used for activity of antioxidant enzymes and oxidative stress markers in liver and western blotting for expression of hepatic GPx1 and GPx4. The SD diet increased mortality at birth; decreased viability and survival indices; and stunted growth, length, and liver development in offspring, thus decreasing hepatic Se levels, GPx, glutathione reductase, and catalase activities, while increasing superoxide dismutase activity and protein oxidation. The SS diet counteracted all the above results. GPx1 expression was heavily regulated by Se dietary intake; however, although Se dietary deficiency reduced GPx4 expression, this decrease was not as pronounced. Therefore, it can be concluded that Se dietary intake is intimately related to growth, length, and directly regulating GPx activity primarily via GPx1 and secondly to GPx4, thus affecting liver oxidation and development. These results suggest that if risk of uterine growth retardation is suspected, or if a neonate with low birth weight presents with signs of liver oxidation, it may be beneficial to know about Se status.

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Introduction

Selenium (Se) is an essential trace metal with beneficial properties and is principally known for its antioxidant capacity, which protects cell from free radicals (Parízek 1990). The biological effectiveness of Se is based upon the integration of selenocysteine (as the 21st amino acid) into the active center of 25 selenoproteins with biological properties. Among these selenoproteins, the glutathione peroxidase (GPx) family is the most studied and is involved in regulating oxidative processes and cell membrane protection (Papp *et al.* 2007), as these selenoenzymes catalyze the reduction of hydroperoxides and lipid peroxides to their corresponding alcohols and water with reduced glutathione (GSH) as the electron donor (Brigelius-Flohé & Maiorino 2013).

Se has multiple functions in the organism; therefore, Se deficiency can lead to a number of physiological disorders in humans and animals. Increasing evidence suggests that this mineral also plays an important role in normal growth and reproduction (Mistry *et al.* 2012). In this context, Se has been related to somatic growth regulation, influencing 3,5,3'-triiodothyronine (T₃) and

the growth hormone (GH)–insulin-like growth factor (IGF) axis (Moreno-Reyes *et al.* 2001). He *et al.* (2006) have reported that a Se deficiency depresses growth by decreasing the activity of the selenoprotein iodothyronine 5'-deiodinase in liver, causing a decrease in the plasma concentration of T₃ and protein turnover. It is suggested that Se is particularly important for IGF bioactivity (Karl *et al.* 2009). However, whether Se intake is important for IGF biological activity has not been studied extensively, despite the relationship between T₃ and the hypothalamic–GH–IGF axis (Miell *et al.* 1993). An association between low Se intake and low IGF1 serum values in adults and children has been reported (Aydin *et al.* 2002, Maggio *et al.* 2010), yet Se supplementation in gestating ruminants does not affect IGF1 concentration in their offspring (Ward *et al.* 2008, Gunter *et al.* 2013).

Several studies have suggested oxidative stress as another agent responsible for impaired reproductive function and intrauterine growth retardation (Biri *et al.* 2007, Hracsko *et al.* 2008, Pathak *et al.* 2011, Mistry *et al.* 2012) by the oxidation of protein, lipids, and DNA, preventing a normal metabolism of these elements,

which in turn leads to a disrupted tissue metabolism. This oxidation is especially harmful during gestation and lactation when the cell membrane proteins are developing. Se is essential for GPx antioxidant activity, preventing the oxidation of the membranes' lipids and proteins (Hefnawy & Tortora-Pérez 2010). Therefore, dams' dietary Se intake during gestation and lactation could alter GPx activity and oxidative balance (Turlo *et al.* 2010) in offspring, which in turn could be related to changes in offspring growth and length at birth, as well as at the end of lactation.

Taking into account that the liver is the main metabolic organ for processing, distributing, and supplying nutrients, as well as releasing IGF to blood, any damage, including oxidation, to this tissue will have an impact on the organism's development. In fact, *in vitro* studies on hepatocytes have found a relationship between GH and IGF1 on GPx activity and antioxidant regulation (Brown-Borg *et al.* 2002). The main objective of this research, therefore, was to study the role of dams' dietary Se intake in the development and growth of offspring during gestation and lactation, analyzing its effect in liver GPx activity and expression and therefore in the oxidative balance as another factor concomitant to correct body development.

Materials and methods

Animals and samples

Male and female Wistar rats (Centre of Production and Animal experimentation, Vice-rector's Office for Scientific Research, University of Seville) weighing ~150–200 g were randomized into three groups: control (C), Se deficient (SD), and Se supplemented (SS). Drinking water and diet (deficient, control, or supplemented) were given *ad libitum* during the pre-gestation (7 weeks), gestation (3 weeks), and lactation (3 weeks) periods. In the week following the pre-gestational period, male ($n=3$) and female ($n=6$) rats were mated to obtain the first-generation offspring for each group. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day 0 of pregnancy; at this moment, pregnant rats were housed individually in plastic cages. The day of parturition, which occurs spontaneously 3 weeks after coitus, was designated as day 1 of lactation. The offspring number was reduced to eight per mother at parturition. The experiments were performed on the offspring of all the three groups 21 days *post-partum*. In this study, we have used eight pups per group to measure all the parameters cited below. These eight pups represent all the litters, as a maximum of two rats per litter, and were allocated to each group taking into account the sex (when this was possible).

The animals were kept at an automatically controlled temperature (22–23 °C) and a 12 h light:12 h darkness cycle (0900–2100 h). Animal care was complied with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

The diets of these rats were prepared according to The Council of the Institute of Laboratory Animal Resources (ILAR 1979), which details known nutrient requirements for most of the common laboratory animals. The diet of the C group contained 0.1 p.p.m. of Se while the SD and SS group diets contained 0.01 and 0.5 p.p.m. of Se respectively. Se was supplemented as anhydrous sodium selenite (an inorganic compound; Panreac, Barcelona, Spain).

The amount of energy consumed (kcal) by dams was estimated by measuring the amount of food consumed by day and multiplying by 3.96 kcal. Se intake was calculated by multiplying the food consumed by p.p.m. of Se in the diets.

Weekly body weight of dams and their pups was controlled until the end of the experimental period. Additionally, cranial–caudal length of pups was measured using a metric caliper. All measures were taken at 0900 h to avoid changes due to circadian rhythms.

At the end of the experimental period, dams and their pups were fasted for 12 h, and feces and urine samples were collected using individual metabolic cages. After that, rats were anesthetized with i.p. 28% w/v urethane (0.5 ml/100 g of body weight). Blood samples were obtained by heart puncture and collected in tubes. The serum was prepared using low-speed centrifugation for 15 min at 1300 g. The abdomen was opened by a midline incision and whole livers were removed, debried of adipose and connective tissues in ice-cold saline, weighed, and stored at –80 °C prior to biochemical determinations.

The amount of milk consumed by the offspring at the end of the lactation period (days 19 and 20) was estimated by subtracting the weight of the pups obtained immediately prior to returning them to the dam from their weight after 30 min of suckling (Subramanian 1995). In order to obtain the maximum amount of milk at day 21 of lactation, 3 h after removing the litters from their mothers, the dams were anesthetized with urethane and milk samples were immediately collected. The milk was obtained by gently massaging the area around each of the 12 mammary glands and then pressing upward from the base of the gland toward the nipple. The amount of milk collected was around 1–1.5 ml/dam.

Gestational and lactation indexes were calculated as follows: female fertility index was calculated as number of pregnancies/number of mating $\times 100$; gestational index as number of successful births/number of pregnancies rats $\times 100$; and live-born index as number of pups born alive/number of pups born $\times 100$. Viability index was calculated as number of pups alive on day 7/number of pups alive and kept on day 4 $\times 100$; weaning index (WI) as number of pups alive at day 21/number of pups alive and kept on day 4 $\times 100$; and lactation survival index (LSI) as (number of total offspring–number of dead offspring/number of total offspring) $\times 100$.

Se analysis

Se levels were determined by graphite furnace atomic absorption spectrometry, using a PerkinElmer AAnalyst 800 high-performance atomic absorption spectrometer with Win-Lab32 for AA Software, equipped with a Transversely Heated Graphite Furnace (THGA) with longitudinal Zeeman effect background corrector and an AS-furnace autosampler

(PerkinElmer, Überlingen, Germany). The source of radiation was a Se electrodeless discharge lamp. The instrumental operating conditions and the reagents are the same as those we used in the previous paper by [Ojeda et al. \(2009\)](#). Samples: serum samples were diluted fivefold in 0.2% v/v HNO₃ and 0.2% Triton X-100 solutions and urine samples were diluted 1:2 v/v. After 72 h at 100 °C dry temperature, feces and milk samples were weighed and digested in a sand bath heater (OVAN, Badalona, Spain) with nitric acid for 72 h, and perchloric acid and hydrochloric acid (6 M) were added. The accuracy of the method has been confirmed using CertiPUR Selenium AA Single Element Standard of National Institute of Standards and Technology and Technology Standard Reference Material (NIST-SRM). The linear concentration range of the calibration curve was 5–100 µg/l, with a correlation coefficient of 0.996. The analytical coefficient of variation (CV) was calculated from the values obtained by measuring the same control at 100 µg/l with each batch of specimens analyzed. The CV of the measurements was of 0.021, the limit of detection was 0.2 ng/l, and the repeatability was of 7.1%.

Biochemical analysis: antioxidant enzymes and oxidative stress markers

In order to measure the activity of antioxidant enzymes as well as the oxidation of lipids and proteins, liver tissue samples were homogenized (100 g for 1 min, 1:10 w/v; Pobel 245432, Madrid, Spain) in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA, and 1 mM dithiothreitol) in an ice bath. The homogenate was centrifuged at 900 g for 10 min at 4 °C. The resulting supernatant was employed for the biochemical assay. GPx activity was determined by the method of [Lawrence & Burk \(1976\)](#), in which GPx catalyzes the oxidation of GSH by hydrogen peroxide. The catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) activities were determined using the methods of [Beers & Sizer \(1952\)](#), [Worthington & Rosemeyer \(1974\)](#), and [Fridovich \(1985\)](#) respectively. Lipid peroxidation was evaluated by the method based on the reaction between malondialdehyde (MDA) and thiobarbituric acid ([Draper & Hadley 1990](#)). Hepatic protein oxidation was measured according to a method based on the spectrophotometric detection of the reaction of 2,4-dinitrophenylhydrazine with protein carbonyl (PC) to form protein hydrazones ([Reznick & Packer 1994](#)). The protein content of the samples was determined by the method of [Lowry et al. \(1951\)](#), using BSA as the standard.

Selenoprotein immunoblotting assays

The expression of the hepatic selenoproteins GPx1 and GPx4 in pups was conducted using the Laemmli method (1970). The samples utilized contained 75 µg protein. Proteins were separated on polyacrylamide gel and transferred onto a nitrocellulose membrane (Immobilon-P Transfer Membrane, Millipore, Billerica, MA, USA) using a blot system (Transblot, Bio-Rad). Membrane nonspecific sites were blocked during 1 h with blocking buffer: TTBS (50 mM Tris-HCl, 150 mM NaCl, and 0.1% (v/v) Tween 20, pH 7.5) and milk powder 3% (Bio-Rad), and thereafter, they were probed overnight at 4 °C with

specific primary antibodies (rabbit polyclonal IgG, Santa Cruz Biotechnology), dilutions: GPx1 (1:20 000) and GPx4 (1:5000). Subsequently, secondary antibody (anti-rabbit IgG HRP conjugate, Santa Cruz Biotechnology) was utilized in dilutions of 1:5000. Monoclonal mouse anti-β-actin (IgG1 A5441, Sigma-Aldrich) was used to detect β-actin, as a loading control, with a dilution of 1:40 000, and a secondary antibody anti-mouse IgG peroxidase conjugate (A9044, Sigma-Aldrich) was used in a dilution of 1:8000. The membrane was incubated for 1 min with the commercial developer solution Luminol ECL reagent (GE Health Care and Lumigen, Inc., Buckinghamshire, UK). The quantification of the blots was performed by densitometry with PCBAS 2.08e Software analysis (Raytest, Inc., Straubenhardt, Germany). The results were expressed as percent arbitrary relative units referred to values in control pups that were defined as 100%.

Statistical analysis

The results are expressed as means ± S.E.M. The data were analyzed using a statistical program (GraphPad InStat 3, San Diego, CA, USA) by ANOVA (one-way ANOVA). The statistical significance was established at $P < 0.05$. When ANOVA resulted in differences, multiple comparisons between means were studied by the Tukey–Kramer test.

Results and discussion

Gestational parameters

In our Se deficiency model, no alteration in reproductive performance was found, except for a slight reduction in the live-birth index ([Table 1](#)). [Ramirez et al. \(2001\)](#) hypothesized that dietary Se deficiency in dairy goat kids could be considered a risk of perinatal mortality of kids (0–7 days), which had an extremely low weight. Our results showed that Se deficiency in dams did not compromise reproductive success as all the rat crosses were fertile and all the pregnancies carried to term. Furthermore, all the dams gave birth within the usual expected 21-day gestation period and there were no significant changes in the number of pups per litter. However, although the calorie intake of the three groups was similar during gestation, the body weights and lengths of their offspring were significantly different ([Table 1](#)). Therefore, the effects observed could only be attributed to the dams' Se intake. In this context, dietary Se deficiency causes intrauterine growth retardation, as pups showed a lower birth body weight and cranial–caudal length. By contrast, a SS diet significantly increased birth body weight and cranial–caudal length compared with C pups. These results are in agreement with the available bibliography cited that points to Se deficiency as a key factor on somatic growth retardation, via T₃ and IGF ([Moreno-Reyes et al. 2001](#)). However in our model, Se supplementation also activated somatic growth. In this context, [Rayman \(2012\)](#) has also reported that a higher Se status has antiviral effects and is essential

Table 1 Gestational parameters in dams and offspring.

	C	SS	SD
Dam energy intake (kcal/day)	75.8±5.8	79.6±5.6	86.32±7.3
Dam Se intake (µg/day)	1.89±0.09	9.95±0.10*	0.24±0.02 ^{†,§}
FFI (%)	100	100	100
GI (%)	100	100	100
LBI (%)	100	100	95.91
No. of offspring per litter	11.0±0.5	13.0±0.6	12.25±1.1
No. of offspring survival	11.0±0.5	13.0±0.6	11.25±0.6
Offspring weight at birth (g)	5.7±0.10	7.0±0.31*	4.7±0.08 ^{†,§}
Offspring cranial–caudal length at birth (cm)	4.91±0.08	5.74±0.12*	4.58±0.05 ^{†,§}

The results are expressed as mean ± s.e.m. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Signification: C vs SS, * $P < 0.001$; C vs SD, [†] $P < 0.001$, [‡] $P < 0.01$; SS vs SD, [§] $P < 0.001$. Female fertility index (FFI) was calculated as number of pregnancies/number of mating×100; gestational index (GI) as number of successful births/number of pregnancies rats×100; and live-born index (LBI) as number of pups born alive/number of pups born×100.

for successful male and female reproduction, as well as reducing the risk of autoimmune thyroid disease. According to the data obtained, *Mistry et al.* (2012) found that low plasma Se concentration could contribute to growth retardation, possibly by lowering the placental antioxidant defense because, as it is well known, Se is a cofactor of the antioxidant enzyme GPx. In preliminary unpublished studies of our research group, we have found that an increase in Se in dams' diet could improve GPx placenta activity and oxidative balance.

Lactation parameters

During the lactation period, dams showed evident differences in energy intake. Therefore, both SD and SS rats showed a significantly higher energy intake than C ones (Table 2). Se intake was eight times higher in SS dams vs C ones and six times lower in SD with respect to C. However, when milk Se levels were analyzed, there were no differences between SS and C dams while SD presented the lowest Se content. This decrease, however, was not proportional to Se intake, as the decrease in Se levels was less pronounced. It could be suggested that despite the fact that the dams consumed different

amounts of Se, they tried to reduce these differences by sacrificing their own demands in order to provide their offspring with a correct amount of Se via milk. These results are in agreement with those reported by *Abd El-Ghany et al.* (2008) in ruminants.

When serum Se levels were analyzed in pups (Table 2), the SS group had the highest value, while SD and C pups had similar amounts of Se in serum. This should be because, Se stored in tissues could be utilized to maintain plasma GPx activity during periods of low Se intake (*Payne & Southern 2005*).

At the end of lactation, SD offspring showed the lowest weight and cranial–caudal length and SS pups the highest weight (Table 2). This lower development in SD pups was a consequence of both the restriction in fetal growth observed at birth and the Se deficiency presented during lactation because despite consuming the same amount of milk as the rest of the groups, this milk's Se content was significantly lower. These results demonstrated that dams' low dietary Se intake affects offspring development and growth during pre- and postnatal life and, that, according to *Hefnawy & Tortora-Pérez (2010)*, abnormally low Se values can cause serious growth disorders. Our results also demonstrated that a SD diet

Table 2 Lactation parameters in dams and offspring.

	C	SS	SD
Dam energy intake (kcal/day)	124.9±12.3	191.2±15.4*	211.7±16.8 [§]
Dam Se intake (µg/day)	3.1±0.31	23.9±2.01 [†]	0.53±0.03 ^{§,¶}
VI (%)	100	100	89.79
WI (%)	100	100	100
LSI (%)	100	100	89.79
Offspring weight at 21 days (g)	32.4±1.24	37.5±1.85 [‡]	23.2±0.55 ^{§,¶}
Offspring cranial–caudal length at 21 days (cm)	10.37±0.21	10.76±0.42	8.95±0.29 ^{§,¶}
Milk intake in 30 suckling (µl/g)	16.97±0.8	16.88±1.2	16.59±1.0
Se in milk (µg/g)	0.124±0.005	0.126±0.003	0.102±0.003 ^{,a}
Se in serum (µg/l)	114.06±3.1	109.75±3.9	217.19±3.74 ^{§,¶}

The results are expressed as mean ± s.e.m. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Signification: C vs SS: * $P < 0.001$, [†] $P < 0.01$, [‡] $P < 0.05$; C vs SD: [§] $P < 0.001$, ^{||} $P < 0.01$; SD vs SS: [¶] $P < 0.001$, ^a $P < 0.01$. Viability index (VI) was calculated as number of pups alive on day 7/number of pups alive and kept on day 4×100; WI as number of pups alive at day 21/number of pup alive and kept on day 4×100; and LSI as (number of total offspring–number of dead offspring/number of total offspring)×100.

significantly decreased the viability and the LSI while it did not affect the WI (Table 2), causing serious damage in some offspring that died during the first week of life. Therefore, at this stage of life, Se deficiency is not only linked to growth retardation but also to mortality. In the same context, and corroborating the effects of a low Se supply on growth during gestation and lactation, a correct Se supplementation in dams promotes development in fetuses and pups, improving the lactation parameters measured. The experimental design used in this work demonstrates that a low dams' dietary Se intake during gestation and lactation is directly related to offspring development. Knowledge of the Se status in women with a risk of uterine growth retardation or in neonates with low birth weight may be beneficial for them. However, during lactation, the parameters for pup weight and cranial–caudal length in the SS group tend to approach to the control values. Therefore, the Se supplementation used in this experiment seems to mainly affect the development of pups during gestation.

Finally, when liver development was analyzed (Table 3), at the end of the lactation period, SS and C pups had similar liver weights in relation to their body weight and protein content. However, SD pups had a lower liver weight and lower protein content, evidence of an underdeveloped liver.

Antioxidative defense and oxidative stress markers in liver

As was expected, Se liver deposits at the end of lactation (Fig. 1) were significantly lower in SD pups and higher in SS ones with respect to C pups. However, the repletion found in SS pups was lower than that expected. These results support the work of Sunde & Raines (2011) who found a sigmoidal response with a plateau in liver Se deposits in weaning rats fed with different amounts of Se (0.08–0.24 µg Se/g diet). This Se content in liver is related to its developmental state. In accordance with these data, GPx activity and Se levels in liver decreased analogously in SD pups and increased at a similar rate in SS pups. This direct relationship between Se and GPx

Figure 1 Hepatic Se levels (µg/g dry weight) in offspring. The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Significance: C vs SS, ^c $P < 0.05$; C vs SD, ^{*} $P < 0.001$; SS vs SD, [#] $P < 0.001$.

activity in liver was also found by other authors in different tissues (Agay *et al.* 2005, Ojeda *et al.* 2012).

In order to investigate whether the pups exposed to different Se intake show alterations in liver antioxidant enzyme activities, a reduction in GR and CAT activity was observed in SD offspring while SOD was greatly enhanced when compared with C pups (Fig. 2). Simultaneously, MDA and PC levels were determined as markers of oxidative stress in liver (Fig. 3). No changes in MDA concentrations were observed in the three groups studied, yet PC levels that resulted in higher protein liver oxidation were significantly higher in the SD group. This induced oxidation indicates that normal protein metabolism was disrupted, resulting in an accumulation of damaged molecules. The protein oxidation is probably due to the remarkable increase in SOD activity. Despite the fact that this is the most important enzyme attenuating free radical-induced oxidative damage, it is acting jointly with inhibited GPx and CAT enzymes that do not fight against the SOD-generated H_2O_2 (Agay *et al.* 2005). This SOD over-activation leads to an accumulation of H_2O_2 , which in the absence of GPx and CAT activities increases Fenton reactions, producing protein oxidation in liver. Therefore, there is a clear relationship between Se deficiency, GPx activity, and protein oxidation in liver, which is also parallel to low liver development. This increase in SOD activity in conditions of Se deficiency has also been detected by other authors in mouse liver (Stýblo *et al.* 2007), as well as in other tissues (Sirota 2010, Erkekoglu *et al.* 2012). This increase in SOD activity could be to compensate the formation of more superoxide generated by mitochondrial dysfunction, caused in part by the increase in reactive oxygen species (ROS; Shi *et al.* 2012), by the decrease in Se levels that are related to a correct mitochondrial function and its biogenesis (Mehta

Table 3 Hepatic morphological parameters in offspring.

	C	SS	SD
Liver weight (g)	1.20 $\pm$ 0.03	1.48 $\pm$ 0.04*	0.79 $\pm$ 0.01 ^{†,‡}
Relative liver weight (g/g body weight (%))	3.7 $\pm$ 0.1	3.94 $\pm$ 0.09	3.40 $\pm$ 0.05 ^{‡,‡}
Protein (mg/g liver wet tissue)	103.18 $\pm$ 3.48	108.61 $\pm$ 5.15	92.52 $\pm$ 3.68 ^{§,‡}

The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, Control group; SS, Se supplemented group; SD, Se deficient group. Signification: C vs SS: ^{*} $P < 0.001$; C vs SD: [†] $P < 0.001$, [‡] $P < 0.01$, [§] $P < 0.05$; SD vs SS: [‡] $P < 0.001$, [‡] $P < 0.05$.

Figure 2 Activity of hepatic antioxidant enzymes in offspring: GPx (A), GR (B), CAT (C), and SOD (D). The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Significance: C vs SS: ^c $P<0.001$, ^c $P<0.05$; C vs SD: ^{*} $P<0.001$; SS vs SD, [#] $P<0.001$.

et al. 2012, Wang *et al.* 2013), or by the decrease in GPx4 expression found in liver, which has a major role in protecting mitochondria from oxidative damage (Cole-Ezea *et al.* 2012).

Dietary Se supplementation in dams counteracts the effects of a Se deficiency, significantly increasing the activity of pups' GPx, GR, and CAT while decreasing SOD activity when compared with SD pups. Comparing these activities with those in controls, increases in the activities of GPx and CAT were proportional to increase in SOD observed. Therefore, the H_2O_2 formed by SOD was successfully removed by the latter two enzymes while no liver oxidation was found. Ahmad *et al.* (2012) also described an increase in CAT and SOD activities after Se supplementation in adult chickens, explaining this effect as being an overexpression of the selenoprotein iodothyronine deiodinase leading to high levels of T_3 , which are closely related to ROS production. This upregulation of the antioxidant activity generated by Se supplementation is an efficient manner of allowing hyperactivity of the thyroid axis without oxidative damage, enabling correct development. This stimulation of the antioxidant activity could also be related to the effects of GH and IGF over the hepatocytes *in vitro* (Aksu *et al.* 2013) and *in vivo* (Brown-Borg *et al.* 2002), appearing to increase oxidation and decrease GPx activity when they act as somatic growth inducers. Therefore, it might point out that Se and GPx, among other selenoproteins, play a role in correct liver development, preventing concomitant oxidation.

Finally, as GSH is the main intracellular endogenous antioxidant molecule (Avanzo *et al.* 2001), the ratio of

reduced glutathione oxidized glutathione (GSH:GSSG) was measured as an indicator of oxidative stress (Fig. 4). A significant decrease in GSH:GSSG ratio was evident in the SD group when compared with C pups. However, Se supplementation increases this value – it is even higher than in C pups. This decreased ratio in SD pups is mainly due to an increase in GSSG (0.023 ± 0.004 $\mu\text{mol/g}$ tissue) when compared with C (0.011 ± 0.0015 $\mu\text{mol/g}$ tissue) and supplemented (0.007 ± 0.0006 $\mu\text{mol/g}$ tissue) groups. This might be due to the overproduction of H_2O_2 provoked by SOD activity, which promotes the Fenton reaction and enhances oxidation.

By contrast, Se supplementation increased the GSH:GSSG ratio with respect to C pups. These results are in agreement with several studies that have indicated that Se supplementation can enhance GSH concentrations and decrease concentrations of its oxidized products (GSSG) in tissues and blood (Hoffman 2002, Richie *et al.* 2012). Therefore, Se supplementation to dams during gestation and lactation prevents oxidative stress in their pups' liver as it increases antioxidant enzyme activities and improves the GSH:GSSG ratio. In conclusion, Se supplementation could be proposed as an antioxidant therapy during pregnancy and lactation

Figure 3 Hepatic oxidation in offspring: levels of MDA (mol/mg protein) in lipid (A) and PC (nmol/mg protein); (B). The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Significance: C vs SD, ^{*} $P<0.05$; SS vs SD, [#] $P<0.001$.

Figure 4 GSH:GSSG ratio in offspring. The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Significance: C vs SS, ^c $P < 0.05$; C vs SD, ^{*} $P < 0.001$; SS vs SD, [#] $P < 0.001$.

promoting growth and development of the offspring and protecting them from oxidative stress.

Selenoprotein expression

Selenoprotein synthesis is highly dependent on Se, and there is a hierarchy of selenoprotein expression in mammals when Se is limited (Driscoll & Copeland 2003); thus, a competition for translation exists between selenoproteins (Schomburg & Schweizer 2009). However, little is known about the expression of the selenoprotein GPx in the offspring of rats that receive a different Se content in their diets during gestation and lactation. The main GPx isoforms delivered in liver are GPx1 and GPx4 (Hoffmann *et al.* 2007; Figs 5 and 6). In this study, GPx1 expression in offspring also responded to Se concentration in diet and in liver, coinciding with liver GPx activity (Figs 5A and 6). Thus, SD pups expressed a very low amount of GPx1 vs C ones, and SS expressed a higher amount than C ones. It is deduced that dietary Se provokes down/upregulations in Se liver deposits, GPx activity, and GPx1 expression. These results agree partially with the results of other authors in adult rats after Se deprivation (Sunde & Raines 2011, Liu *et al.* 2012). Taking all these data into account, it could be suggested that the antioxidant activity determined in the liver of pups depends primarily on GPx1 expression.

The isoform GPx4 presents specific and essential functions for life, as it is the only GPx enzyme that is able to reduce hydroperoxides within membranes, avoiding membrane oxidation (Brigelius-Flohé & Maiorino 2013). In this study, GPx4 expression also decreased in SD

pups; however, this decrease was lower than that found in GPx1 expression (Figs 5B and 6). By contrast, SS pups expressed more GPx4 than C ones, but this increase was proportionally much higher than that observed in GPx1 expression. Analyzing these results, we conclude that, in nursing pups, GPx4 is preferentially sustained in comparison to GPx1, as is the case in adult rats (Sunde & Raines 2011). Thus, as in adult animals, selenoproteins featured a different degree of Se dependence for their biosynthesis in pups, probably related to the physiological importance of each protein (Schomburg & Schweizer 2009). As GPx4 acts protecting against oxidation of the membrane phospholipids, it could play an important role during lactation (when there is an intense phase of cell formation), protecting the newly synthesized phospholipids. Taking into account that in this period of life the amount of phospholipid vs triglycerides is higher, the lipid profile is different to that found in adulthood (Ojeda *et al.* 2008); the increase in GPx4 could be related to the absence of lipid oxidation in the groups studied. Based on this information, it can be concluded that GPx1 is very sensitive, and GPx4 is highly resistant, to dietary Se deficiency in offspring, and according to this, as GPx1 expression is fully committed, in SD pups protein oxidation occurs; however, as GPx4 expression is not completely decreased, phospholipid

Figure 5 Hepatic selenoproteins GPx1 (A) and GPx4 (B) expression in offspring. The results are expressed as mean $\pm$ S.E.M. and analyzed by a multifactorial ANOVA (one-way ANOVA) followed by the Tukey's test. The number of animals in each group is 8. Groups: C, control group; SS, Se supplemented group; SD, Se deficient group. Significance: C vs SS: ^c $P < 0.001$, ^c $P < 0.05$; C vs SD: ^{*} $P < 0.001$; SS vs SD: [#] $P < 0.001$.

Figure 6 Hepatic selenoproteins GPx1 and GPx4 expression in offspring.

oxidation does not take place. This resistance of GPx4 is probably due to having an important physiological function in these pups, as [Brigelius-Flohé & Maiorino \(2013\)](#) described in their review, a knockout of GPx4 is the only knockout of a GPx that is embryonically lethal; therefore, GPx4 cannot simply be an antioxidant enzyme but must have specific functions that are essential for life and cell proliferation, and in the SD pups of this study is decreased.

Conclusions

In conclusion, maternal Se intake during gestation and lactation alters offspring oxidative response and development. This effect is related to Se liver deposits and, in part, to GPx activity, which primarily depends on GPx1 expression and secondly to GPx4. When Se is supplemented in diet, pup development is improved as antioxidant enzymes are overexpressed, avoiding the oxidative action of somatic growth. When the Se supply is deficient, intrauterine growth retardation appeared together with a higher risk of death during lactation. In this case, Se is depleted in liver, GPx activity decreased, and protein oxidation appeared. These effects are linked to an almost total suppression of GPx1 expression and to a partial of the GPx4. If a risk of uterine growth retardation is suspected, or neonates with low birth weight present signs of liver oxidation, knowledge about their Se status may be of great benefit. In conclusion, Se supplementation could be proposed as an antioxidant therapy during pregnancy and lactation promoting growth and development of the offspring and protecting them from oxidative stress.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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