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Placental oxidative status in rural residents environmentally exposed to organophosphates

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ABSTRACT

The impact of environmental organophosphate pesticide exposure on the placenta oxidative status was assessed. Placental samples were collected from women residing in an agricultural area during pesticide pulverization period, non-pulverization period and from control group. Carboxylesterase activity was significantly decreased in pulverization period group. Enzymatic and non-enzymatic defense system, the oxidative stress biomarkers and the nuclear factor erythroid 2-related factor levels showed no differences among groups. However, in the pulverization period group, an inverse association between catalase activity and placental index, a useful metric for estimating placental inefficiency, was found. This result suggests that catalase may serve as a potential placental biomarker of susceptibility to pesticides. Further studies designed from a gene-environment perspective are needed.

1. Introduction

Organophosphorus (OP) compounds are a class of widely used pesticides which represent the most commonly chemical applied in agricultural pest control programs. The primary acute toxicological effect of OPs is associated with inhibition of acetylcholinesterase (AChE) and pseudocholinesterase enzymes. Cytochrome P450 family enzymes have been shown to carry out activation of phosphorothionate and phosphoroditionate compounds to the oxon products (Chambers et al., 2010; Ojha et al., 2011). The oxon derivatives are the actual powerful inhibitors of type "B" esterases such as AChE and carboxylesterase (CaE) (Barata et al., 2004), whereas the products of the dearylation reaction and the oxon hydrolysis represent the main detoxification metabolites (Buratti et al., 2007). The balance between OP activation and detoxification establishes their risk to humans.

In addition to esterase enzyme inhibition, several studies provide evidence that OP exposure induces oxidative stress affecting different systems and organs such as the immune system (Richter et al., 2009), brain (Mekail and Sharafaddin, 2009), liver (Mink et al., 2012; Mostafalou et al., 2012), hematological system (Van der Oost et al., 2003), and reproductive system (Agarwal et al., 2012).

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Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS: superoxide, H_2O_2 and $^{\bullet}OH$) and the ability of antioxidant enzymes to scavenge ROS (Myatt and Cui, 2004). The participation of ROS in pesticide toxicity may occur at different levels: by enzymatic conversion to secondary reactive products and/or ROS, depletion of antioxidant defenses, impairment of antioxidant enzyme function (Franco et al., 2009), or by alterations of metabolic links, which indirectly increase ROS generation (Mekail and Sharafaddin, 2009; Southorn and Powis, 1988). In fact, agriculture workers exposed to OP presented a depletion in erythrocyte antioxidant enzymes (Myatt and Cui, 2004) and an increase in the products o lipid peroxidation (López et al., 2007; Rastogi et al., 2009).

Normal pregnancy is a state close to the limit at which oxidative stress may become pathological (Roberts and Hubel, 2009). Decreased antioxidant capacity and increased ROS have emerged as likely promoters of several pregnancy-related disorders such as low birth weight, preeclampsia, and preterm birth (Al-Gubory et al., 2010; Roberts and Hubel, 2009) as well as with lower fetal growth in normal pregnancies (Kim et al., 2005). Evidence for a weak correlation between urinary stress oxidative biomarkers of midterm pregnancy and oxidative stress levels of placental delivery with birth size were also reported (Min et al., 2009). Moreover, Luo et al. (2006) hypothesized that "oxidative stress may be the key link between adverse insults and fetal or developmental programming of the metabolic syndrome".

Despite the recognition that the health of the placenta is a prerequisite for the health of fetus (Gupta, 2007) and that this organ may function as an OP temporary depot (Abdel-Rahman et al., 2002; Abu-Qare et al., 2000), few studies have focused on placental OP toxicity. Microscopic examination of placenta samples derived from OP exposed women showed atypical characteristics of tertiary villi (Levario-Carrillo et al., 2001) and alterations in the maturity homogeneity within placental tissue (Acosta-Maldonado et al., 2009). We have previously demonstrated increased AChE and catalase (CAT) activities (Rovedatti et al., 2012; Souza et al., 2005) and changes in mitochondrial and nuclear lipid profiles (Vera et al., 2012) in placenta of women living in agricultural area exposed to OP applications. Recently, we have reported up-regulation of enzymes which are implicated in tissue repair associated to the inhibition of placental CaE (Bulgaroni et al., 2013). Nevertheless, studies exploring the extent to which OP environmental exposure during pregnancy affect the placental oxidative condition are very few.

The present study was conducted to assess the potential impact of environmental pesticide exposure on the placenta oxidative status of women residing in an agricultural area. The enzymatic and nonenzymatic antioxidant defense parameters as well as the oxidative stress indicators were studied. Additionally, the nuclear factor erythroid 2-related factor 2 (Nrf2) levels which plays a crucial role in the cellular redox homeostasis (Niture et al., 2010) and was associated with the pathogenesis of preeclampsia (Chigusa et al., 2012) was explored.

2. Materials and methods

2.1. Chemicals

Reduced glutathione (GSH), 5′-dithiobis (2-nitrobenzoic acid), glutahione reductase, nicotinamide adenine dinucleotide phosphate-reduced tetrasodium salt (NADPH), and bovine serum albumin (BSA) were purchased from Sigma Co. (St. Louis, MO, USA). All the other reagents used were of analytical grade.

2.2. Antibodies

Polyclonal rabbit Nrf2 (C-20) antibody was purchased from Santa Cruz. Mouse monoclonal anti β-actin and horseradish peroxidase-conjugated donkey anti-rabbit antibodies were from Sigma.

2.3. Participant recruitment and sample collection

The study included seventy healthy pregnant women (15–35 years old), enrolled through 2009–2011, who delivered single healthy babies at term by vaginal mode. Forty-six women recruited in the public Hospital in General Roca City, Rio Negro, situated in the northern part of Argentine Patagonia were included in the rural group. They are residents of farms or small rural communities surrounding fruit cultivation areas of the Río Negro River Valley where OPs such as azinphos methyl, phosmet, chlorpyrifos, and dimethoate, are applied 3 months per year. Pesticides are usually finely dispersed as droplets or particles at the time of pulverization and aerial drift from the target area is frequent, increasing the potential environmental exposure of this population. Samples collected from October to December were considered as pulverization period samples (PP), and those collected from April to August were considered as non-pulverization period samples (NP). Both groups were matched for self-reported passive smoking. Another group of pregnant women with no history of pesticide exposure, attended to the Castro Rendón Hospital in Neuquén City ($n = 24$), was considered as control group (CG).

A brief questionnaire to each woman was requested to document about occupation, education, smoking habits, housing, diet, and household use of pesticides. Obese women and those with anemia, overweight, X-ray exposition, on medication, or suffering from any complications including pregnancy complications or with documented infection were excluded from the study. Maternal clinical background data were taken from medical histories. We also restricted the study to mothers who had not smoked, used drugs or drunken alcohol during the pregnancy and the labor lasted less than 10 h. Informed consent was obtained prior to the enrollment in the study. The study protocol was approved by the ethical committee of the local Advisory Committee of Biomedical Research in Humans.

For analytical determinations, sample tissues were obtained from the central area of the placenta maternal side to avoid variation in the antioxidant enzyme activities (Hempstock et al., 2003). Placental samples were snap frozen in liquid nitrogen and transported to the laboratory where they were stored at −80 °C until analysis.

2.4. Enzyme assays

CaE (EC 3.1.1.1) activity: was measured using α-naphthyl acetate, as described previously (Morgan et al., 1994). Briefly, placenta samples were homogenized in sodium phosphate buffer (0.1 M, pH 6.5) containing 0.5% Triton X-100. Then, samples were centrifuged at 20,000 × *g* for 10 min at 4 °C and the supernatant was collected for enzyme activity determination. The hydrolysis of α-naphthyl acetate was monitored at 550 nm.

CAT (EC 1.11.1.6) activity was determined recording the continuous decrease in hydrogen peroxide (H_2O_2) absorbance at 240 nm at 25 °C (Beers and Sizer, 1952) and calculated using the extinction coefficient ($\varepsilon = 40\ mM^{-1}\ cm^{-1}$). Placenta samples were homogenized in sodium phosphate buffer (50 mM, pH 7.0) containing 0.5% Triton X-100 and centrifuged at 2600 × *g* for 5 min at 4 °C. The supernatant was collected for enzyme activity determination. Enzyme activity was expressed as $U\ mg^{-1}$ of protein. One unit is defined as the amount of the enzyme, which decomposes 1 μmol of substrate per min at 25 °C.

Glutathione peroxidase selenium-dependent (GPx) (EC 1.11.1.9) activity was measured recording NADPH oxidation at 340 nm and calculated using the extinction coefficient ($\varepsilon = 58{,}857\ mM^{-1}\ cm^{-1}$) according to Czarniewska et al. (2003). Placenta samples were homogenized in sodium phosphate buffer (50 mM, pH 7.4) and centrifuged at 10,000 × *g* for 20 min at 4 °C. The resulting supernatant was diluted in potassium phosphate buffer (50 mM, pH 7) containing 0.3 mM H_2O_2, 1 mM GSH, $0.5\ IU\ mL^{-1}$of glutathione reductase, 0.21 mM NADPH and 1 mM sodium azide. The optical density values were corrected by unspecific NADPH oxidation. The enzyme activity was expressed as $mU\ mg^{-1}$ of protein.

Linear conditions for all the enzyme activities were previously adjusted.

2.5. GSH content

GSH was determined by the method described by Tietze (1969), based upon the development of the relatively stable yellow color on addition of 5,5′-dithiobis (2-nitrobenzoic acid) to compounds containing sulfhydryl groups. Briefly, placental tissue was homogenized in $HClO_4$ 0.5 M and centrifuged at 10,000 × *g* for 10 min at 4 °C. Acid soluble thiol groups were quantified using the extinction coefficient ($\varepsilon = 13.6\ mM^{-1}\ cm^{-1}$). GSH content was expressed as $nmol\ mg^{-1}$ of protein.

2.6. Protein carbonyl content

For the assessment of protein carbonyl groups plasma proteins were derivatized with 2,4 dinitrophenyl hydrazine (DNPH) and measured as described by Nicholls-Grzemski et al. (2000). Briefly, placental tissue was homogenized in Krebs–Ringer buffer pH 7.2 and centrifuged at 2600 × *g* for 5 min. The resulted supernatant treated with DNPH was then precipitated with trichloroacetic acid (10% final concentration) and extensively washed with ethanol/ethyl acetate After centrifugation at 2600 × *g* for 5 min, the pellet was resuspended in urea/Tris–Cl/EDTA 8 M and this solution was measured spectrophotometrically at 370 nm. Protein carbonyl content was calculated using the extinction coefficient of DNPH ($\varepsilon = 2.1 \times 10^4\ M^{-1}\ cm^{-1}$) and was expressed as $nmol\ mg^{-1}$ of protein.

2.7. Lipid peroxidation content

Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reactive substances as described by Ohkawa et al. (1979). Placental samples were homogenized in Krebs–Ringer buffer pH 7.2 and then precipitated with trichloroacetic acid 20%. Sample supernatants were then allowed to react with thiobarbituric acid at 100 °C. After extraction with butanol the absorbance was recorded at 532 nm and lipid peroxidation was calculated by using a calibration curve performed with malondialdehyde. Results were expressed as $nmol\ mL^{-1}$ of protein.

Total protein content was quantified by the Lowry method (Classics Lowry et al., 1951). All the above determinations were carried out with a UV/vis 1603 Shimadzu Spectrophotometer and conducted in triplicate.

2.8. Western blot analysis

Nine placenta samples (500 mg) chosen at random in each group were homogenized in ice bath using 1 mL RIPA buffer (1% Triton X-100, 0.5% sodium deoxicholate, 9% SDS, 5% DTT, 1 mM sodium ortovanadate, 10 μg phenylmethylsulfonyl fluoride, 30 μg aprotinin). Equal amounts of protein (60 μg) were diluted in SDS sample buffer, boiled at 100 °C for 5 min and loaded onto a 10% SDS-PAGE gel and run at 150 V for 1 h. After migration, proteins were electrotransferred to nitrocellulose (Bio-Rad Laboratories) at 100 V for 1 h. The membrane was blocked in Tris buffered saline (25 mM Tris, 150 mM NaCl, 2 mM KCl, pH 7.4) containing 0.2% Tween 20 and 5% non-fat dry milk, washed and incubated overnight with the following primary antibodies: anti-Nrf2 (1:500), anti β-actin (1:3000) with shaking. After washing the blots were incubated with secondary antibodies anti-rabbit peroxidase conjugated (1:5000) at room temperature for 1 h. Protein antibody complexes were visualized by an enhanced chemiluminescence detection system. Actin protein was used as an internal standard.

2.9. Morphometric parameters

Placenta was weighed immediately after normal vaginal delivery. Information about the status of the newborn at birth (weight, length, head circumference, gender and gestational age) was collected from medical records. Weight, length and head circumference were adjusted according to gestational age and gender using the standardized Z-scores table of the Argentine Society of Pediatrics (Lejarraga and Fustiñana, 1986).

2.10. Statistical analysis

Power calculations to determine the required sample size were based on available data of the analytical variables. Results were expressed as means ± SD. Comparison between groups was performed using one-way ANOVA followed by the post hoc Tukey's multiple comparison tests. Statistical significance

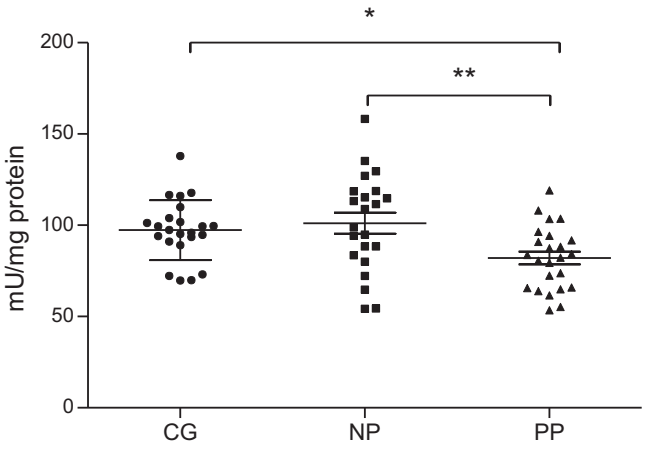

Fig. 1 – Activity of OP exposure biomarker. Carboxylesterase enzyme activity in placental extracts obtained from control (CG, $n = 23$) and rural residents in non-pulverization period (NP, $n = 22$), and pulverization period (PP, $n = 24$) groups. Significant differences between groups were evaluated by one-way analysis of variance followed by a Tukey's multiple comparison test as a post hoc analysis. P values are indicated on the top of the scatter plot. Values represent the mean ± SD. $^{*}p < 0.05$; $^{**}p < 0.001$.

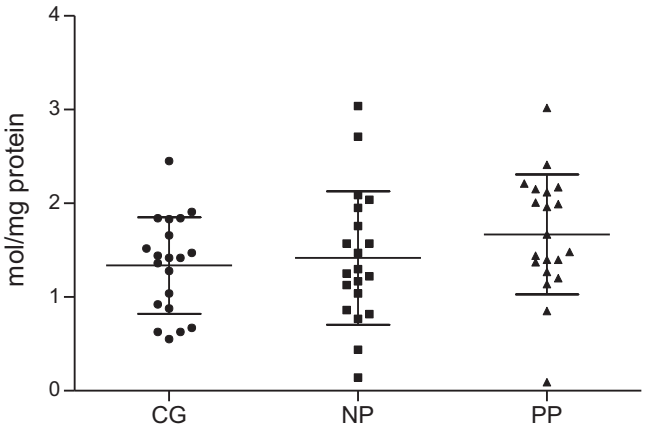

Fig. 2 – Reduced glutathione level in placental extracts obtained from control (CG, $n = 20$) and rural residents in non-pulverization period (NP, $n = 20$) and pulverization period (PP, $n = 20$) groups. Differences between groups were evaluated by one-way analysis of variance followed by a Tukey's multiple comparison tests. Data represent mean ± SD. No significant differences were found.

was assumed as $p < 0.05$. Statistical analysis Categorical variables were compared using the Pearson's chi-squared test (χ^2). The associations between analytical variables and morphometric parameters were estimated by calculating the Pearson's correlation coefficient.

3. Results

The demographic characteristics of the subjects that participated in this study are given in Table 1. The comparison of the demographical characteristics and habits of women resident in the rural area that constituted the NP and PP groups indicates that they were a homogenous group. While, there were some differences between pregnant women from rural and urban populations. In fact, the most notably were regarding to the groundwater consumption, indoor pesticide use, and smoker status. Nevertheless, these differences were not statistically significant.

Considering that CaE inhibition is a recognized secondary oxon target (Casida and Quistad, 2004) and it persists for days to weeks in animal tissues (Chanda et al., 1997) we analyzed the effects of pesticide exposure during pregnancy by the determination of placental CaE activity. Placental samples collected during PP showed a significant decrease in CaE enzyme activity with respect to those collected in NP (19%; $p < 0.001$) or to the CG samples (16%, $p < 0.05$) (Fig. 1). Thus, these data clearly indicate that the placental tissues of PP group were exposed to OP. However, no significant differences in the morphometric parameters of newborns and placenta (gestational age, gender, placenta weight, and height, weight, and circumference head of the newborn) were found among groups (Table 2). Furthermore, the placenta weight to newborn weight (pw/nw) ratio, an indicator that describes the placental impact on fetal growth (Burkhardt et al., 2006), also showed no significant changes among groups. Mean values and variability of newborn birth weight, placenta weight, and pw/nw reported in the present study are in line with reference compilations (Burkhardt et al., 2006; Haeussner et al., 2013).

To determine whether the diminution in the CaE activity was accompanied with changes in the antioxidant/oxidant balance GSH levels (Fig. 2), CAT (Fig. 3A) and GPx (Fig. 3B) enzyme activities as well as stress biomarkers such as the lipoperoxide and protein carbonyl content (Fig. 3) were evaluated. The data showed no significant changes in the antioxidant/oxidant status in placenta samples among groups. Additionally, Nrf2 protein levels assayed by western blot in placenta tissue from all groups were similar (Fig. 4).

Finally, we performed a correlation among the obtained values of GSH, lipoperoxide content, protein carbonyl groups, and CAT, CaE, GPx enzyme activities with placental or newborn morphometric parameters. Only a moderate association of placental index and CAT activity in PP group of the rural population was found ($p = 0.023$; $r = -0.48$) (Fig. 5).

4. Discussion

This is one of the few studies performed so far addressing effects of OP exposure during pregnancy on placental oxidative status in rural residents.

Exposed subjects (PP group) of rural areas enrolled 2009–2011 presented significant lower CaE activity than those of NP or CG in agreement with our previous reports (Bulgaroni et al., 2013; Vera et al., 2012). This esterase is a recognized secondary oxon target (Casida and Quistad, 2004) that binds to OP and is considered a sensitive indicator of environmental OP exposure (Wheelock et al., 2005, 2008). Moreover, the inhibition of CaE can be important in cumulative toxicity with exposure to multiple anticholinesterase pesticides (Cohen, 1984;

Table 1 – Demographic characteristics of study participants.

Demographic characteristics	Control ($n=24$)	Non-pulverization period ($n=22$)	Pulverization period ($n=24$)
Age (years)[a]	23.6 ± 5.6	24.6 ± 5.9	21.9 ± 5.9
Parity[a]	1.2 ± 1.7	1.6 ± 1.7	1.1 ± 1.5
Instruction level (%)[b]			
Illiterate	0	0	5
<High school	60	80	79
High school	40	20	8
Graduate degree	0	0	8
Smoking status (%)[b]			
Active smoker	0	0	0
Passive smoker	4	23	25
Groundwater consumption (%)[b]	0	15.4	8.3
Self-reported indoor pesticide use (%)[b]	0	11.5	16.7

Data are expressed as mean ± SD or as percentage when indicate.
[a] $p > 0.05$ by ANOVA and Turkey post-test.
[b] $p > 0.05$ by chi-square test.

Karanth et al., 2001). Thus, our results clearly indicate during PP, certain amount of OP compounds reached the placenta and their metabolic activation to the oxon products has taken place.

It was reported that in utero exposure to OP may impact on birth length and weight (Perera et al., 2003; Whyatt et al., 2004), on length of gestation (Eskenazi et al., 2004; Souza et al., 2005), and on fetal head circumference in some susceptible subpopulations (Berkowitz et al., 2004), even if a recent review did not identify any strong associations exhibiting consistent exposure-response patterns. Evidences show that the placenta is a recognized target organ for pesticide toxicity (Magnarelli and Guiñazú, 2012). However, little is known about OP-induced oxidative stress in placental tissue as an underlying mechanism and consequences for human fetal development.

In regards to humans, there is a lack of information about OP residues in placenta in nonlethal poisoning. One obvious reason for this is the inability to collect the sample shortly after pesticide application (Rovedatti et al., 2012). Because OPs have relatively short half-lives and are quickly metabolized, and excreted from the body, estimating the internal doses in tissues or fluids is particularly challenging (Wessels et al., 2003). A study of the toxicokinetics of labeled OP administered to pregnant rats showed that the placenta is a poor barrier against these compounds. However, data of relative residence of OP with respect to the maternal plasma (which reflects OP tissue relative exposure), revealed that the placenta may function as a temporary OP depot. In fact, detectable amounts of OP residues were found in placentas collected on the 21st day of gestation from rats intoxicated orally from gestation (Abu-Qare et al., 2000).

Biomarkers may provide insight into the potential mechanisms of contaminant effects (Van der Oost et al., 2003). The decreased placental CaE activity may be associated not only to OP incorporation and bioactivation but also to concomitant ROS production (Barouki, 2010; Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003; Ojha et al., 2011). However, we did not found significant differences in the concentration of both protein carbonylation and malondialdehyde oxidation products among the studied groups.

Placental tissue has a redox system among which GSH plays the first line of defense against oxidatively generated damage. GSH has an important function both as a substrate for GPx and cofactor for the phase II detoxifying enzyme, glutathion-S transferase (Hayes and McLellan, 1999). As shown in Fig. 2, no significant differences in the GSH content were associated to PP group.

Table 2 – Morphometrical parameters of newborns and placenta.

Characteristics	Control ($n=24$)	Non-pulverization period ($n=22$)	Pulverization period ($n=24$)
Newborn weight (kg)[a,c]	3.313 ± 0.49	3.437 ± 0.34	3.209 ± 0.38
Newborn height (cm)[a,c]	48.28 ± 3.22	49.12 ± 1.65	48.91 ± 2.77
Head circumference (cm)[a,c]	35.40 ± 3.46	34.76 ± 1.12	34.38 ± 1.06
Placental weight (g)[c]	515.80 ± 88.49	510.77 ± 98.00	494.92 ± 104.52
Placental index[b,c]	0.16 ± 0.02	0.15 ± 0.03	0.15 ± 0.02
Ponderal index[c]	3.05 ± 1.13	2.91 ± 0.37	2.75 ± 0.31
Gestational age[c]	38.72 ± 1.34	38.27 ± 1.22	39.29 ± 1.16
Male newborns[d]	64	54	29
Female newborns[d]	36	46	71

Data are expressed as mean ± SD.
[a] Data were corrected by gestational age and sex.
[b] Data were expressed as placenta weight (kg)/newborn weight (kg).
[c] $p > 0.05$ by ANOVA and Tukey post-test.
[d] $p > 0.05$ by chi-square test.

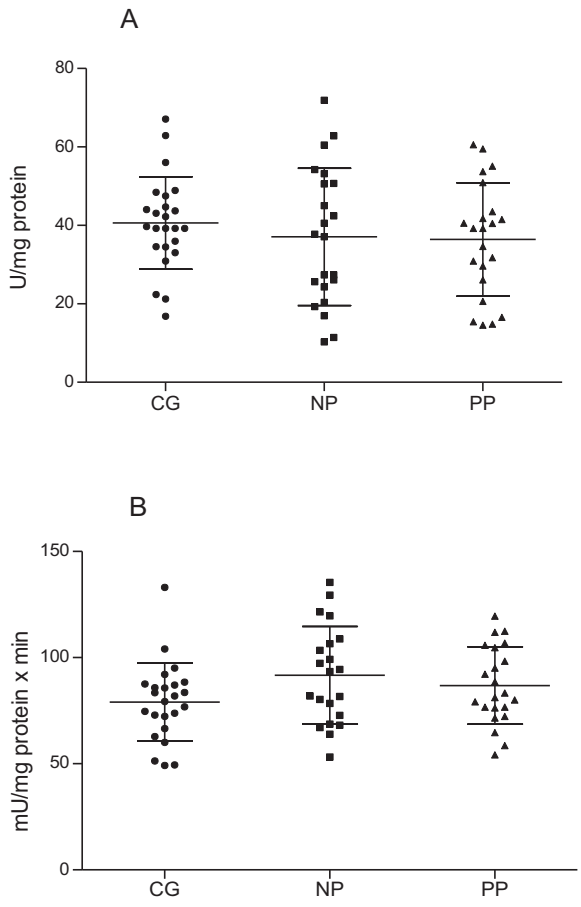

Fig. 3 – Activity of enzymatic antioxidant defense system in placental extracts. (A) Catalase activity was assessed in samples obtained from control (CG, *n* = 24) and from rural residents in non-pulverization period (NP, *n* = 22) and pulverization period (PP, *n* = 22) groups. (B) Glutathione peroxidase selenium-dependent enzyme activity was assessed in samples obtained from control (CG, *n* = 24) and from rural residents in non-pulverization period (NP, *n* = 21) and pulverization period (PP, *n* = 22) groups. Differences between groups were evaluated by one-way analysis of variance followed by a Tukey's multiple comparison tests. Data represent mean ± SD. No significant differences were found.

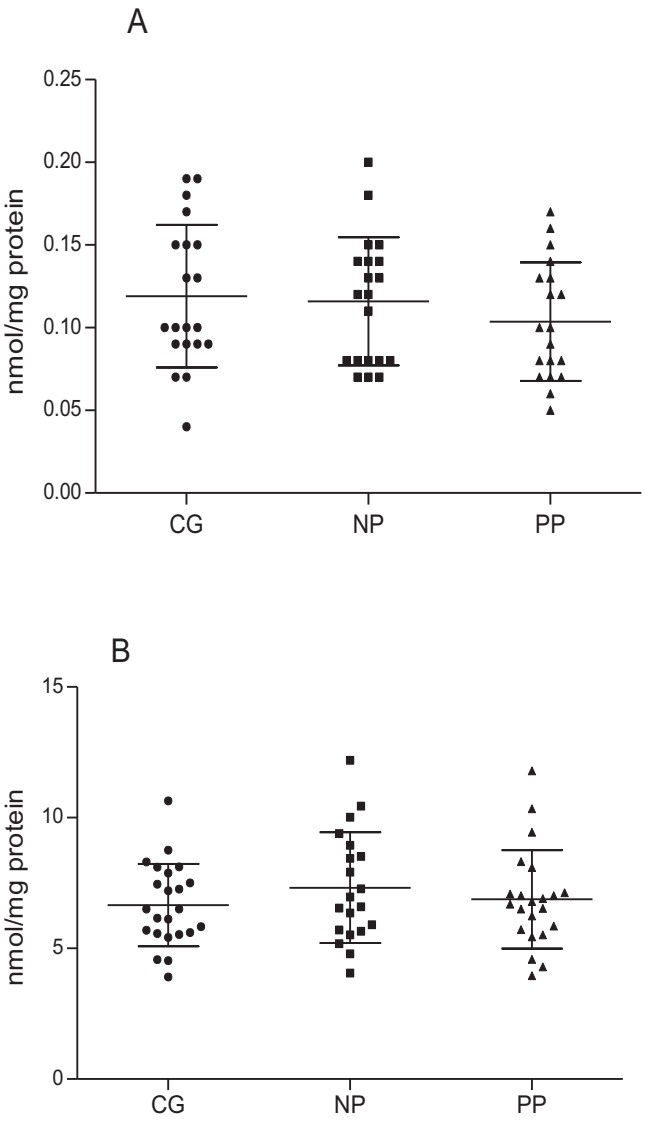

Fig. 4 – Biomarkers of oxidative stress in placental extracts. (A) thiobarbituric acid reactive substance levels were assessed in samples obtained from control (CG, *n* = 20) and from rural residents in non-pulverization period (NP, *n* = 20) and pulverization period (PP *n* = 19) groups. (B) Carbonyl protein content was assessed in samples obtained from control (CG, *n* = 23) and from rural residents in non-pulverization period (NP, *n* = 20) and pulverization period (PP, *n* = 22) groups. Differences between groups were evaluated by one-way analysis of variance followed by a Tukey's multiple comparison tests. Data represent mean ± SD. No significant differences were found.

Even though we have previously reported increased CAT placental activity associated to OP exposure in a rural population of the Río Negro River Valley (Souza et al., 2005), in the present report in which the studied population was enrolled through 2009–2011 we failed to demonstrate changes in CAT and GPx activities in PP group. Our different results may be explained by the drop in the volume and/or the frequency of OP applications in the last five years consistent with changes in the food safety standards (Rovedatti et al., 2012).

It is well documented that Nrf2 over-expression in experimental systems protects against oxidatively generated damage elicited by several agents including pesticides (Chiapella et al., 2013; Li et al., 2011). Nevertheless, in consonance with the lack of up regulation of GPx and CAT, no changes were observed in Nrf2 levels in the exposed placental tissues.

All together, our results suggest that placental tissues were able to handle the prooxidant conditions that might have been generated by the OP exposure. In agree with these data, we have recently demonstrated that trophoblast-derived JEG-3 cells are able to attenuate the oxidative stress induced by CPF through the adaptive activation of the Nrf2/ARE pathway

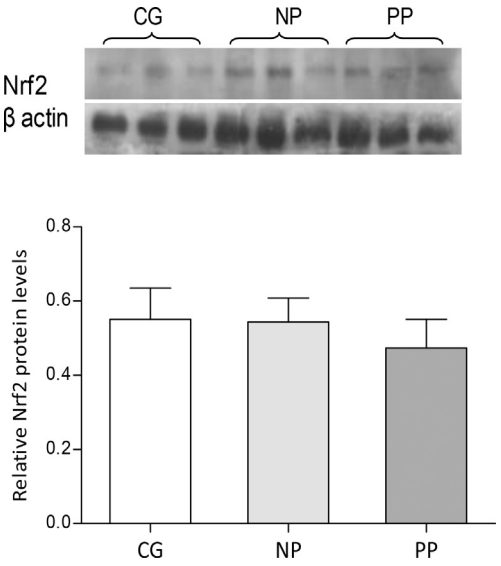

Fig. 5 – Western blot analysis of protein extracts prepared from placenta lysates obtained from control (CG) and from rural residents in non-pulverization period (NP) and pulverization period (PP). Assays were performed using anti-Nrf2 and β-actin antibodies as described in Section 2. The bar graph represents the densitometric quantification (mean ± SD) of Nrf2 protein levels relative to β-actin of nine samples from each group placenta. No significant differences were found between groups.

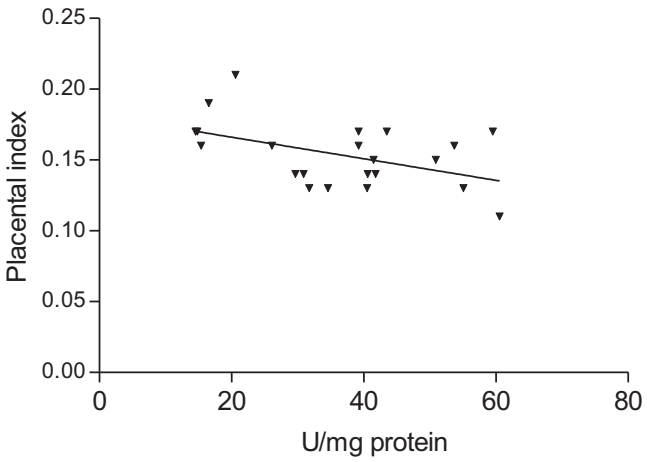

Fig. 6 – Relationship between placental index (placenta weight/newborn weight) vs. placental catalase activity of pulverization period (PP) group. Line represents linear regression ($p = 0.023$; $r = -0.48$).

(Chiapella et al., 2013). Besides the well-known limitations of in vitro models, it must be noted that these effects were produced at concentrations 5 and 10 fold higher that those considered representative of OP human environmental exposure (Buratti et al., 2007). In line with this, it was reported that the viability of isolated cytotrophoblasts from human placenta was not affected by a concentration of 500 μM H_2O_2 (Aris et al., 2009). Indeed, trophoblast-derived JAR cell line has been shown to resist a concentration of 1000 μM H_2O_2 (Hallmann et al., 2004).

In the current study, we found a negative moderate association between CAT activity and placental index, only in the PP group (Fig. 6). This inverse association might be interesting as prognostic for chronic health conditions to follow-up this cohort as these children develop to adolescence and beyond. Interestingly, OP exposure in this population took place at the end of pregnancy, a critical window to intrauterine growth which is associated to placental delivery oxidative stress levels (Min et al., 2009). Placental index which is independent of the fetal sex (Haeussner et al., 2013) is considered a useful metric for estimating the conceptual cost of fetal growth (Angiolini et al., 2006; Fowden et al., 2009). Placental efficiency is genetically determined, in part, but is also responsive to environmental conditions during pregnancy (Fowden et al., 2009). Moreover, in pregnancy complicated by undernutrition, melatonin, a potent endogenous antioxidant, may improve placental efficiency and birth weight by upregulating placental antioxidant enzymes (Richter et al., 2009). Changes in placental efficiency can occur by alterations in the weight of fetus, or placenta or both (Fowden et al., 2009). However, no significant changes in these morphometric parameters or in the placental index among groups were observed (Table 2). In consonance, a work recently performed by Bulgaroni et al. (2013) showed no changes in these parameters. However, these authors found an increase in the IL-13 expression frequency that could be related to a compensatory mechanism in response to OP-induced injury in some of the OP exposed placentas.

Our results should be interpreted in light of some study limitations. First, some investigators have observed higher maternal oxidative stress before and after delivery compared with women with elective cesarean (Hung et al., 2011), while Roberts et al. (2009) found no differences in placental oxidative and nitrative stress biomarkers regarding to the mode of delivery. In addition, Cindrova-Davies et al. (2007) observed that several placental markers of oxidative stress were increased as a result of long term labor. In this sense, the current study was restricted to those patients with <10 h labor. Second, it is known that oxidative effects depend on diet as well as polymorphisms of the antioxidant and detoxifying enzymes (Al-Gubory et al., 2010). Related to this, inter-individual differences in the expression of embryonic and fetal CAT likely constitute an important determinant of risk for ROS-mediated developmental pathologies (Abramov and Wells, 2011).

5. Conclusions

We speculate that low CAT activity would predispose to placental inefficiency and propose that placental CAT may have a potential as a placental biomarker of susceptibility to pesticides. To prove these hypothesis further studies designed from a gene-environment perspective to examine OP potential oxidative effects in placenta are required.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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