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Metals content in placentas from moderate cigarette consumers: correlation with newborn birth weight

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Abstract

Cigarette consumption during pregnancy produces deleterious effects in both, mother and fetus, some of them due to the presence of toxic elements in cigarette smoke, such as cadmium. Placenta constitutes a dual-purpose specimen for evaluating the pollutant burden exerted on the mother as well as on the fetus. The main objective of this study was to establish a correlation between placental concentration and distribution of some metal elements and birth weight of neonates delivered by mothers, who were either moderate smokers or nonsmokers. Forty nonsmoking and moderate smoking pregnant women paired per age, parity, weight, height and body mass index were selected. Smoking was assessed by self-reported cigarette consumption during pregnancy and urine cotinine concentration before delivery. Placental metal concentrations were evaluated by atomic absorption spectrometry (copper and cadmium) and neutron activation analysis (zinc and iron). Newborns from smokers had lower birth weights compared to infants from nonsmokers. Birth weights were correlated with placental cadmium concentrations in both, smokers and nonsmokers. Placental zinc and cadmium of smokers were mainly located at the maternal side and their levels were higher than those found in nonsmoker's placentas. In addition, all metal nutrient/pollutant ratios were decreased in the smoker group. In this first study performed in our region, we found that moderate smoking mothers deliver neonates with decreased birth weight and highly correlated to placental cadmium concentration. Decreased metal nutrient/pollutant ratios, a condition here found in smokers, may indicate a placental dysfunction, contributing to impair birth weight.

Introduction

The increase in environmental pollution created by anthropogenic socio-economic activities has caused a serious public health concern, especially the impact of pollutants on essential mineral status of population from developing countries. Cigarette smoke, a main pollutant, has been associated with several adverse outcomes of pregnancy such as low birth weight, spontaneous abortion, prenatal and neonatal mortality and long-term defects in physical and mental development of the offspring (Haworth *et al.* 1980; Wen *et al.* 1990; Cliver *et al.* 1995; Kendrick *et al.* 1996). Increased levels of cadmium, as one of the innumerable compounds present in cigarette smoke, have been reported in plasma and placenta of smoking pregnant women (Kuhnert *et al.* 1987; Bush *et al.* 2000a). In addition, placental zinc/cadmium concentration ratio at term has been positively associated to newborn birth weight (Kuhnert *et al.* 1988a). Mothers who smoke have altered ratios of placental zinc to placental cadmium, mainly in older and

multiparous mothers, a condition that may be relevant to fetal growth and development (Kuhnert *et al.* 1988a). Since zinc has been previously associated to mammalian growth (Salgueiro *et al.* 2002), it is quite possible that high levels of placental cadmium may result in retention of zinc in smokers, and thus contributing to reduce fetal growth (Kuhnert *et al.* 1988b). Cadmium influence on placental zinc retention may be mediated by metallothionein (MT), a low molecular weight protein able to strongly bind zinc, cadmium and copper inside the cells (Bremner 1991). This protein contributes to cellular homeostasis of trace essential element, and it has been also suggested to be involved in cellular zinc transport through the plasma membrane (Bremner 1991). In this regard, it has been shown that cadmium clearly stimulates MT synthesis in isolated trophoblasts (Lehman & Poisner 1984). Then, potentially increased MT production in smoker's placentas might be able to capture more zinc, which in turn may result in decreased bioavailability of this element. An altered zinc bioavailability in the fetus-placental unit of smoking mothers has been related to lower infant birth weight (Kuhnert *et al.* 1987).

The interaction between zinc and cadmium has been previously determined in placentas from smokers, and their relationships with birth weight have been already discussed. Recently, interactions between iron and copper in development and pregnancy outcome have been reviewed (Gambling *et al.* 2003). However, the incidence of placental levels and distribution of all these elements on fetal growth has not yet been evaluated.

In this study, we describe concentrations and main location of zinc, iron, copper and cadmium in placentas from both, smoking and nonsmoking mothers, with the purpose of correlating these concentrations with neonate birth weight as an expression of fetal growth. Both groups of mothers had similar characteristics of age, parity, nutritional status, body mass index and gestational age, to avoid influence of external factors in the metal elements content determination. Findings of this study, obtained in a limited Chilean population, gives further support for the detrimental effects of smoking in birth weight. Main results demonstrate that neonates delivered by smoking mothers have lower birth weight than neonates delivered by nonsmokers. In addition, among all the metal elements evaluated in placental tissue, only cadmium was highly correlated with newborn birth weight in smoking mothers.

Materials and methods

Placentas were obtained upon delivery in the maternity ward at Sótero del Río Hospital in southern Santiago. Inclusion criteria included healthy young parturients with normal pregnancies and without history of alcohol or drugs. All mothers had normal nutritional status evaluated as previously described (Atalah *et al.* 1997). The ethical committee of our institution approved the investigation and the questionnaire, which included medical and dietary history as well as data on occupational and possible environmental sources of metal exposure. The assessment of smoking was based on self-reported cigarette consumption during pregnancy and urine cotinine determination immediately before delivery. Parturients were divided into two groups: women who had never smoked (nonsmokers) and women who smoked at least 5 cigarettes per day throughout the entire pregnancy (smokers).

Sample preparation

Immediately after delivery, the entire placenta was weighed and placed in a polyethylene plastic bag and frozen at −70 °C until laboratory transport (Iyengar & Rapp 2001a). To determine trace elements, half of the partially thawed placenta was thoroughly washed and lyophilized at low temperatures using a programmed cycle of temperature and pressure: −25 °C; −20 °C (1.03 mbars); −10 °C; 20 °C (0.02 mbars) and 30 °C for 32 h (Christ Delta 1–20 kD). To determine metal elements location in placental tissue, three samples from maternal-facing surface (deciduas) and from fetal-facing surface (chorionic plate) ($n = 20$) were lyophilized as described above. Subsequently, samples were grinded and homogenized, constituting the stock placental material for metal element determinations.

Urine cotinine

A urine sample was taken just before delivery and maintained frozen at −20 °C until cotinine evaluation which was carried out by radio-immunoanalysis

(RIA) (Diagnostic Product Company, Los Angeles, CA, USA).

Determination of metal elements

Zinc and iron levels were determined by instrumental neutron activation analysis (INAA) at the Neutron Activation Analysis Laboratory of the Chilean Commission for Nuclear Energy. Samples were irradiated for 24 h at thermal neutron flux of 1×10^{13} neutrons/sec/cm^2 and after 20 days, samples were measured with a high-resolution gamma ray spectrometer.

Cadmium and copper were determined by atomic absorption spectrometry (AAS 5 E-A) with a graphite furnace device for solid samples (SS-GFAAS, Carl Zeiss Technology). Results were expressed as μg/g of dry weight tissue. The references samples were supplied by the National Institute for Standards and Technology (NIST, MD, USA).

Statistics

Statistical analyses were performed using a statistical software package for Windows (Statistica for Windows, Release 4, 5, Statsoft Inc. 1993, USA). Data were expressed as mean $\pm$SD. The Student-*t* test was applied to compare results obtained in both groups: smokers and nonsmokers, and significance was assumed at $P < 0.05$. The Pearson test was utilized to establish correlation between elements content present in placentas and neonates birth weight.

Results

Maternal characteristics of smokers and nonsmokers are described in Table 1. No differences in age, parity, height, body mass index, placental weight and nutritional status between both groups were observed. However, as expected, cotinine levels in women urine were significantly higher in smokers than in nonsmokers (Table 1). Smokers reported a consumption of 5–10 cigarettes per day. Newborn characteristics are described in Table 2. They were delivered at similar gestational ages in both groups, being all of them adequate for gestational age as judged by the *z* score. As shown, the only significantly different parameter between both groups was the birth weight. Thus, infants from smokers have approximately 280 g less birth weight than those infants delivered by nonsmoking mothers. Although the *z* score for birth weight and height was within normal ranges for both groups, a significantly lower birth weight *z* score for smoking mothers-related newborns was obtained. Total placental zinc and cadmium concentrations were significantly higher in smokers than in nonsmokers (Table 3); copper levels were similar in both groups, while iron levels were decreased in smokers. These results were concordant to metal concentrations observed in maternal and fetal sides of placentas, with the only exception of iron (Table 4). In nonsmokers as in smokers, zinc was preferentially located at the maternal side of placenta (Table 4). Interestingly, only placentas from smoking mothers were able to concentrate cadmium at the maternal side.

Table 1. Clinical characteristics of smokers and nonsmokers.

Maternal Characteristics	Nonsmokers ($n = 20$)	Smokers ($n = 20$)
Age (years)	27 ± 7	28 ± 9
Body weight initial (Kg)	62 ± 8	58 ± 6
Body weight at delivery (Kg)	72 ± 7	71 ± 5
Height (cm)	158 ± 5	155 ± 6
BMI initial (Kg/m^2)	25 ± 4	24 ± 2
BMI at delivery (Kg/m^2)	29 ± 4	29 ± 2
Parity	1.4 ± 1.2	1.3 ± 1.2
Weight gain (g/weeks)	313 ± 59	360 ± 113
Cigarette number (per day, self-reported)	0	5–10
Cotinine (urine)	13 ± 11	710 ± 419 *
Placental Weight (g)	558 ± 102	603 ± 103

BMI: body mass index, *$P < 0.01$.

Table 2. Clinical characteristics of newborns.

Newborn status	Nonsmokers ($n = 20$)	Smokers ($n = 20$)
Birth weight (g)	3564 ± 205	3282 ± 458*
Birth weight (z score)	−0.06	−0.71**
Birth height (cm)	49.8 ± 1.5	48.4 ± 2.6
Birth height (z score)	−0.06	−0.42
Ponderal index (g/cm^3)	2.8 ± 0.2	2.6 ± 0.6
Cranial circumference (cm)	34.4 ± 0.9	34.2 ± 1.8
Gestational age (weeks)	39.3 ± 0.8	38.9 ± 2.2
Intrauterine growth	AGE	AGE

AGE = adequate for gestational age, *$P < 0.01$, **$P < 0.02$.

Table 3. Total placental concentration of zinc, copper, iron and cadmium (μg/g dry wt).

Group	Zn	Cu	Fe	Cd
Nonsmokers ($n = 20$)	53 ± 9	5.7 ± 0.9	603 ± 149	0.02 ± 0.01
Smokers ($n = 20$)	62 ± 8	5.4 ± 1.1	550 ± 101	0.06 ± 0.02
P	0.01	0.289	0.01	0.01

Table 4. Concentration of zinc, copper, iron and cadmium (μg/g dry wt) in maternal and fetal side of the placenta.

Group	Zn		Cu		Fe		Cd	
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
Nonsmokers ($n = 20$)	53 ± 6*,**	45 ± 5*,**	5.9 ± 0.9	5.3 ± 0.6	622 ± 189**	627 ± 198**	0.03 ± 0.01**	0.03 ± 0.01**
Smokers ($n = 20$)	60 ± 6*,**	54 ± 5*,**	6.0 ± 1.1	5.6 ± 1.8	540 ± 101**	589 ± 169**	0.05 ± 0.02*,**	0.04 ± 0.01*,**
P	0.02	0.01	0.06	0.21	0.47	0.22	0.01	0.02

*$P < 0.01$ maternal vs fetal, **$P < 0.01$ smokers vs nonsmokers.

Homogeneous placental distribution was observed for copper and iron. Placental cadmium levels were better correlated to newborn birth weights from smokers than from nonsmokers ($r = 0.8$ and 0.4 respectively; Figure 1). Correlation between newborn birth weight and placental levels of zinc (Zn), copper (Cu) or iron (Fe), either in smokers or nonsmokers was not found. Placental Zn/Cd, Fe/Cd and Cu/Cd ratios of smokers were lower than the corresponding ratio values obtained for nonsmokers (1.05 ± 0.36 vs 2.67 ± 0.84; 8.1 ± 3.9 vs 29.8 ± 12.1 and 0.1 ± 0.03 vs 0.3 ± 0.1 respectively; mean ±SD, $n = 20$ per group; Figure 2). Additionally, in smokers as in nonsmokers, any of these metal nutrient/pollutant ratios did not show important correlation with birth weight.

Discussion

The study of placenta as an appropriate biological tissue to evaluate metal exposure has been already considered elsewhere (Iyengar & Rapp 2001a). Smoking is considered as a source of cadmium to the body burden and represents a good model to know effects of cadmium on fetal development. Several studies have reported effects of smoking on fetal growth and development, but specific mechanisms involved in those effects have not been clearly described (Kuhnert *et al*. 1987; Roquer *et al*. 1995).

It has been long recognized that smoking increases cadmium levels in plasma and placenta of smokers (Kuhnert *et al*. 1987; Bush *et al*. 2000a; Piasek *et al*. 2001). Also, smoking mothers deliver newborns with reduced birth weight, a fact that has been related to the relation of placental zinc/cadmium levels (Cliver *et al*. 1995; Roquer *et al*. 1995; Lindley *et al*. 2000). The interaction of both elements in placental tissue seems to be relevant for fetal development, since placental zinc/cadmium ratio at term has been positively associated to newborn birth weight (Kuhnert *et al*. 1988a). In the present study, it is shown that smoking

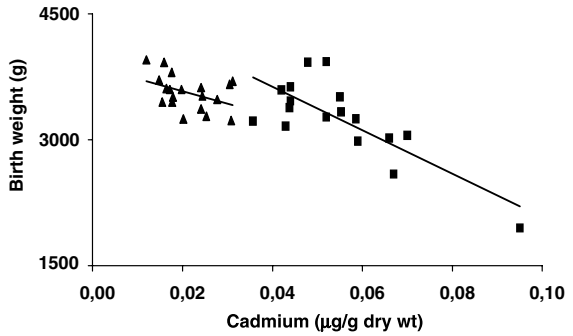

Figure 1. Correlation between birth weight and placental levels of cadmium in a population of (▲) nonsmokers and (■) smokers. Pearson correlation coefficients were $r = 0.4$ and $r = 0.8$ for nonsmokers and smokers respectively. $N = 20$ for both groups.

mothers delivered neonates with decreased birth weights in comparison to nonsmokers.

Other factors such as maternal age, parity, height and pre-pregnancy weight are considered potential constitutional determinants, playing important roles for fetal growth and developmental (Hindmarsh *et al.* 2000; Mamelle *et al.* 2001). At this point, it is important to mention that smoking and nonsmoking mothers involved in the present study, had similar age, weight, height, parity and nutritional status.

In this work we have found elevated placental zinc and cadmium concentrations and reduced iron levels in smokers. Placental levels of cadmium were inversely correlated to newborn birth weight, independent to the smoking habit, although the correlation became higher by two folds in smokers. Correlation between placental levels of zinc, copper or iron at term, and newborn's birth weight from both groups was not obtained. Although it is well known that zinc is needed for fetal growth in animals (Salgueiro *et al.* 2002), a relationship between maternal or fetal zinc status and newborn birth weight in humans has not always been found. Nevertheless, in earlier stages of human fetal development, high levels of zinc in fetal tissues and plasma have been previously reported (Chaube *et al.* 1973; Nasrat *et al.* 1992). Therefore, findings of this and previous reports demonstrating high concentrations of zinc in smoker's placentas may reflex a sustained accumulation and/or retention of the metal at this level. This situation may lead to a decreased bioavailability of zinc to the fetus, thus affecting fetal growth during a crucial period of development, which in turn is reflected in decreased newborn weight at term. Indeed, decreased zinc levels in cord vein red blood cells previously found in infants of smokers may indicate decreased placental zinc transport (Kuhnert *et al.* 1988b). Accumulation and/or retention of zinc in smoker's placentas may be due to the elevated cadmium levels observed in this tissue. The mechanism by which cadmium could be involved in placental accumulation/retention of zinc is not yet understood, but may be associated to an increased synthesis of a cadmium-induced metallothionein (MT). Cadmium is able to increase MT

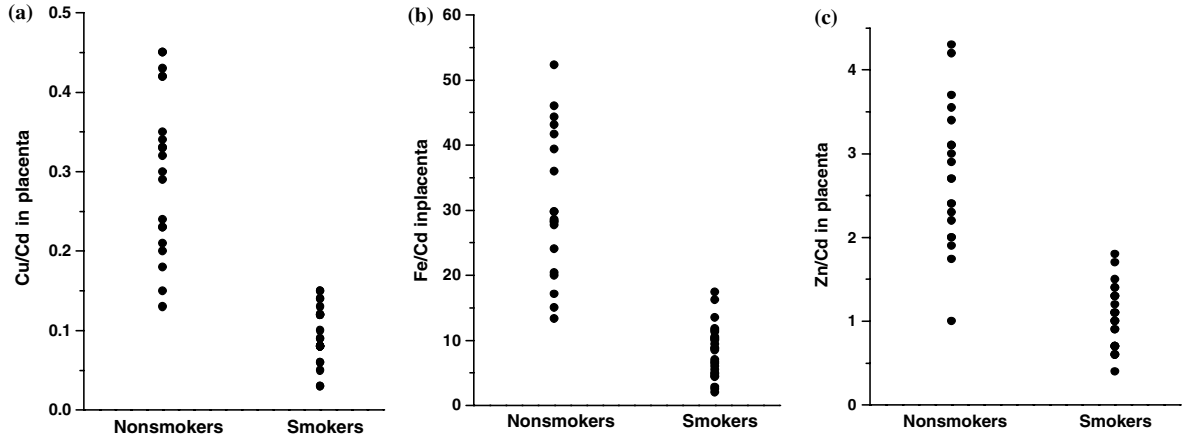

Figure 2. Distribution of placental copper/cadmium (Cu/Cd, a), iron/cadmium (Fe/Cd, b) and zinc/cadmium (Zn/Cd, c), ratios in nonsmokers and smokers. Cadmium was expressed in ng/g dry weight and zinc, copper and iron in µg/g dry weight. In each case, comparison of mean ±SD ratio values of smokers and nonsmokers were significantly different ($P < 0.001$; Friedman Anova).

production in isolated trophoblasts (Goyer & Cherian 1992) and its direct effect in smoker's placentas has recently been demonstrated (Ronco *et al*. 2004). Therefore, since MT is able to bind zinc and cadmium, it may be suggested that high levels of cadmium-induced MT will lead to an increased binding of zinc to this protein, which in turn will result in placental accumulation/retention of this metal (Ronco *et al*. 2004).

Recently, Piasek *et al*. (2001), reported significant higher cadmium levels and reduced iron levels in placentas from smoking mothers, but no differences in placental zinc contents in comparison to nonsmokers. Those elevated cadmium and reduced iron levels in smoker's placentas are in agreement with results reported in the present study. Nevertheless, we found increased zinc content in smoker's placentas, a result that is in agreement with those previously reported by Kuhnert *et al*. (1988a). Discrepancies in metal element evaluations among different authors may arise from methodological aspects to assess smoking. In our experimental group, the assessment of smoking was not only based on self-reported individual cigarette consumption, but also on the detection of urine cotinine levels immediately before delivery. Although other works have based their results on self-reported assessment of smoking (Miller *et al*. 1988; Demir *et al*. 1994; Clausen *et al*. 1999; Kantola *et al*. 2000), detection of nicotine metabolites like cotinine might lead to a more objective and reliable assessment of cigarette consumption. For this reason, here the smoking group included the self-reported smoking habit and values of cotinine concentrations higher than 200 ng/ml in urine. Self-report indicated a homogeneous consumption of 5–10 cigarettes per day in the whole smoker group. However, a wide range of urine cotinine was obtained (250–1200 ng/ml), suggesting a high dispersion in cigarette consumption. Wide range in urine cotinine may be likely due to the variable time between last cigarette smoked and urine sample collection just before delivery. In addition, it must be also taken into account that during pregnancy, cotinine half-life is reduced from 17 to 8 h (Dempsey *et al*. 2002). Although, it may be concluded that urine cotinine concentration just before delivery is not a suitable indicator to quantify real cigarette consumption, it corroborates self-reported smoking during pregnancy (Vio & Salazar 1997). It is worth to mention that a urine cotinine concentration over 200 ng/ml established as an inclusion criteria in the smokers group was a highly significant different value compared to the cotinine concentration found in nonsmoker's urine (13 $\pm$ 11 ng/ml).

The effect of iron and copper interactions in development and pregnancy outcome has been recently reviewed (Gambling *et al*. 2003), indicating that iron deficiency during pregnancy conducing to anemia could be a cause of lower birth weight (Allen 2000) and increased risk of cardiovascular disease in adulthood (Godfrey *et al*. 1991). Although copper deficiency during human pregnancy is less common, in experimental animals copper deficiency during pregnancy has been associated with cardiovascular disease, brain abnormalities and fetal growth retardation (Keen *et al*. 1998).

In this study, placental iron levels found in smokers were lower than those found in nonsmokers, a finding that is in agreement with previous reported data (Piasek *et al*. 2001). Nevertheless, no correlation between placental iron concentrations and birth weights was obtained. The reduced content of placental iron in smokers should not be a consequence of maternal iron deficiency, considering that this group had an adequate nutritional status. A possible explanation for this finding may be related to the expected elevated levels of nickel in placentas from smokers, a metal that may disrupt placental iron homeostasis. Cigarette smoke contains nickel that once introduced into the respiratory tract may reach, cross and accumulate in human placentas (Chen & Lin 1998; Torjussen *et al*. 2003). The syncytiotrophoblast is the most frequent site for nickel deposit (Reichrtova *et al*. 1998a), a fact that may be related to the presence of the divalent metal transporter 1 (DMT-1) in this tissue (Georgieff *et al*. 2000). In several cells DMT-1 has been involved in the uptake of several divalent metals, included nickel (Garrick *et al*. 2003; Tallkvist *et al*. 2003). In placenta as in other cells, DMT-1 has been also shown to be involved in endosomal iron exit (Georgieff *et al*. 2000; Garrick *et al*. 2003). Thus, it could be hypothesized that nickel may disrupt iron homeostasis in placental syncytiotrophoblast cells because of its interaction with DMT-1, thereby decreasing endosomal iron exit, which may lead to a chronic reduction in iron absorption from maternal serum (Georgieff *et al*.

2000). Nickel may also disrupt iron/DMT-1 interactions in placental Hofbauer cells, a condition probably involved in decreased iron storage in this tissue (Georgieff *et al*. 2000). Iron deficiency in placenta could also mimic iron removal effects associated with a cellular hypoxic state (Templeton & Liu 2003). This state might be additive to the known smoking-induced chronic hypoxia (Bush *et al*. 2000b), thus increasing expression of several genes involved in iron homeostasis, through the hypoxia-inducible factor-1 (HIF-1) (Schneider & Leibold 2003). Additionally, iron deficiency/chronic hypoxia interaction may activate stress-related hormones, thus altering metabolic processes associated to placental functions related to intrauterine fetal growth (Allen 2001).

Localization of metal elements in placentas has raised discrepancies in the literature; some reports describe a homogeneous distribution, while others indicate a preferential localization of these elements (Manci & Blackburn 1987; Lagerkvist *et al*. 1996; Reichrtova *et al*. 1998b; Piasek *et al*. 2001). In our study, zinc was concentrated at the maternal side, both in placentas from nonsmokers and smokers. Interestingly, cadmium was preferentially concentrated at the maternal side only in smoker's placentas. Copper and iron were homogeneously located in the whole placenta. This situation may indicate that zinc and cadmium could be retained at the maternal side of placentas, and as already stated, decreasing the chance of transference to the fetus. It has been described that mechanisms operating at the maternal surface of placental syncytiothrophoblast membranes are considered to be limiting for maternal-fetal transfer of zinc (Aslam & McArdle 1992). Nevertheless, it is not known if these mechanisms respond to changes related to maternal zinc status and/or to changes related to fetal zinc necessities during growth and development.

The presence of zinc and cadmium in placenta is a good example of nutrient-pollutant interaction, and seems to be a key factor for fetal development. In fact, placental zinc/cadmium ratio at term has been positively associated to newborn birth weight (Kuhnert *et al*. 1988a). In the present study, although nonsmoker's zinc/cadmium ratio was higher and significantly different to the ratio found in smokers, a clear correlation between placental zinc/cadmium ratio and birth weight was not found. Other metal nutrient/pollutant interactions such as copper/cadmium and iron/cadmium ratios were also higher in nonsmoker's placentas.

Several hypotheses have been raised to explain an abnormal fetal development leading to low birth weight (IJzerman *et al*. 2002; Jones & Jose 2004; Wallace *et al*. 2004). Nevertheless, it is suggested here that, although a correlation between birth weights and any of these metal nutrient/pollutant ratios was not found, low placental nutrient/pollutant ratios could be considered as an additional detrimental factor affecting fetal growth.

To our knowledge, this study represents the first of this type in an urban population of a South American city (Santiago de Chile) and placental range concentrations of all studied elements found here agree with those previously reported in other studies from different countries (Iyengar & Rapp 2001b). Cadmium levels in placentas from the nonsmoking group are within the range of previous studies for this toxic element (Iyengar & Rapp 2001c). However, in this study, cadmium levels in smoker's placentas were higher than those previously reported; suggesting higher levels of cigarette consumption in our smoking group (Piasek *et al*. 2001). In addition, in contrast to other published works, we found that smoking mothers delivered newborn with lower birth weights than nonsmokers. This clear interaction could be due to our more suitable evaluation of pregnant women smoking habit. A positive correlation between nutritional essential elements and birth weight was not found. Nevertheless, placental cadmium levels were highly and inversely correlated to newborn weights, mainly in smokers. In summary, our results provide further evidence to suggest detrimental effects of maternal smoking on fetal growth with still unknown consequences in the adult life.

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