Environmental Contamination and Infant Development in a Bolivian Mining City

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A todas las mujeres y niños de Oruro

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Han pasado cuatro años, grandes experiencias y largos viajes por el mundo desde que empecé a trabajar en esta tesis. En agosto del 2007 viajaba a Bolivia sin saber muy bien con qué ni con quién me encontraría. Como atraída por una aventura desconocida me involucré en el proyecto ToxBol después de que Núria me comentara que un investigador francés, Jacques Gardon, buscaba a alguien para comenzar una cohorte de niños en una ciudad minera de Bolivia. Así fue que aterricé a 4000 metros en un país nuevo, con una cultura nueva y donde pasaría dos de los años más enriquecedores de mi vida. ¡Quién habría dicho que en Bolivia me esperaba un trabajo apasionante, unos compañeros increíbles, unos amigos de por vida, un marido y una tesis!

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ABSTRACT

Background: Oruro is a mining city in the Bolivian highlands. Most of its citizens are exposed to polymetallic cocktails. This study aims to determine whether the neuropsychological development of one year-old children can be associated with 1) polymetalic exposures and/or 2) growth patterns.

Methods: The thesis builds from data collected by the ToxBol multidisciplinary project. The health task was centred on the follow-up of a birth cohort that set to evaluate child development. Children were examined at 11 and 12 months of age using the Bayley Scale of Infant Development (BSID).

Results: 1) Analyses revealed no high concentrations of metals in the blood of pregnant women. 2) No neuropsychological anomalies were observed in association with metal concentrations or growth patterns. 3) A positive association was observed between low lead values in blood and the neuropsychological development of children. 4) During the first 6 months of life, growth rate was negatively associated with weight at birth and positively associated with the BMI at 12 months of age.

Conclusion: Although heavy metallic pollution was demonstrated by environmental studies, unexpected low levels of exposure were registered from pregnant women. Neither the level of exposure to metals nor the growth patterns appear to have an impact on child neuropsychological development. We argue that the positive effect that was observed in association to lead might be caused by factors such as diet.

RESUMEN

Antecedentes: Oruro es una ciudad minera del altiplano boliviano. Muchos de sus habitantes están expuestos a un cóctel polimetálico. El objetivo principal es el de determinar si existe una asociación entre desarrollo neuropsicológico de los niños de un año y 1) la exposición polimetálica y/o 2) los patrones de crecimiento.

Métodos: Esta tesis está basada en los datos recogidos del Proyecto multidisciplinario ToxBol. Se realizó el seguimiento de una cohorte de niños para evaluar su desarrollo. Para evaluar el desarrollo neuropsicológico de los niños se utilizaron las escalas de Bayley de desarrollo infantil (BSID).

Resultados: 1) No se observaron altas concentraciones de metales en sangre de las mujeres embarazadas. 2) No se observaron anormalidades neuropsicológicas en relación con concentraciones de metales o patrones de crecimiento. 3) Se observó una asociación positiva entre exposiciones bajas de plomo y el desarrollo neuropsicológico del niño al año de vida. 4) La velocidad de crecimiento, desde el nacimiento hasta los 6 meses, se asoció negativamente con el peso al nacer y positivamente con el BMI a los 12 meses.

Conclusión: A diferencia de lo demostrado en otros estudios epidemiológicos, se observó una inesperada baja exposición. No se observó una relación entre el grado de exposición a metales- o los patrones de crecimiento -y el desarrollo neuropsicológico del niño. El efecto positivo del plomo creemos que se debe a otros factores como la dieta.

RESUM

Antecedents: Oruro es una ciutat minera del altiplà Bolivià. La població es troba es contacte amb fonts de contaminación polimetàlica. L'objectiu principal es el de determinar si existeix una associació entre el desenvolupament neuropsicològic dels nens d'un any i 1) la exposició polimetálica i/o 2) patrons de creixement.

Mètodes: Esta tesi està basada en dades del Projecte multidisciplinari ToxBol. La tasca de salut es va centrar en el seguiment del desenvolupament d'una cohort de nens. El desenvolupament neuropsicològic infantil es va avaluar amb les escales de Baley de Desenvolupament infantil (BSID).

Resultats: 1) No es van observar concentracions altes de metalls en sang de les dones embarassades. 2) No es va observar anormalitats neuropsicològiques en realció amb les concentracions de metalls o patrons de creixement. 3) Es va observar una associació positiva entre concentracions baixes de plom en sang i el desenvolupament neuropsicològic dels nens d'un any. 4) La velocitat de creixement, des del naixement fins els 6 mesos, es va associar negativament amb el pes al néixer i positivament amb el BMI als 12 mesos.

Conclusió: A diferencia del que esperàvem, es van registrar baixes concentracions de metalls a les dones embarassades. Tampoc s'observa una relació entre el grau d'exposició a metalls, o patrons de creixement, i el desenvolupament neuropsicològic del nens. El efecte positiu que observem del plom, pensem que es deu a altres factors com la dieta.

PREFACE

This thesis is the result of extensive fieldwork in Bolivia, further analysis in Spain, and additional writing and research in Canada. Fieldwork was conducted under the auspices of the Institut de recherche pour le Development (IRD) during two years in La Paz and Oruro, Bolivia (2007-2009). The ToxBol project (Toxicidad en Bolivia/Toxicity in Bolivia), emerged out of this two-year fieldwork research, and so did the health subproject *Mine Niño*. Fieldwork was conducted under the supervision of Dr Jacques Gardon. Funding for this fieldwork was appreciated by IRD and the Agence Nationale de la Recherche (ANR, France). Work in Spain was conducted at the Centre for Epidemiological Research in Barcelona (CREAL) under the supervision of Dr Jordi Sunyer (2009-2011). Funding in Spain was appreciated by the IMIM Foundation full doctoral scholarship programme. Final writing and revising were done in Quebec, Canada, while completing a research associateship at the Department of Psychology of the Université Laval. During this associateship, I was offered the opportunity to do fieldwork in three communities situated in the Nunavik region as part of a research project focusing on Inuit communities, health and education (Inujkuak, Kuujjuarapik, and Kangiqsualujjuaq). This experience allowed me to engage in matters of child neuropsychological development in Quebec’s Great North, and thus engage in comparative studies (i.e., public health and infant neuropsychological development in Quebec and Oruro). Funding for this fieldwork was appreciated Centre de recherche du CHUQ (CRCHUQ).

This doctoral work is presented following the “thesis by publication” normative as established and defined by the Doctoral programme in Biomedicine at the Department of Experimental Science and Health, Universitat Pompeu Fabra. The thesis includes an abstract, an introduction outlining the thesis justification, objectives, methods, and results, copies of two articles submitted to international peer review journals (one is already published and the other is under review; the present Ph.D. candidate is lead author in both papers), together with a discussion, and a bibliography. Both articles draw from the ToxBol project. The present doctoral thesis is presented as part of larger study lead and subsidized by the IRD and the ANR, France.

A part of the present doctoral project was selected to compete in the Rolex Awards for Enterprise 2010 Young Laureates Programme. The project was shortlisted (out of 200 competing projects) by an international committee and I was invited to present it in Rome in Novermber 2009. Included with this thesis is the video of my project entitled ‘*Contaminated Environments, Ailing Children: on the contaminated environment of a mining city in Bolivia and its direct effects of child development*’ (see enclosed CD).

The contributions of the present Ph.D. candidate in the articles discussed and presented in this thesis include the writing of the articles, fieldwork in Bolivia, statistical analysis, and bibliographical review.

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1. INTRODUCTION

1.1 Oruro: A mining city in the Bolivian highlands

Oruro (17° 58′ S–67° 06′ W) is one of the largest mining cities in Bolivia. The city is located in the Andean flat highlands (also known as Altiplano), at an altitude of ~3700 m.a.s.l., and about 300 km south of La Paz **(Fig. 1)**. Living conditions at high altitude are challenging with an arid, cold and windy climate during the winter months (June to September) and heavy rains during the summer (December to March).

Fig. 1 Map of Bolivia (Nathan, 2008)

The Bolivian economy relies in great part on the exploitation and exportation of natural resources (Smolders et al., 2003; Van Damme et al., 2008). Oruro is particularly rich in polymetallic ore deposits such as Ag, Cu, Pb, Sn, and Zn. An increase in the demand in minerals in the last two decades has contributed in the opening (and reopening) of many mining industries in Bolivia. Today, the mining industry in Bolivia is operating at full capacity. In 2008, the mineral production in Oruro registered a record extraction production of 30000 tons of Zn, 10 000 of Sn, 2 500 of Pb, 500 of Sb and 130 of Ag and W (Instituto Nacional de Estadística, 2010). With little efforts deployed to the safeguard of the environment, the Bolivian mining industry now registers an alarmingly high environmental impact. Safety measures are also often rudimentary. In spite of these issues, the Bolivian mining industry remains an important source of employment and as such a main player in the Altiplano economy.

The main Oruro mines are *San José*, *Colorada*, and *Itos*. Except for a brief period between the mid-1980s and the early 1990s, San José has been extracting minerals for over 400 years. The city also hosts the largest smelter complex in the country.

Fig. 2 Mining acid drainage and households on mining wastes© ToxBol Team

The environmental contamination in Oruro is a complex issue, counting several important sources of contamination that directly affect the local population **(Fig. 2).** In addition to the contamination associated to the city mines (extraction processes, smelting, transformation, and transport), other types of contaminants are to be taken into account such as those generated by local textile, ceramic and steel industries (Goix et al., 2011, Goix, 2012) **(Fig. 3).** The mines of *San José* and *Itos* form the main mining area of Oruro, situated on the northeast and southwest of the city, respectively. Chemical and steel industries also operate within this mining area. The city centre encompasses a small but highly dense residential district together with an assortment of commercial, industrial and craft productions. The eastern area of the city includes the three industrialized districts where most of the smelting activities take place, including the *Fundición de Vinto* – the most important tin smelting industry in Bolivia (Goix et al., 2011).

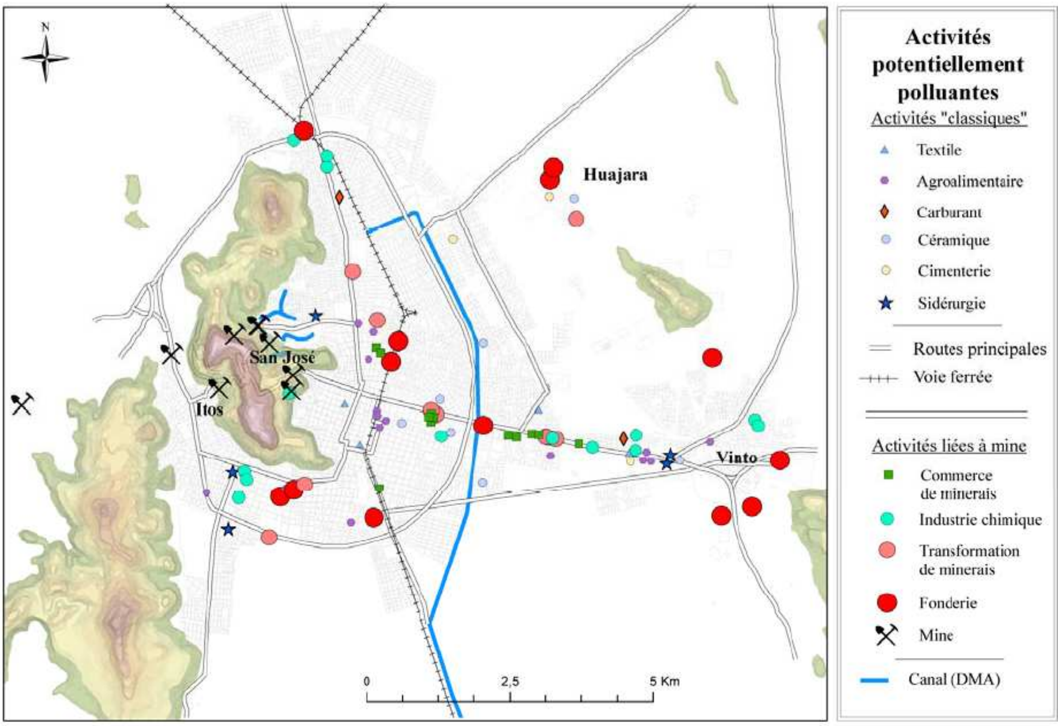

Fig. 3 Representation of the most important industries in Oruro (Goix, 2012).

The presence of important mining wastes, smelters and ore dressing industries makes Oruro comparable to La Oroya (Peru), one of the most contaminated cities in the world (Fraser, 2006, 2009). Many Oruro families live in close proximity to the city mines with very little, if any, awareness of the possible health risks associated to prolonged exposure of environmental contaminants. Nor do the authorities have the proper knowledge to be able to develop efficient tools to educate local populations (Tapia et al. 2010). Fortunately, both the local populations and the authorities have begun to express some concerns. All agree that this is an urgent problem for many more families around the globe face similar environmental risks.

1.2 Demography and Sanitation

Oruro has a population of ~220000 residents. Women's population structure is expansive, emphasising a wide based pyramid. It is also a young population, counting 36% under the age of 15. About 20% of women are within reproductive age. The fertility rate is high, registering an average of 3.6 children per women in 2008 (MEASURE, 2008).

The nutritional situation is also of central concern. The prevalence of health and malnutrition problems is high, and even more so if we compare with the situation in developed countries. According to the strategic plan published by the OPS (Organización Panamericana de la Salud) for the 2003-2007 period, Bolivia is placed in the 'priority list' based on the fact that is sanitary results have been deemed inadmissible by the member states of the OPS (PAHO, 2011). Although percentages have reduced in the past 5 years, malnutrition in Bolivia (based on 5yr-old children) reach 27% according the WHO – amongst the highest in Latin America. Within Bolivia, the highlands register the highest percentages of malnutrition (30 to 44%). Nearly 8% of the highland population shows severe delayed growth patterns while acute malnutrition and global malnutrition represent 1% and 4%, respectively. 12% of women living in the highlands have a height inferior to 145cm (which is usually an indication of nutritional and obstetric risks), and 48% of women suffer from a type of anaemia.

Anaemia in children (severe, moderate, or minor) reaches 73% in Bolivia, while the child mortality rate is the second highest in the Americas (50 per 1000 in 2008) after Haiti (**Table. 1**). Based on a study conducted by the OPS in 2001, high neonatal mortality rates in Bolivia were associated with a high prevalence for infectious diseases (32%), disorders related to prematurity and low weight at birth (30%), together with respiratory disorders related to the perinatal phase (22%) (PAHO, 2007). In the Oruro region, specifically, child mortality reaches 56 per 1000, whilst perinatal mortality is maintained roughly at 31 per 1000. Additionally, the incidence of long-term chronic diseases such as obesity, diabetes, and cardiovascular diseases are also increasing (Pérez-Cueto, FJA. et al., 2004, 2009; Pérez-Cueto, A. et al., 2005). In 2008, the proportion of overweight or obese women had increased to the point of reaching 50%. The health situation in Bolivia is also particularly challenging because of the coexistence of both generalized malnutrition and sedentary lifestyle diseases (Bermudez et al., 2003; De Onis et al., 2000; Morales et al., 2004).

País	Mortalidad infantil	Mortalidad en la niñez
Haití 2000	80	119
Haití 1994-95	74	131
Bolivia 2008	**50**	**63**
Bolivia 2003	**54**	**75**
Bolivia 1998	**67**	**92**
Guatemala 1998-99	45	59
Guatemala 1995	51	68
Perú 2004-06	21	29
Perú 2000	33	47
Perú 1996	43	59
Honduras 2005-06	25	32
Honduras 2001	34	45
Nicaragua 2001	31	40
Nicaragua 1998	40	50
Brasil 1996	39	49
República Dominicana 2007	32	36
República Dominicana 2002	31	38
República Dominicana 1996	47	57
Colombia 2005	19	22
Colombia 2000	21	25
Colombia 1995	28	36

Table 1 Child and infant mortality rates in Latin American countries (MEASURE, 2008)

1.3 Mining pollution and human toxic metals Exposure

The Bolivian mining industry exploits and exports a great variety of metal resources such as tin, antimony, copper, and silver (Smolders et al., 2003; Van Damme et al., 2008). According to the EPA (Environmental Protection Agency), metals have unique characteristics. 1) They are found naturally and in different concentrations. 2) All environmental media have naturally occurring mixtures of metals, and metals are often introduced into the environment as mixtures. 3) Some metals are essential to human biology. 4) The environmental chemistry of metals strongly influences their fate and effects on human and ecological receptors.

5) Each metal has a toxicokinetic and a toxicodynamic that depends on the type of compound and the capacity of the exposed organism to regulate or store it (EPA, 2007).

Metals can be classified into three groups depending of their impact on health (Goyer, 2004): nutritionally essential (e.g., cobalt, iron, copper, zinc), non-essential with a possible beneficial effect at low levels of exposure (e.g., nickel, boron), or non-essential with no beneficial effects (e.g., arsenic, lead, silver, cadmium, antimony, aluminium, mercury). The latter group is the most dangerous for human health and the environment, especially when the concentration of the exposure is high and/or chronic. Toxicity associated to non-essential metals includes neurologic, cardiovascular, hematological, gastrointestinal, musculoskeletal, immunological, and epidermal effects (EPA, 2007).

Mining industries located in close proximity to urban populations increase the human exposure to non-essential compounds and the probability of toxic effects on human health (Hakan et al., 2008; Liu et al., 2005; Moreno et al., 2010; Rojas et al., 2007). Exposure to metals usually results from: inhalation of the ambient air (suspended particles), diet, contaminated water, skin contact (although usually of limited impact) or through contaminated soil. The effects of the exposure varies according to factors such as age (children as opposed to adults), life stage, life style, gender, nutritional status, genetic variability (polymorphism) and preexisting underlying diseases (Fraser, 2009; Goyer, 2004).

Children may be more vulnerable than adults to some toxicants. A child's exposure to chemicals can be different, and often greater, than that of an adult. The difference in exposure is mostly explained by differences in behaviour, diet, as well as metabolic and physiologic characteristics. Behaviour patterns increase the risk of exposure. A typical hand-to-mouth action is such a behavioural pattern where hands that might have touched contaminants in the soil are often left unwashed. Children also eat, drink, and breathe more air everyday than adults in terms of body/weight ratio. Finally, the metabolic pathways of children are at an immature stage which means that they are necessarily more exposed and little prepared to withstand the effects of a toxic agent (Landrigan et al., 2004; Faustman et al., 2000). Alterations in the early stages of life also imply more time for chronic diseases to increase. In fact, exposure to metals such as lead, arsenic, and methylmercury during the first stages of development can have severe effects such as cerebral damage; whereas a similar exposure on an adult would have little to no effect (Grandjean and Landrigan, 2006). The potential sources of contamination and how they affect the health of young children, specifically, are therefore of central importance in the field of epidemiology.

1.4 Lead: its effects on health

Lead contamination is one kind of exposure whose effects are most damaging for human health (with As, Hg and Cd). Exposure to lead remains one of the most researched topics both in contexts of developing and developed countries – although the highest contamination levels to lead and the fewest preventive measures have been registered in developing countries (Domínguez-Cortinas et al., 2012; Acosta-Saavedra et al., 2011; Riddell et al., 2007; Jacoby, 1998; Astete et al., 2009; Conklin et al., 2007; Pebe et al., 2008). Like other types of metals, lead is found in its natural state often in combination with other minerals and inorganic salts (Goyer, 2004). Lead enters the organism mostly through the process of inhaling trace metal particles as well as water and/or food ingestion. Once having reached the lungs, lead is then distributed in other parts of the body (through the blood stream) which can potentially generate serious effects on adult health (hematological, gastrointestinal, cardiovascular, renal, neurological, reproductive), and most especially in the health of children, including anaemia, renal alterations, neurological development, low weight at birth or delayed growth (EPA, 2007). The level of toxicity and the effects associated to lead depend on a number of factors including concentration, time of exposure, and/or the age. Because of their different absorption rates the various forms of lead in minerals are known to condition the hazard for humans. In some studies a direct association in blood lead levels was related to mining pollution and dust exposure (Gulson et al., 1994; Malcoe et al., 2002). Yet, other studies found no association between soil lead concentration and blood lead levels in people living in a polluted mining context (Danse et al., 1995; Berglund et al., 2000). Amid the reasons for the absence of such associations is the metal low bioavailability registered in some studies (Davis et al., 1993).

While the risks associated to lead exposure are now better recognized, it is not so long ago that high lead concentration products were used on a daily basis such as certain types of paints, ceramics, and gasoline. The use of such products was heavily criticized in various studies in epidemiology. Although discontinued in most parts of the world, lead-based products remain commonly used in some countries (Rubin et al., 2002; Su et al., 2002). Studies continue to identify and demonstrate the important risks associated to various kinds of environmental contaminants, even at low concentrations (Needleman, 2004; Needleman et al., 2004). In fact, the limit at which lead is considered toxic has been lowered on various occasions by international health organizations. For instance, in 1971 lead was considered toxic beyond the 40 μg/dl mark. Two decades later, in 1991, the limit was lowered at 10μg/dl (Center for Disease Control, 1991).

Whereas most studies focus on the neurotoxic effects of lead, important also are other recorded adverse effects of lead toxicity on growth, the immune system, and pregnancy (Grandjean and Landrigan, 2006). Lead exposure also significantly disrupts the development of the nervous system throughout childhood and alters cellular differentiation, myelinisation, and the formation of the synapses between neurons (Philip et al., 2004; Alfano and Petit, 1982; Mendola et al., 2002; Johnston and Goldstein, 1998). Recent studies in epidemiology now even show the adverse effects on child neurodevelopment of lead exposures at values below 10μg/dl

(Bellinger, 2008; Needleman, 1991; Kordasa et al., 2006; Lanphear et al., 2005; Surkan et al., 2007; Jedrychowski et al., 2007; Canfield et al., 2003) – **Fig. 4**.

The toxic effects from lead exposure are of special concern during pregnancy. Toxicity can reach beyond the placenta barrier and affect the neurosystem of the foetus. Studies have shown that pregnant women with a previous prolonged exposure to lead are most susceptible to expose the foetus to lead toxicity. This is due to a release of accumulated lead particles in bones, also referred to as "skeletal lead stones" (Wigle et al., 2007; Gulson et al., 1997; Miranda et al., 2010). Jedrychowski et al. (2009), Shen et al. (1998), and Tellez-Rojo et al. (2006) all reported the neurotoxic impact of prenatal lead exposure on young children, even at very low levels. Hu et al. (2006) also confirmed the adverse effects of foetal lead exposure during the first trimester of pregnancy on mental development at 24 months of age. Plusquellec et al. (2007) observed a negative effect of blood lead levels on language, memory, fine motility, and certain aspects of behaviour (Plusquellec et al., 2007). Amongst the reported negative effects on child neurodevelopment, both prenatal and postnatal exposures include the reduction of the IQ values, learning difficulties, mental disorders, attention deficit hyperactivity disorder (ADHD), and antisocial behaviour (Bellinger et al., 1987; Needleman, 1990; Nevin, 2000). However, results are less evident in cases where samples were taken from children younger than 12 months of age (Jedrychowski et al., 2009; Bellinger, 2008a). This is partly due to the fact that it is very difficult to 1) have access to direct samples (e.g., blood) from new born babies (e.g., refusal from mothers) and 2) to evaluate the damages that occur at such an early stage of development (Counter et al., 2000; Counter et al., 1998).

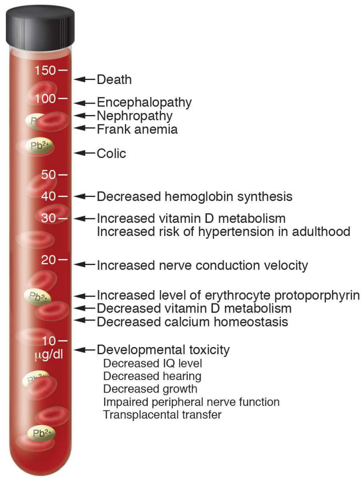

Fig. 4 Effects of inorganic lead (µg/dL) exposure in children. (Bellinger and Bellinger, 2006).

1.5 Child Neurophysiological Development

Child neuropsychological development can be affected by a number of environmental, genetic, and social factors. The most common environmental factors include chemical compounds such as metals, solvents, and pesticides (Grandjean and Landrigan, 2006). High toxicity metals include lead, arsenic, and methyl mercury (Landrigan et al., 2004). Metals that reach the placenta barrier during pregnancy (the placenta is not an effective physical barrier) expose the foetus to a number of toxic effects (Andersen et al., 2000). Important also is that the “blood-brain barrier” does not fully form until a few months after birth. Prior to this time the brain remains exposed and vulnerable to toxic agents (Adinolfi, 1985; Saunders, 1986). There also remains the possibility that neurotoxic compounds can be secreted during lactation (Andersen et al., 2000; Michaelsen, 2009); although the long-term positive effects of lactation on neurodevelopment will often compensate the negative effects of the exposure (Ribas-Fito et al., 2003).

The development of the nervous system is a complex and lengthy process encompassing different stages of development during the lifespan of an individual (Rodier, 1995; Rice et al., 2000). Throughout the crucial periods of development (e.g., foetus development and infancy), the nervous system is most vulnerable and thus more susceptible to toxic exposures; and it is during those periods that exposures can cause permanent damage and a negative impact of adult life (Grandjean and Landrigan, 2006). It is precisely because of the fact that new born babies are more vulnerable by toxic contaminants that studies like the present thesis are necessary in order to understand the neurological system.

In addition to the known neurotoxicity caused by chemical agents, other factors can have a negative effect on the development of a child. Precarious living conditions, malnutrition, and a high prevalence of infectious diseases are such factors; and these are commonly found amongst vulnerable child populations in the Bolivian highlands (MEASURE, 2008).

1.6 ToxBol Project

Toxbol is a multidisciplinary project encompassing geochemists, medical doctors, epidemiologists, geographers, and sociologists affiliated with the *Institut de recherche pour le développement* (IRD France-Bolivie) and the *Universidad Mayor de San Andrés* (La Paz, Bolivia). The aim of the project is to study the origin and impact of polymetallic contamination on the environment, health, and society of the mining city of Oruro situated on the Bolivian Altiplano. The project is delined into four research axes: health, human geography, geochemistry, and aquatic ecology. Each axis includes various research subprojects. ToxBol contributes in providing much needed data on the level of contamination of the city of Oruro and its possible effects of children's health. The project is most valuable as it provides the necessary tools for understanding the environmental situation to which the Oruro citizens are exposed.

a) Environmental context

ToxBol was preceded by a pilot project in 2006 whose aim was to evaluate the level of environmental contamination and child exposure in Oruro. Hair and dust samples were collected. A metallic contamination exposure pattern in household dust was reported. The results revealed that the levels of metals were significantly different in homes located in close proximity to the city mines and mineral processing facilities by comparison to those located in the outskirts of town, especially in the cases of lead and antimony (**Table. 2**) (Fontúrbel et al., 2010). In addition to proximity, the behavioural habits of workers also contributed in increasing the indoor metal concentrations (e.g., workers returning home while still wearing their work clothes). The mean of lead indoor dust contamination in the San José mining district was extremely high (1290 mg/kg).

In parallel to the pilot project, the geochemistry ToxBol team focused on environmental matters and demonstrated the impact of the mining industry on air, water and soils pollution. Metal concentrations were measured in aerosols. Measurements were also taken from Tillandsias and used as bioindicator (plants that grows on electric cables). The results revealed an impact of the Vinto smelting area with especially high values of As, Cd, and Sb in the PM2.5 fraction of aerosols and in the concentrations observed in Tillandsias. Top soil dust was found to reach 16 000µg/g of lead in some football fields used by children, but was generally low in aerosols particles. Arsenic attained 200 ng/m3 in PM10 near the smelter area, with a high bioavailability, as estimated by UBM method (Bioaccessibility Research Group of Europe) (Goix et al., 2011; Goix, 2012). Based on data drawn from the aerosols and Tillandsias, three sources of atmospheric contamination were identified and associated to mining activities: city mines, smelter emissions, and traffic (Goix, 2012).

Metallic element	Mining district			Peripheral district		
	Arithmetic mean (Standard error)	Geometric mean	Min–Max value	Arithmetic mean (Standard error)	Geometric mean	Min–Max value
As	143.38 (19.33)	90.28	12.90–635.55	42.50 (1.40)	41.68	30.19–68.75
Cd	15.96 (2.49)	11.48	4.60–101.34	6.05 (0.20)	5.94	4.19–10.31
Cu	114.30 (12.43)	88.97	30.30–471.25	77.02 (15.27)	56.56	23.90–617.20
Pb	1289.78 (235.36)	619.11	66.60–8413.45	221.43 (60.59)	133.66	48.51–2415.25
Sb	494.44 (138.50)	152.91	21.50–6199.35	46.43 (5.93)	39.23	18.11–203.65
Sn	114.13 (21.73)	76.39	19.60–1172.35	36.21 (2.16)	33.90	19.49–76.87
Zn	583.75 (64.69)	442.91	119.30–2295.22	252.06 (21.00)	228.34	132.00–843.38

Table 2 Metal concentrations (ppm) determine in domestic dust in Oruro. (Fontúrbel et al., 2011).

On the contrary, metal concentrations in drinking water did not reach toxic levels as defined by WHO – although some water samples in the Vinto region showed levels of arsenic and antimony that were well above toxic limits. This occurrence did not appear in the mean values. All water samples taken from Oruro were below toxicity levels (**Table. 3**). However, results emphasised by Fontúrbel et al. (2010) and Goix et al. (2011) from soil and dust samples clearly show the existence of high environmental risk to which Oruro citizens are exposed.

	Al	Cr	Mn	Ni	Cu	As	Mo	Cd	Sb	Ba	Pb	U
Norme OMS	200	50	400	70	2000	10	70	3	20	700	10	15
Moyenne total (n=30)	2,9 ±0,7	1,0 ±0,1	1,3 ±0,3	0,5 ±0,2	4,8 ±0,8	3,2 ±0,5	0,7 ±0,1	0,029 ±0,006	4,5±2	82 ±2	0,4 ±0,1	5,8 ±0,3
Maximum	19,6	1,6	6,4	6,9	21,7	12,9	1,8	0,14	49,3	100	3,2	8,0
Moyenne Oruro (n=25)	1,6 ±0,2	1,15 ±0,05	0,8 ±0,2	0,5 ±0,3	5,0 ±0,9	2,21 ±0,08	0,54 ±0,01	0,023 ±0,004	0,47 ±0,03	86±1	0,4 ±0,1	6,4 ±0,1
Maximum	5,1	1,6	3,9	6,9	21,7	3,1	0,6	0,10	1,2	98,6	3,2	8,0
Moyenne Vinto (n=5)	9,3 ±3,1	0,23 ±0,04	3,6 ±1,0	0,30 ±0,02	3,8 ±1,4	8,2 ±2,0	1,3 ±0,3	0,06 ±0,02	25±7	62±10	0,4 ±0,1	3,2 ±0,7
Maximum	19,6	0,3	6,4	0,4	8,1	12,9	1,8	0,14	49,3	99,6	0,8	4,5

Table 3 OMS normative and Oruro mean values ± SE of metals concentrations in tap water samples (µg/L).

b) Child exposure

The ToxBol pilot project (2006) primarily consisted of analysing hair samples from children in local schools in order to determine traces of contamination. Samples were collected from 7–12 year old children from primary schools located in and around the Oruro mining districts (Barbieri et al., 2010). Schools were selected based on geographical position in relation to mines and smelters. The concentration of essential and non-essential elements was evaluated using ICP-MS (Inductively coupled plasma mass spectroscopy, IPEN São Paulo ISO 17025). Children from schools near the mining and smelting areas were 4 to 14 times more exposed to non-essential metallic compounds than children from schools in the city centre and suburban areas. High levels were registered of arsenic, lead, cadmium, and antimony. These results supported the hypothesis of a moderate to high contamination (Barbieri et al., 2010). (**Table. 4 and Table. 5**)

	Rural school		Suburb school		Downtown school	
	Geometric mean (SD)	P5–P95	Geometric mean (SD)	P5–P95	Geometric mean (SD)	P5–P95
Pb	1.57 (1.33)	1.10–2.64	2.03 (2.29)	0.39–8.05	2.32 (2.98)	0.33–10.19
As	0.07 (6.73)	0.00–0.44	0.42 (2.5)	0.11–1.74	0.39 (2.31)	0.11–1.24
Cd	0.05 (1.53)	0.02–0.12	0.08 (1.95)	0.03–0.35	0.08 (2.11)	0.03–0.19
Hg	0.13 (1.93)	0.07–0.90	0.13 (1.48)	0.08–0.21	0.15 (3.03)	0.05–0.50
Sn	0.05 (1.68)	0.02–0.09	0.08 (1.75)	0.03–0.17	0.12 (2.20)	0.04–0.38
Sb	0.02 (9.47)	0.00–0.08	0.09 (1.57)	0.04–0.21	0.08 (3.75)	0.02–0.35
Ni	0.18 (3.13)	0.05–1.05	0.52 (1.85)	0.24–2.11	0.13 (6.42)	0.00–0.85
Bi	0.01 (3.73)	0.00–0.18	0.02 (3.12)	0.01–0.11	0.05 (3.98)	0.01–0.51
Ag	0.05 (1.48)	0.03–0.11	0.12 (2.28)	0.02–0.51	0.07 (3.31)	0.01–0.76
Mn	1.26 (2.14)	0.27–5.48	1.53 (1.93)	0.48–3.86	0.56 (6.33)	0.00–2.81
Cr	0.05 (1.69)	0.02–0.12	0.09 (1.57)	0.04–0.14	0.07 (2.81)	0.01–0.32
Cu	7.76 (1.29)	4.04–11.76	7.40 (1.34)	4.54–11.20	6.89 (1.62)	3.53–15.44
Co	0.01 (2.05)	0.00–0.05	0.02 (1.72)	0.01–0.04	0.01 (1.79)	0.00–0.03
Se	0.57 (1.58)	0.33–1.21	1.10 (1.96)	0.45–3.77	0.90 (2.12)	0.34–3.89
Zn	145.40 (1.31)	85.1–210.1	105.87 (1.75)	35.1–190.3	85.28 (1.57)	32.9–146.3

Table 4 Trace Elements Concentration in the Rural, Suburban and Downtown Schools. Results in µg/g of hair (Barbieri et al., 2010).

	Mine school		Smelter school	
	Geometric mean (SD)	P5–P95	Geometric mean (SD)	P5–P95
Pb	14.08 (2.72)	3.24–65.4	21.22 (3.30)	2.67–208.8
As	0.79 (2.79)	0.10–3.30	1.32 (3.01)	0.28–5.55
Cd	0.07 (16.72)	0.00–2.03	0.03 (15.93)	0.00–0.65
Hg	0.49 (3.68)	0.09–8.44	0.50 (2.57)	0.13–2.55
Sn	0.20 (2.20)	0.05–0.67	0.72 (3.15)	0.18–4.86
Sb	0.10 (56.16)	0.00–4.14	0.62 (4.02)	0.08–5.44
Ni	0.36 (9.55)	0.00–6.94	0.64 (4.26)	0.12–2.82
Bi	0.06 (3.41)	0.02–0.72	0.11 (3.77)	0.02–0.88
Ag	0.33 (3.54)	0.04–3.11	0.29 (2.87)	0.08–2.04
Mn	1.34 (6.75)	0.03–8.18	2.41 (2.91)	0.63–9.58
Cr	0.12 (4.12)	0.00–0.83	0.11 (5.51)	0.00–0.69
Cu	11.37 (2.57)	3.17–44.89	11.77 (1.47)	6.69–22.26
Co	0.00 (35.77)	0.00–0.08	0.00 (36.45)	0.00–0.05
Se	1.08 (2.39)	0.28–5.23	1.27 (2.51)	0.24–5.84
Zn	123.66 (2.14)	31.47–357.3	142.38 (1.76)	57.3–385.4

Table 5 Trace Elements Concentration in the Mine and the Smelter Schools. Results in µg/g of hair. (Baribieri et al., 2010).

The indoor dust pollution was found to be strongly associated with the school age children’s level of exposure to non-essential elements such as Pb, As, Sb. A contrasted situation was found in industrial and non-industrial districts with a 1/6 to 1/10 ratio depending on non-essential elements for human exposure and environment pollution. These observations were considered to be of sufficient concern to justify an evaluation of exposure based on blood samples and a health impact study.

c) Effects on Health

Based on the registered environmental contamination in Oruro, the ToxBol project aimed to determine its possible toxic impact on the neuropsychological development of children living in the city. This thesis presents the results of the *Mine Niño* (My Child) subproject lead by the ToxBol health team. The research took place in the city of Oruro (Bolivia) from 2007 to 2009. The aim was to study the impact of trace metal "cocktails" in child health (including neuropsicologic development, growth, and development of the immune system together with the susceptibility to infections). Follow-up of a birth cohort was conducted at two local hospitals: the *Barrios Mineros*, located near the San José mine, and the *Caja Nacional de Salud*, located nearer the city centre. Both hospitals were selected based on their location, the first for its proximity to the contaminated mining areas and the latter for its proximity to the city centre. Selection was made in order to provide a more general perspective of the Oruro health portrait. A month by month follow-up was performed throughout the child's first year of life in order to document diseases and treatment (if and when occurring), behavioural habits, neuropsychological development, and potential problems related to nutrition. Blood samples (taken during pregnancy) as well as hair samples from all mothers were taken in order to assess the cohort's level of exposure to environmental contaminants. Additional blood samples were taken from the umbilical cord at birth. The two articles presented in this thesis build from the data collected during the two years of the *Mine Niño* project. Two scales were applied at different stages of development of the birth cohort in order to evaluate a possible association between the neuropsychological development of the children and their exposure to trace metals (present at least in the air, dust, and soil). The Brazelton Neonatal Behavioral Assessment (BNBAS) scale was applied at 2 weeks, 4 weeks, and 8 weeks following birth. The Bayley Scale for Child Neurodevelopment was then applied at

11 and 12 months. The present thesis presents the results of the latter Bayley Scale.

d) Involvement and Contribution to the Toxbol project

I spent two years completing fieldwork in the Bolivian highlands. I was initially offered a contract to join the ToxBol team in 2007 after the completion of my M.Sc. in Public Health during which I focused on the impacts of contaminated environments on child development. As an active member of ToxBol and subsidized by the IRD, I participated in the conducting of most of the fieldwork and consultations with the mothers and their children.

After a prenatal consultation with a physician (at the end of the second or the beginning of the third trimester), pregnant women were invited to participate in an information session. During these sessions, my colleagues and I provided the pregnant women both with general and specific details about the study objectives and the follow-up process. The women who agreed to participate signed an informed consent form in our presence. Upon enrolment, we interviewed each woman individually using detailed questionnaires in order to obtain information on socioeconomic status, general health status, as well as nutritional status (e.g., nutrition in the 24 hours preceding the interview). Once all interviews completed, we created a personal file for each woman (‘nutritional status’ files) in which were included all details registered during the consultations such as anthropometric data, bio-impedancemetric data (% of body fat, body mass index, % of water, and so forth), as well as densitometry (bone scan). We then proceeded to compile the data drawn from the analysis of the blood samples (the analysis was performed at the hospital laboratory) in order to measure haemoglobine and haematocrite. In parallel to the above, the first babies of our birth cohort were born. We took turns in order to ensure a presence of a ToxBol member during each of the births so as to take samples of the umbilical cords. Samples were sent to La Paz where some were kept for immunological analyses while others were forwarded to Montpellier (France) for metal concentrations measurements.

Two weeks following birth the mothers were contacted again for a first examination of their baby's neurodevelopment using the Brazelton scale (the test was performed by a medical doctor). The test was repeated at 4 weeks and 8 weeks. In addition to the neurodevelopment tests, we conducted further interviews with each mother in order to obtain various data including baby nutrition, baby health problems and treatments, if any, as well as neurological development, weight, size, head circumference, etc. Interviews were conducted monthly basis until the child would reach 12 months of age.

The Bayley Scale of Infant Development (BSID) was performed on children at ages of 11 and 12 months. I had received prior training in Sabadell (Barcelona, INMA Cohort) on the BSID procedures before departing for Bolivia. Upon arriving in Bolivia, I trained my colleague, and with her we performed all BSID tests.

One of the main challenges of our fieldwork was the difficulty to keep track of all participants. Some had phone numbers, others not; some had actual physical home addresses, others not. My colleagues and I visited all families in their homes. We did so primarily for two reasons: 1) to locate the GPS coordinates of all the homes and 2) to discuss with the participating women the importance of the follow-up process so as to increase participation.

Finally, we organised additional information sessions in various strategic points throughout the city in order to further inform local authorities of our project, its aims and importance.

1.7 Fieldwork in Bolivia

Conducting fieldwork in Bolivia is a challenging (but most rewarding) experience. Localised riots and road blocks are common in the highlands. They can (and often do) considerably delay ongoing research projects, especially those projects that depend on frequent transfers of personnel (staff, volunteers, etc.) and/or materials (biological samples, food supplies, etc.) between cities – as it was the case in our study.

In Bolivia, the mining industry is a vital source of employment, especially in the highlands. In fact, working in the mines remains for many a matter of family tradition. It is therefore understandable that any discussion or endeavour to emphasise the environmental contamination produced by the mining industry may be perceived by some as a threat to their livelihood. Such reticence can be explained by the fact that, without the mines, there is little chance of ensuring a steady family income. In such context, one of the main challenges of conducting research projects in mining cities like Oruro is not to appear as a threat to one of the main economic sources of the country.

Of pressing matter is the fact that there is a serious lack in the general population’s knowledge and awareness of the environmental problems and their repercussions on their health (and their children’s health). Given that contamination in and of itself is not a ‘tangible’ problem, it is difficult for many to fully understand the implied long term risks on their health. Also, and as a consequence of the above, contamination is often not considered an urgent problem. Quite the contrary, it appears to be of low priority. This remains a problem as many families the world over are exposed to environmental contamination without being fully aware of the risks on their health.

Local traditions play a significant role in any type of fieldwork. Understanding the traditional (spiritual) beliefs of the local communities and their approach (and strong ties) to traditional medicinal practices will go a long way in reducing misunderstandings and frustrations that can emerge from fieldwork. Without necessarily engaging any anthropological theory, an important first step is to acknowledge the cultural background of each mother. This information, given on a voluntary and discretionary basis only, allows us to better understand each mother’s cultural background and thus understand why their approach to medicinal practice and methods might differ from ‘ours’. In other words, these ‘cultural differences’ play a great role in how we inform, treat, and expect the mothers to participate in our project. It also allows us, as foreigners interacting with local families on a daily basis, to better understand their ‘way of life’ and thus create stronger professional ties. More importantly, it allows us to treat them efficiently without interfering or compromising their beliefs and cultural ethics. Specifically, the above mainly concerns the fact that local populations strongly believe in a number of illnesses that are inflicted by the natural forces of nature (rather than, for instance, being the product of environmental contaminants – as modern western medicine would assume). Our awareness (and sensitivity) towards these local traditional practices will go a long way in determining our approach in developing our methods and tools so as to examine and inform the Oruro families (see also *Paper 1*, p.61).

2. JUSTIFICATION/ RATIONALE

In recent years, environmental studies dedicated to world health issues have grown to be more aware of the direct relationship between environmental contamination and individual health/overall physical and mental wellbeing. Until very recently, in fact, environmental and health sciences were developing as parallel disciplines with very little interaction. Fortunately, the gap between the two disciplines is everyday thinner.

The possible effects of heavy metal exposure on health are becoming better documented. However, the majority of studies in epidemiology have been conducted in developed countries where the levels of exposure to environmental contaminates are relatively low or diminishing. In developing countries, high levels of exposure to environmental contaminants are an issue of central concern for public health. This is in addition to the vulnerability of populations (poverty, precarious living conditions, malnutrition), economic difficulties, and delays in the development and application of safety measures and awareness programmes. Children are most vulnerable to levels of exposure and the most notable effects are usually related to neuropsychological and/or physical developmental problems.

Few studies in environmental epidemiology have been conducted in countries like Bolivia. Oruro is a mining city where a large portion of its population lives in close proximity to mining wastes. Among the listed environmental contaminants are metals such as lead, arsenic or antimony whose toxic effects have been extensively documented. The presence of all these metals further underscores the polymetallic exposure of this particular population. In addition to the documented presence of important contaminants in the environment, there are additional issues in Oruro related to sanitation, infant mortality, malnutrition, and delayed growth. In this context, the ToxBol birth cohort provides a good opportunity to shed further light on the neurodevelopment and growth patterns of children in Oruro.

Oruro, as a case-study, provides much information and significant progression on our global knowledge of the causal effects of contaminated environments on health. While Oruro may be a relatively small city, there exist many more small cities around the globe with similar environmental problems. A better assessment and understanding of the problems in Oruro will go a long way in understanding and addressing the problems that many other small cities are facing elsewhere. Additionally, the present work contributes in providing further data on the Oruro environmental context. The analysis of such data will no doubt create awareness toward this environmental problem.

3. STUDY OBJECTIVES

3.1 General objective

Study the neuropsychological and physical development of children living in the mining city of Oruro between the ages of 0 to 12 months.

3.2 Specific objectives

a) Study the effects of heavy metal exposure on the neuropsychological development of children at ages of 11 and 12 months of age.
b) Describe the growth patterns of children during their first year of life.
c) Determine whether there is a relation between the growth rate of new born children and their cognitive development having reached the age of 11 and 12 months.

4. METHODS

This thesis builds from data collected by the ToxBol projet A total of 346 child births were registered in the two hospitals where the study was based. 256 children completed the Bayley test (BSID) at least once between the ages of 11 and 12 months. Of these children, 246 had an associated blood sample taken from their mother during pregnancy and 175 had a monthly follow-up of their growth. The data documented from the 246 children is discussed in the first paper submitted in this thesis entitled ‘Child Neurodevelopment in a Bolivian Mining City’, while the data from the 175 is discussed in the second paper entitled ‘Infant Growth Patterns and Child Cognitive Development in a Bolivian Mining City’.

The sources of information used were the following: detailed questionnaires administered to the mothers during interviews, biological samples (mother’s blood, umbilical cord bloom, hair, urine, buccal mucosa), physical exams, ultrasounds (bones and thymus), neuropsychological tests and environmental measures.

4.1 Analysis of trace metals

During the prenatal consult, a blood sample was taken from the mothers who agreed to participate in the study. In most cases, blood samples were taken either at the end of the second term or at the beginning of the third. The samples were analysed in order to assess the level of exposure to metals and metalloids (Pb, As, Cd, Sb, Cs, Zn, Fe, Cu, Se, Rb y Sr). Additional umbilical cord samples were taken at birth (natural or caesarean). Analyses of the digested whole blood samples from pregnant women were performed using ICP-MS at the IRD's Hydrosciences Montpellier Laboratory (HSM), France.

4.2 Neuropsychological evaluation

Child neuropsychological development was assessed using the Bayley Scales of Infant Development (Bayley, 1997; Spanish Edition). The BSID is one of the most valid psychometrical measurements to examine infant mental, motor, and behavioural development from 1 to 42 months of age. The mental scale consisted of 163 items that assessed age-appropriate cognitive development in areas such as performance ability, memory, and first verbal learning. The psychomotor scale consisted of 81 items assessing fine and gross motor development. Of the selected 246 children, 74.8 % performed the first test at ages varying between ≥10.5 and <11.5 months and 81.7 % did the second test at ages varying between ≥11.5 and <12.5 months. The BSID was administered at both hospitals by two trained examiners who did not know the children's degree of metallic exposure.

An additional variable was generated (BSID Testing Quality) and divided into three non-hierarchical categories to evaluate the quality of the BSID conditions: Normal Conditions; Behaviour Problems; and Special Situations. Behaviour Problems included situations where the test could not be completed properly due to the child's fearful or frantic behaviour. Special situations included events such as the child's tiredness prior to the BSID, hunger, or temporary sickness, all of which were considered firsthand factors that might contribute in generating falsely deficient test results.

4.3 Monthly follow-up of the child's growth

Growth and duration of breastfeeding was documented for each child on a monthly basis from birth until the age of 12 months. Anthropometric measurements: weight was measured in naked infants using an electronic scale (SECA®) to the nearest 10 g; supine length with knees extended was measured with an infantometer to the nearest 0.1 cm. A total of 175 children with the complete monthly growth follow-up were included in the analysis.

4.4 Statistical Methods

In the first paper were included only subjects with complete neuropsychological development data and maternal blood measures. In the second paper were included subjects with complete neuropsychological development data and growth follow-up. Preterm children and children with mothers with levels of lead extreme (n= 4) were excluded from the study.

The main outcomes were child neuropsychological development measured using the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). Rapid growth between birth and 6 months of age was defined as a change in weight z-score > 0.67 and slow growth was defined as a z-score change of < -0.67. Z-score for weight at birth and 6 months were calculated separately by sex. The entire cohort of children was used as the reference (internal standardization of the sample).

Covariates included birth characteristics (birth weight, birth length, caesarean section, and prenatal risk), maternal characteristics (age, education, smoke habit, alcohol consumption, marital status, parity, trimester of pregnancy), infant growth characteristics (weight, length, BMI) as well as other characteristics (number of adults at home, hospital of birth).

Multivariate linear mixed models were performed using metal concentration as the exposure variable in order to evaluate the potential association between metals concentration and rapid or slow growth on child neurodevelopment. All metal concentrations except zinc were natural log transformed. Mixed models were used to account for repeated measurements of neurodevelopment in children aged between 10.5 and 12.5 months and to maximize statistical power.

Confounders included key predictors of test scores such as examiner administering the test, the quality of the test and the number of the test (first at 11 months or second at 12 months). Others such as caesarean section, child gender, parity and number of adults in the household were also included in the adjusted final model. The potential non-linearity of the relationship between neuropsychological development and weight gain or metals concentrations was determined using Generalised Additive Models (GAM).Analyses were performed using STATA 10.0.

5. RESULTS

5.1 PAPER 1

Child neurodevelopment in a Bolivian mining city

María Ruiz-Castell, Pamela Paco, Flavia-Laura Barbieri, Jean-Louis Duprey Joan Forns, Anne-Elie Carsin, Rémi Freydier, Corinne Casiot, Jordi Sunyer, Jacques Gardon

Environmental Research 2012;112:147-54.

Ruiz-Castell M, Paco P, Barbieri FL, Duprey JL, Forns J, Carsin AE, Freydier R, Casiot C, Sunyer J, Gardon J. Child neurodevelopment in a Bolivian mining city. Environ Res. 2012;112:147-54

5.2 PAPER 2

Infant Growth Patterns and Child Cognitive Development in a Bolivian Mining Region

Ruiz-Castell M, Carsin A-E, Barbieri F-L, Paco P, Sunyer J, Gardon J. Infant Growth Patterns and Child Cognitive Development in a Bolivian Mining Region. *American Journal of Human Biology*, Submitted.

Child Growth Patterns and Cognitive Development in a Bolivian Mining City

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Abstract.

Objectives: This study aims to 1) follow up and characterize infant growth patterns during the first year of life in Bolivia, and 2) determine whether there exists an association between weight gain and cognitive development in children living near contaminated mining industries.

Methods: Data on 175 children participating to the ToxBol (Toxicity in Bolivia) birth cohort were analyzed. Rapid-growth during the first 6 months was defined as a change in weight z-score > 0.67 while slow-growth was defined as a weight z-score change of < -0.67. Neurodevelopment was evaluated using the Bayley Scales of Infant Development (BSID) at10.5–12.5 months of age. Mixed models were used to examine the association between cognitive development and weight gain.

Results: Rapid growers weighed less at birth ($P < 0.01$). However, they revealed a higher BMI at 12 months of age (0.70 ± 0.73, $P < 0.01$). After adjustment for confounding, rapid growth was not associated with cognitive development (coef=0.49, 95% confidence interval= -4.10, 5.08).

Conclusions: In this Bolivian cohort, children born smaller were more likely to grow/develop faster and attain greater weight and size. Their cognitive development was not affected by their growth patterns.

Key Words. Weight gain, BMI, child neurodevelopment, BSID, Bolivia.

Introduction

Anthropometric indices at birth are useful to predict child development in terms of health and nutritional status. Various studies have emphasized the importance of weight and size of new born babies as determinant factors directly affecting their health during adult life. For instance, low weight at birth is associated with various sets of complications; some of which have immediate impact on development while others are chronic in time (Bergvall et al., 2006; Geva et al., 2006; Sørensen et al., 1997) .Other types of growth patterns are also relevant for evaluating children's development and health such as neonatal catch-up growth and early growth patterns during the first year of life.

In countries like Bolivia, child malnutrition and growth problems continue to persist. According to WHO standards, chronic, acute and global children's malnutrition (under 5 years) in Bolivia are 27.1%, 1.4% and 4.3%, respectively. Highest percentages are registered in mining cities such as Oruro and Potosi located in the Bolivian highlands. In Oruro, values reach 33.4% (chronic), 1.7% (acute) and 7.7% (global) (MEASURE, 2008).

Although data exists on birth rates, relatively little is known about growth patterns in Bolivia. Weight gain patterns may vary according to a child's growth rate. By definition, an increased growth pattern is defined by a growth rate that exceeds the normal growth-time vector. This increase typically follows a delayed growth pattern such as a growth intrauterine restriction or the lack of proper nutrition following birth. In cases of children smaller for their respective ages, a rapid catch up may reduce child morbidity and mortality thus providing it with a good chance to regain its adequate weight (according to his age), improve his nutritional status, better resist infections, and increase life expectancy (Weaver, 2006). However, such catch up presents various sets of potential problems (Weaver, 2006). Irregular acceleration rates have been linked with long-term chronic complications in adult life such as obesity, diabetes and cardiovascular diseases (Goodell, 2009; Holzhauer et al., 2009; Ong and Dunger, 2004; Yanovski, 2003). The incidence of such diseases is increasing in developing countries (Kelishadi, 2007; Martorell et al., 2000; Misra and Khurana, 2008; Prentice, 2006; Rivera et al., 2004) and Bolivia is no exception (Pérez-Cueto et al., 2005, Pérez-Cueto et al., 2009, 2004). The present paper argues the importance to further evaluate issues of obesity and overweight in Bolivian populations as growth patterns seem to be different. Delayed growth patterns among Bolivian populations may have been underestimated by failing to consider the specificity of the population (Baya Botti et al., 2010). The health situation in Bolivia is particularly challenging, primarily because of the coexistence of both generalized malnutrition and sedentary lifestyle diseases (Bermudez and Tucker, 2003; De Onis et al., 2000; Morales et al., 2004).There also remains a need to establish whether this adverse association can also be linked with cognitive and motor development. In cases of low birth weight or birth small for gestational age (SGA), the association could be compensated with potential beneficial effects including better developing cognitive functions (Brandt et al., 2003; Casey et al., 2006; Franz et al., 2009). However, other studies have observed that rapid or slow growths during the first year of life are associated with lower values in cognitive tests (Belfort et al., 2008). Accordingly, this study aims to 1) follow up and characterize child growth (weight, size, Body Mass Index (BMI), weight gain) during the first year of life in a Bolivian mining region and 2) to determine whether there exists a positive association between growth rates in children born at term and their cognitive development.

Methods

Study Population and Recruitment

The ToxBol study (Toxicity in Bolivia) was conducted in Oruro (Bolivia) between May 2007 and November 2009 at the prenatal care units of two hospitals – Hospital Barrios Mineros (HBM) and Caja Nacional de Salud (CNS). Pregnant women were enrolled during the end of the second or the beginning of the third trimester. The study was approved by the National Ethics Committee of Bolivia, La Paz (Bolivia). Detailed questionnaires were administered to each woman in order to obtain information concerning their socioeconomic status, as well as their general health and nutritional status. Growth patterns and duration of breastfeeding were documented for each child on a monthly basis from birth until the age of 12 months. Medical assistants documented anthropometric indices: weight was measured in naked infants using an electronic scale (SECA®) to the nearest 10 g; supine length with knees extended was measured with an infantometer to the nearest 0.1 cm. For 42 children, weight at 6 months and at 12 months was missing. Using the full repeated-measurements based on our entire sample (*n*=137), sex-specific growth curves were constructed for each child using mixed models with random intercept and slope after transformations based on fractional polynomials (Royston and Wright, 1998). Using these curves weight at 2 times point (6 and 12 months) we estimated for those infants for which weight records were not available. The method was accurate and the correlation between prediction and estimation in infants with measurements were: 0.90 at birth and 0.97 at 6 months for boys; and 0.95 at birth and 0.96 at 6 months for girls. Between 10.5 and 12.5 months of age, neurological development was evaluated twice using the Bailey Scale of Infant Development (BSID). After exclusion of preterm births (*n*=29) and children with behavioural problems during the first (*n*=8) or second (*n*=6) tests, a total of 175 children were included in the analysis with complete monthly growth follow up and BSID test performed at least once.

Neurodevelopmental Testing

Child neurodevelopment was assessed using the BSID (Bayley, 1977). The present paper focuses in particular on the cognitive development. The mental scale consisted of 163 items which assess age-appropriate cognitive development in areas such as performance ability, memory, and first verbal learning. The BSID was administered at both hospitals by two trained examiners in the presence of the mother.

An additional variable was generated (BSID Testing Quality). The variable was divided into three non-hierarchical categories in order to evaluate the quality of the BSID conditions: Normal

Conditions; Behaviour Problems; and Special Situations. Behaviour Problems included situations in which the test could not be completed properly due to the child’s fear or frantic behaviour. Special Situations included events such as the child’s fatigue, hunger or temporary illness prior to the BSID.

Statistical analysis

Z-score for height and weight at birth and 6 months were calculated separately by sex. Our entire cohort of children was used as the reference (internal standardization of the sample). Rapid growth between birth and 6 months of age was treated either as a categorical variable (slow growth/medium growth/rapid growth) or as continuous variable. Rapid growth was defined as a change in z-score > 0.67 and slow growth was defined as a z-score change of < -0.67 (Monteiro and Victora, 2005). These limits correspond to having 50% of our children population defined as average/medium. Additionally, BMI z-scores at 12 months were estimated as a continuous variable.

Child cognitive development was analyzed using the Mental Development Index (MDI) and treated as a continuous outcome variable standardized to a mean of 100 points with a standard deviation of 15.

Associations between covariates and child cognitive development were tested in univariate analysis using Student’s t-test for dichotomous variables in order to detect potential confounders. Associations between covariates (parental socio-demographic situation and children anthropometric measurements at birth, 6 months and 12 months) and growth rate were tested using ANOVA.

The association between neurodevelopment and weight gain were investigated using mixed model adjusted by examiner in addition to other potential confounders, e.g., gender, caesarean section, parity. Mixed models were used to account for repeated measurements of cognitive development in children at age between 10.5 and 12.5 months. The reason for this was to maximize statistical power because of our limited sample. Co-variables were selected a priori based on previous literature (Chandola et al., 2006; Fewtrell et al., 2001; Mendez et al., 2011; Ong and Dunger, 2004) and included in a multivariate model if the *P* of association in the bivariate analyses was $P < 0.2$. The potential non-linearity of the relationship was determined using Generalised Additive Models (GAM) (Hastie et al., 1990). Analyses were performed using STATA 10.0.

Results

Birth weight and height of the 175 children included in the analysis were within normal range (3 282±372, g and 49.4±1.76, cm). **Table 1** describes the main anthropometric patterns: birth weight and BMI at 12 months; z-score for child weight gain from birth until 6 months of age; weight and height at 6 months; distribution of cognitive development. **Table 2** presents mother–child characteristics. After restriction to the 175 children with complete data, 77 (45%) children fell into the medium growth, 50 children into the rapid growth, and 48 children into the slow growth. Comparison of anthropometric indices between groups suggest that there were significant differences in birth weight, weight at 6 and 12 months, weight for height z-score at 6 and 12 months, length at 12 months, length for age z-score at 12 months and BMI z-score at 12 months ($P < 0.001$). There was no significant difference between parental characteristics (maternal age, maternal height, maternal education, smoking habits, and parity), prenatal risk or birth by caesarean section. Rapid growers weighed, on average, less at birth. However, at 6 and 12 months of age, these infants were on average heavier and taller than other children. Children with slower growth were born heavier and bigger but their weight and height did not continue to grow following the same trajectory as those in the medium group **(Fig. 1)**.

There was a negative association between weight gain, from birth until 6 months, and birth weight **(Fig. 2)**. However, the association between weight gain and BMI z-score at 12 months was positive **(Fig. 3)**. Both these associations remained significant after adjusting for covariates ($b_{birth\ weight} = -0.47$, $P < 0.001$) and ($b_{BMI} = 0.61$, $P < 0.001$).

In multivariate linear mixed models, there was no association between growth rate during the first 6 months and the cognitive development at 12 months. After adjusting for potential confounders the effect remained the same for slow and rapid growers (**Table 3)**.

Discussion

Birth weight of children born at term is found well within normal range. Children born with a smaller size were also those registering a faster growth rate in addition to greater weights and sizes at ages of 6 and 12 months. Weight gain was not associated to child cognitive development.

According to the National Demographic and Health 2008, 4% of cases in Oruro register low birth weight (birth weight <2 500 g). In the present study, the birth weight of children is found within the normal range – with only one case <2 500 g. The low percentage of children with low birth weight may be explained by the following two patterns: 1) the fact that preterm children were excluded and 2) women leaving the study before completion generally had a lower education level (Ruiz-Castell et al., 2012). On the other hand, 43.3% of children were born with a size lower than 49cm. However, the range of size at birth is also within normal values, with only 4 registered cases of < 46 cm. At 6 and 12 months of age, size and weight measurements also appear to be within normal ranges according to age.

Weight and size variations during the first year of life are factors of equal importance as weight and size at birth. Early infant growth patterns are associated with health issues during adulthood. These issues can be caused by a slower or faster rate of growth during the first six months of life. Health problems can be associated to a nutritional problem in cases of those with slow growth rate (Martins et al., 2011; Vorster and Kruger, 2007) and an increase in the BMI in cases of children with faster growth rate (Fabricius-Bjerre et al., 2011; Kelishadi, 2007; Yanovski, 2003). Both cases reveal an important repercussion on the cognitive development at various stages of the child’s life (Baker-Henningham et al., 2009; Halkjaer et al., 2003; Li et al., 2008). As in other studies (Holzhauer et al., 2009; Mendez et al., 2011), we observed that children born with a smaller size also registered a faster growth rate together with greater weights and sizes at ages of 6 and 12 months. By contrast, children born heavier and with slower growth register a lower weight throughout their development and a smaller size. In this Bolivian highland population, the association between weight, BMI and growth rate was maintained. This occurred in a country where the transition of breastfeeding from exclusive to complementary (around 6 months of age) still indicates a moment of nutritional vulnerability and morbidity (Hoare et al., 1996; Weaver, 2006). Our results suggest that even in a specific population as seen in the present study and with such young children, rapid growth is strongly associated with high BMI at 12 months. This further emphasizes possible prematurely detected problems related with increases in BMI. However, we are unable to determine whether or not rapid weight gain is associated with future problems in adulthood, as observed in other studies (Beardsall, 2009; Monteiro and Victora, 2005;Yanovski , 2003).

There is relatively little data on child growth and cognitive development in Andean populations. Various known factors might play an important role in the child's growth, both physical and mental. Precarious living conditions, malnutrition and a high prevalence of infectious diseases are such factors commonly found among vulnerable child populations in Bolivian highlands (MEASURE, 2008; Nathan, 2008). The next step would be to follow up the child cohort until adulthood in order to obtain a broader perspective, and better understand the impact of rapid weigh gain during childhood in Bolivian adults.

In our study, we did not observe a significant association between growth rate and cognitive development at 12 months. We would expect that slow and fast growers have negative associations with child cognitive development. On the contrary, children that grew slower had positive values for the cognitive development test at 12 months. A similar positive tendency was observed among faster growing children. Two characteristics seem to have a significant impact on the cognitive development during the first year of life in Oruro: 1) child born with normal weight and 2) child born with a lower weight and then rapidly recuperated their adequate sizes according to age. However, such observations are based on children between the ages of 0 to 12 months. Further studies based on children at ages beyond 12 months would be necessary to determine whether slow or faster growth rates can have a greater impact on cognitive development. Several reasons may explain our results. Those are based on a small sample size therefore reducing our capacity of detecting significant statistical associations. Also, it was difficult to obtain data regarding the intellectual, psychological and occupational status of the mothers (Ruiz-Castell et al., 2012) which is likely to have an influence on child neurodevelopment. On the other hand, we were able to follow the growth of children on a monthly basis which allowed for a good characterization of their physical development during their first year of life.

Being born with a normal weight and size is fundamental for predicting child health, yet not necessarily an indication of a normal development from birth to 12 months of age. Our results suggest that data registered at birth cannot be taken as face value to predict the child's development. This study argues the need to focus on how children develop during their first year of life. It is our contention that there should be further studies examining the effects of growth variations in development at later stages of life (i.e., post 12 months of age). This study describes the growth patterns of a population in highland Bolivia. It argues that weight gain during the first 6 months of life is 1) positively associated with BMI at 12 months and 2) negatively associated with weight at birth. In our study, no association with cognitive development at 12 months and weight gain was observed.

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Table 1. Children growth characteristics and cognitive development

	Minimum	Maximum	Mean ± SD	*n*
Birth characteristics				
Birth weight, g	2200	4200	3282.17 ± 371.76	175
Birth height, cm	42	53	49.43 ± 1.76	169
Infant growth characteristics				
Weight at 6 mo, g	4851.18	10820	7531.07 ± 808.70	175
Weight gain z-score from 0 to 6 mo	-2.98	2.89	0.02 ± 1.04	175
BMI z-score at 12 mo	-2.3	2.09	0.17 ± 0.84	175
Cognitive Development				
MDI	59.75	138.03	99.93 ± 15.22	175

Abbreviations: BMI, body mass index.

Table 2. Characteristics of rapid, medium, and slow growers

Characteristic	Slow Growth (*n*=48)	Medium Growth (*n*=77)	Rapid Growth (*n*=50)	P [1]
Male sex	23 (47.92)	36 (46.75)	23 (46.00)	0.98
Birth characteristics				
Birth weight, g	3493.75 ± 386.93	3263.90 ± 313.96	3107.2 ± 344.45	<0.01*
Birth height, cm	49.98 ± 1.83	49.23 ± 1.70	49.23 ± 1.72	< 0.05*
Low birth weight [2]	1 (2.08)	1 (1.30)	2 (4.00)	0.61
Low birth height [3]	12 (26.09)	35 (47.30)	29 (59.18)	<0.01*
Prenatal risk	11 (22.92)	23 (31.94)	19 (38.78)	0.24
Caesarea section	12 (25.00)	24 (31.17)	17 (34.00)	0.61
Infant growth at 6 and 12 mo				
Weight at 6 mo, kg	7.00 ± -0.70	7.49 ± 0.61	8.11 ± 0.81	< 0.01*
Weight for age z-scores at 6 mo	-0.79 ± 0.80	-0.213 ± 0.69	0.47 ± 0.77	< 0.01*
Weight at 12 mo, kg	8.31 ± 0.77	8.86 ± 0.65	9.59 ± 0.88	< 0.01*
Weight for age z-scores at 12 mo	-1.02 ± 0.78	-0.46 ± 0.70	0.23 ± 0.72	< 0.01*
Length at 6 mo	64.91 ± 2.58	65.33 ± 1.72	66.02 ± 2.34	< 0.05*
Length/height-for-age z-scores at 6 mo	-0.83 ± 1.04	-0.68 ± 0.75	0.23 ± 0.72	< 0.05*
Length at 12 mo	71.65 ± 2.46	72.45 ±1.83	73.64 ± 2.50	< 0.01*
Length/height-for-age z-scores at 12 mo	-1.29 ± 0.93	-0.99 ± 0.82	-0.50 ± 0.95	< 0.01*
BMI for age z-scores at 12 mo	-0.35 ± 0.77	0.16 ± 0.74	0.70 ± 0.73	< 0.01*
Parental characteristics				
Maternal age, y	24.69 ± 4.84	26.67 ± 5.76	25.49 ± 5.71	0.14
Maternal height, cm	1.52 ± 0.05	1.54 ± 0.06	1.54 ± 0.06	0.30
Maternal education				
Primary or less	3 (6.25)	9 (11.69)	3 (6.00)	
Secondary	26 (54.17)	36 (46.75)	23 (46.00)	
Tertiary	19 (39.58)	32 (41.56)	24 (48.00)	0.65
Maternal smoke habit	7 (14.58)	9 (11.69)	5 (10.00)	0.78
Parity				
0	21 (43.75)	28 (36.36)	24 (48.00)	
1	19 (39.58)	32 (41.56)	16 (32.00)	
≥2	8 (16.67)	17 (22.08)	10 (20.00)	0.69

Data are represented as mean ± SD or *n* (%). [1] P-value of difference between groups. * P<0.05. [2] Low birth weight refers to birth weight <2500 g. [3] Low birth height refers to birth height ≤ 49.7 cm. Abbreviations: BMI, body mass index.

Table 3. Association between weight gain and children cognitive development

	COGNITIVE DEVELOPMENT (*n*=175)			
	Crude		Adjusted [1]	
Variables	coef ± SE	95% CI	coef ± SE	95% CI
Growth [2]				
Slow	2.77 ± 2.45	(-2.02-7.57)	1.82 ± 2.41	(-2.91-6.54)
Rapid	-0.04 ± 2.42	(-4.80-4.71)	0.49 ± 2.34	(-4.10-5.08)

Values represent the coefficients (coef) from multivariate linear mixed models and the 95% confidence interval.

[1] Adjusted for child gender (male/female), examiner, Caesarean section (yes/no), number of Bayley test (1/2), parity (1/2/≥3), birth weight, mother age, maternal education level (primary/secondary/tertiary or more).

[2] Growth refers to birth until 6-mo

Figure 1. Weight patterns of rapid, medium, and slow growers. Values are means ± SD

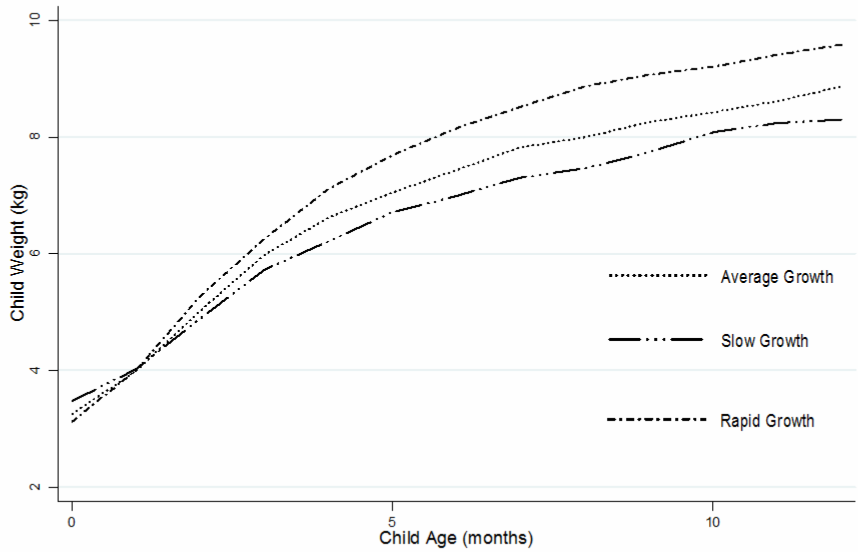


Figure 2. Association between birth weight and children weight gain z-score (from 0 to 6 months). *n*= 175

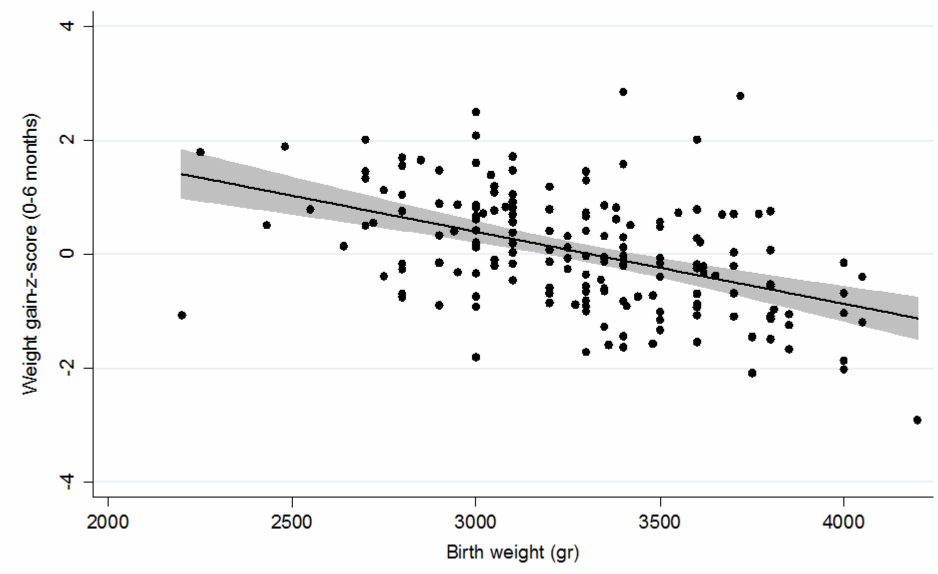
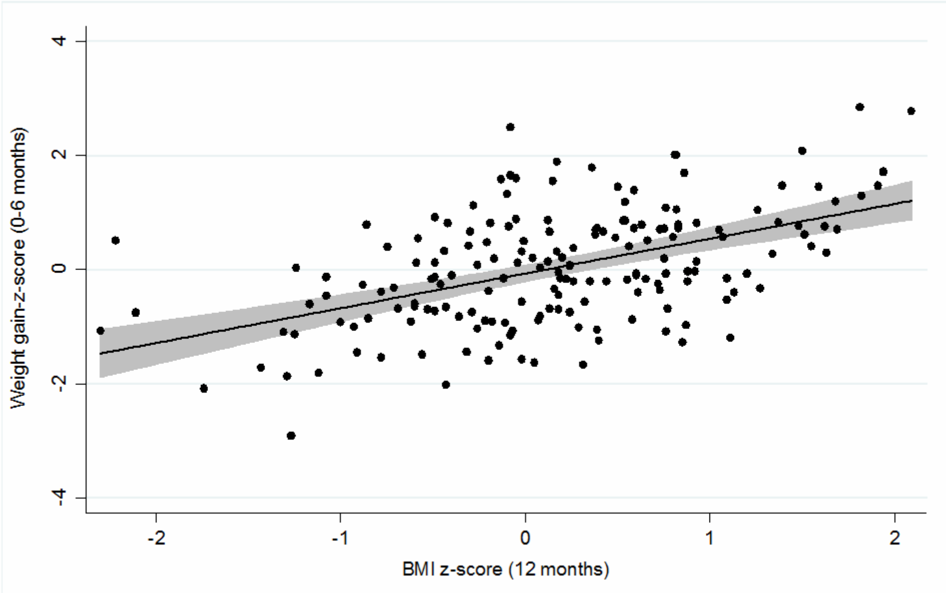


Figure 3. Association between children BMI z-score (12 months) and children weight gain z-score (from 0 to 6 months). *n*=175



6. DISCUSSION

This thesis has examined the neuropsychological development of Bolivian children at 12 months of age. Specifically, it addressed two possible associations: 1) neuropsychological development and metal exposure (i.e., metal toxicity as a consequence of environmental contamination); and 2) neuropsychological development and growth patterns. The thesis also characterised growth patterns during the first 6 months of life.

6.1 Exposure to heavy metals

Blood samples are among of the most effective ways to determine exposure to metals such as lead (Ashton et al., 2009; D'Haese et al., 1996; Diplock, 1993; Ikeda et al., 2011; Levy et al., 2007). In this thesis, blood samples (taken from the pregnant women) were used to measure the level of exposure of the foetus during pregnancy. Samples were taken both during pregnancy and at the moment of birth (taken from the umbilical cord). They provided an indication of the level of each child's exposure.

Based on the results of the present study, essential and non-essential types of metal concentrations were both at non-toxic levels, with the exception of antimony. In general, the values were also fairly homogeneous. We were particularly interested in focusing on lead concentrations in blood and their documented neurotoxic effects, even at low concentrations. From the children examined using the BSID, four of their mothers showed lead levels higher than 10 mg/dl - the level considered as the intervention threshold by many agencies (CDC, 1991). By eliminating these four cases from the study (which revealed extreme values (> 5SD)) we observed that all other registered levels were at non-toxic levels: participants had a median lead level of 1.76 mg/dL (P25-P75 ie 1.34-2.48 mg/dL).

Our initial hypothesis was that metal concentrations in blood would be high. This was based in part on the fact that other studies have registered high metal exposures in the Oruro region based on soil, dust, air, and children's hair samples (Barbieri et al., 2010; Fontúrbel et al., 2011; Goix et al., 2011). Also, because we included in the study women from two distinct hospitals (one located in a highly contaminated area, while the other not) we were expecting a greater variability in the registered levels. However, the registered concentrations were low and homogeneous to the point of being comparable to the results described in other studies based on unexposed populations (Gundacker et al., 2010; Schell et al., 2004).

Nevertheless, contradictory data have also been published elsewhere, especially studies that examined correlations between environmental samples and biomarkers. For instance, Gulson et al. (1994) and Malcoe et al. (2002) demonstrated a correlation between lead levels in blood samples and lead concentrations in dust samples. By contrast, Davis et al. (1993), Danse et al. (1995) and Berglund et al (2000) found no association between lead concentrations in soil and blood samples. Yet, all of the above studies were conducted in similar mining contexts with populations that were directly exposed to environmental contaminants (as was the present study).

The lack of an apparent correlation between the results in Barbieri et al. (2010) and Fonturbel et al. (2011) and the results in the present study (from blood samples) may be explained by factors such as low bioavailability of metals in dust – as also argued by Danse et al. (1995) as well as Gulson et al. (1994). This suggests that just a small fraction of the totality of metals found in dust would actually find itself in the blood stream.

6.2 Child Neuropsychological Development

Our results suggest that neither the exposure to cocktails of metals nor the different growth patterns have a negative effect on the neuropsychological development of the children that participated in our study. In fact, the factors that do appear to have a negative effect were preterm births and births by caesarean. Conversely, gender and parity appeared as factors producing a positive effect on cognitive development.

Important in the present study is the absence of a risk associated with a prenatal exposure to lead. Such observation is in fact contrary to other studies in epidemiology that have demonstrated the negative effects of lead on the neuropsychological development (Hu et al., 2006; Jedrychowski et al., 2009: Shen et al., 1998; Tellez-Rojo et al., 2006). The negative effects of lead have been well documented, and even at low levels of exposure such as those registered in this thesis (see Needleman, 2004; Grandjean and Landrigan, 2006).

In our view, various factors may account for the fact that no negative effects were observed in this study. For instance, the known difficulty to detect neuropsychological problems from children at such young ages (≤12 months). Indeed, neuropsychological problems are most easily detected in children older than 12 months of age (Counter et al., 2000, 1998). As discussed in the introduction of this thesis, behavioural factors (e.g., playing outside and then putting unwashed fingers in mouth) account for the fact that the exposure levels of children may differ from those of the mothers. Accordingly, the results of our neuropsychological evaluation would be associated with postnatal exposure instead of prenatal exposure. Additionally, the Bayley scale may actually provide a too general reading of child's neuropsychological development. The scale can fail to provide specific data on different areas of the neurodevelopment such as language, memory, and motility (Plusquellec et al., 2007). Accordingly, the choosing of more precise methodologies could allow us to further identify specific areas of neurodevelopment that may have been altered. Such methodological steps include the moment during which samples are taken. In this study we analysed blood samples taken from women during pregnancy. Such samples may not have provided entirely accurate readings of the child's level of exposure at the moment the test was performed because they actually measured the prenatal level of exposure instead of the moment during which most of the high exposure occurs (i.e., temporal ‘décalage’).

Other unexpected results in our thesis include the appearance of a positive association between lead levels and the neuropsychological development of children at 12 months of age. However, this association is only observed at very low lead concentrations. The positive association begins to change when including the four extreme values that were excluded from the study (**Figs. 5 - 6**). In our view, the positive association observed at low levels is very likely a false positive – especially in light of the overwhelming literature on the neurotoxic effects of lead. It is our contention that the observed positive effect is probably an indication of the presence of another factor whose effect may be beneficial and have a greater influence on the child’s neuropsychological development. Perhaps a particular component in the child’s diet could be interacting with metals such as lead or even compensating for the neurotoxic effects of lead.

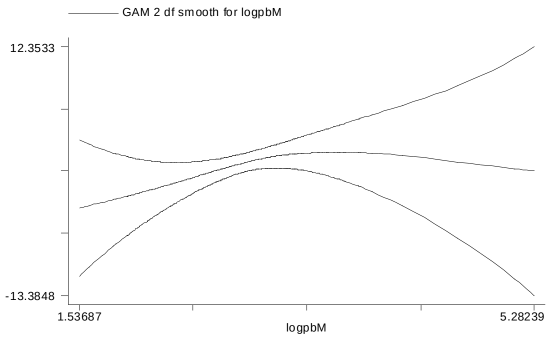


Fig. 5 Cognitive development at 12 months and lead exposure (GAM model)

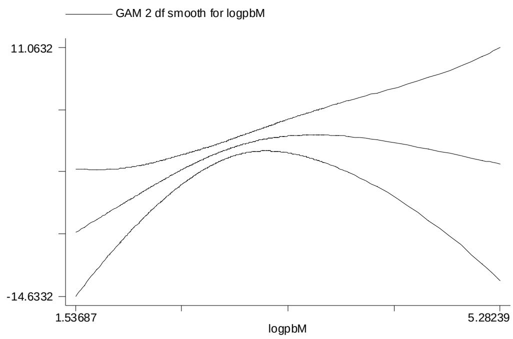


Fig. 6 Psychomotor development at 12 months and lead exposure (GAM model).

As a perspective, it would be interesting to determine whether low metal concentrations have any negative effect on other constituents of health (e.g., respiratory) and at other ages (i.e., >12 months). Very important also is the fact that we observed ~80 pregnant women with high lead concentrations. Unfortunately, we lost these women during the follow-up and thus could not include them in our analyses. Although these women were not showing any particular geographic pattern (they were rather dispersed throughout the city), they did however live in close proximity to smelting industries or mineral storing facilities, and in some cases kept minerals in their homes. These cases emphasise a portion of the population that is in fact at risk of a neurotoxic exposure – even though no particular geographical pattern was identified in association to the high levels.

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We also expected that slow and rapid growth rates would have a negative effect on the child's cognitive development. Our expectation was based on the fact that variations in growth rates have been known to have repercussions on the cognitive development of children at various stages of their life (Li et al., 2008; Halkjaer et al., 2003; Baker-Henningham et al., 2009). In the present study, we have reported different observations which are consistent with others detailed elsewhere, such as an association between rapid growth rates and an increase of the BMI (Holzhauer et al., 2009; Yanovski, 2003; Goodell et al., 2009). Halkjær et al (2003) also observed an inverse association between IQ scores, BMI changes and risks of obesity. Li et al., (2008) observed similar negative effects associated with increases in BMI and child cognitive function in school age children (visuospatial organization and general mental ability). On the other hand, neurodevelopment problems can also be related to a nutritional problem in cases of those with slow growth rate such as poor cognitive development or changes in behaviour (Baker-Henningham 2009, Bergen DC. 2008).

In our study, however, we did not observe an association between growth rates (slow or rapid) and cognitive development at 12 months of age. Perhaps those children with rapid growth patterns may have had a capacity to readily recuperate their normal weight which in turn may have compensated possible problems normally associated with chronic low weight. Regarding those children with high birth weights and slow growth patterns, it may be inferred that weight at birth has likely a greater overall neuropsychological impact than the actual rate of growth. In situations where illnesses during infancy can have mortal repercussions, normal weight according to age in weeks (during the first six months of life) appears to be a determinant factor for a good cognitive development – independently of the rate at which a child develops (growth rate). However, it would be interesting to continue the follow-up in order to see if this association continues after six months of age or, on the contrary, if some of the problems that we were expecting begin to appear.

Although the protective effects of breastfeeding have been well documented elsewhere, we did not include this variable in our final model. As we also discuss in the *Materials and Methods* section of this thesis, monthly information regarding breastfeeding was well documented. We observed that practically all the children were breastfed (were not breastfed: only 2 out of 197 children with Bayley at 12 months; and only 2 out of 181 children with Bayley at 11 months – the same two children in both cases). Since practically all mothers breastfed we did not observe any patterns between children that were breastfed and children that were not. We also considered possible differences associated with breastfeeding duration. Nearly all mothers breastfed until (at least) 12 months of age – data was here again insufficient to highlight any possible difference. Accordingly, this further explains our interest in categorising the breastfeeding variable, both Exclusive (only breastfeeding, without water or other liquids) and/or Predominant (when other liquids were included – but no solids). As an outcome of our recommendations to the mothers, nearly all began feeding their babies with solid foods from 6 moths onward. The nutritional behaviour of the children was therefore somewhat 'regulated' (homogenised).

6.3 Infant Growth Patterns

In the second paper presented in this thesis we discuss three growth patterns during the first six months of life: 1) regular growth; 2) rapid growth; 3) slow growth. Various studies have emphasised the impact of rapid and slow growth patterns on health (Martins et a., 2011; Vorster et al., 2007; Fabricius-Bjerre et al., 2011; Kelishadi , 2007; Yanovski, 2003). The rate at which the child develops is linked with weight at birth and, later, with the BMI. In our study, children with low birth weights appear to grow more rapidly, perhaps as a mode of natural compensation. At one year of age, these children also usually have higher BMI values than the children with regular growth patterns. This however does not necessary mean that all children born smaller (low weight at birth) have had high BMI values throughout their development. Authors observed that higher than average BMI values can have effects on illnesses that occur later in life (teenage or adulthood) such as obesity and/or cardiovascular diseases (Kelishadi , 2007; Yanovski, 2003; Fabricius-Bjerre et al., 2011). On the other hand, lower than average BMI values can also have significant negative effects on health such as poor mental development, physiological, and social alterations, cardiovascular diseases (Martins et al., 2011; Vorster and Kruger, 2007)

Growth patterns identified in our study seem to match those observed elsewhere. The association between weight at birth, growth rate, and BMI has been observed both in the few weeks following birth and after 12 months of age. Thus, the factors that have influenced growth rate remain unclear. Some studies have observed an association between an exposure to endocrine-disrupting chemicals and an increase in growth rate (Mendez et al., 2011). Our recommendation as a venue for future research would be to measure other contaminants in order to address this potential effect.

6.4 Limitations

The main limitation of the present study is the attrition of the cohort. The ToxBol project began with ~400 participating pregnant women. However, various problems throughout the follow-up considerably reduced the number of participants. Participation was difficult to obtain for the following reasons: 1) women's mistrust of health care professionals; 2) lack of an established practice of regular health check-ups; 3) difficulty to fully understand the health issues related to environmental contamination; 4) pressure from family members (husbands and in-laws, especially) who discourage the women to attend local hospitals for no apparent medical reason; 5) traditional medicine practices and beliefs contradicting modern medicine and its practices (e.g., traditionally, women secure their new born babies in tight textile wraps (fajas); such practice is usually not recommended by medical doctors practicing modern medicine); 6) families migrating to other regions of the country; 7) difficulty to locate the participating women during the trial (lack of phone numbers, no official street names or addresses, etc.).

Another limitation was the difficulty to obtain information (variables) that was directly associated with our study objectives. Accurate details regarding the socioeconomic status were amongst the list of essential missing variables. Most family members undertake multiple jobs contracts at the same time. Their status is therefore difficult to assess and categorise. The intellectual status of the mothers would have also facilitated further understanding of our cohort. Unfortunately, the lack of a permanent psychologist on staff did not allow for an accurate gathering of such data. Finally, missing from our anticipated data are indications of weight gain during pregnancy – because of the difficulty to convince the women to return to the hospital for regular monthly follow-ups before birth.

Limitations can also be listed from the data that was collected (only in some cases) such as the lack of accurate indications regarding alcohol consumption, drugs, sexual habits (e.g., birth control), sexually transmitted diseases, prior abortions, and so forth. With regards to the blood samples, specifically, it would have been useful (provided sufficient resources) to collect samples during each trimester of the pregnancy (given that concentrations can change according to the trimester). Equally useful, although arguably and understandably unfeasible, would have been blood samples taken directly from the children at one year of age instead of the mothers.

Finally, and with regards to the actual test of neuropsychological development, the ToxBol project used the first version of the Bayley Test for Child Development. More recent editions of the test have been published where the materials have been revised. The revised material might have been useful in detecting more subtleties in our data sample.

6.5 Highlights

This is the largest study to date on the effects of metals on neurodevelopment in a mining area of Bolivia. The complexity of pursuing epidemiological studies in Oruro resides in the fact that local populations are exposed to various sets of metals in the environment (polymetallic cocktail). This study contributes in providing a contextual and systematic analysis of all of the metals. The toxic effects of certain metals can be (and often are) amplified when in combination to other metals. Our study allows for an inclusive understanding of the complexity of the environmental contamination in Oruro by dealing with each metal both individually and in association with other metals. It provides a comprehensive analysis and identification of the potential toxic effects of metals in the Oruro environment, both individually and as combinations.

A variety of samples (blood, umbilical cord, hair) were taken at various stages to assess the level of exposure (metals) of women. Additional blood samples were taken from a small sample of participating women in order to verify (and compare) metal levels. The correlation of samples taken at different moments in time has contributed in further substantiating the results.

The children's physical and neuropsychological development follow-up was conducted by hospital staff members that received specialised training prior to initiating the study (provided by other ToxBol members). Staff members that conducted both the interviews and the follow-up of the children were the same throughout the project. Such practice contributed in establishing a better rapport and confidence between staff members and families which, in turn, also allowed us to gather, in time, more reliable information from the interviews. Although the attrition of the cohort was significant, a systematic and fully documented monthly follow-up was performed on the participating children – documenting growth patterns, illnesses, neuropsychological development, and nutritional patterns.

The data collected during the course of this project is a significant contribution toward a better understanding of the environmental situation in Bolivia and its possible effects on health, particularly in mining contexts. The thesis therefore contributes in further bridging matters of health and environmental contamination in developing countries. Likewise, it allows us to engage child neurodevelopment more generally.

6.6 Contributions to the field and venues for future research

Further examinations of the bioavailability of metals found in similar environmental contexts may clarify why highly exposed adult populations appear unaffected. It would be useful to obtain blood samples from school age children to determine whether the exposure pattern persists or if, on the contrary, concentrations are much higher. For instance, it would be interesting to repeat the ToxBol project on populations with similar characteristics such as in Potosí. The mining city of Potosí is also situated in the Bolivian highlands, roughly 300 km southeast of Oruro. Life near the Potosí mines is similar that of Oruro (high environmental contamination, poverty, malnutrition, high infant mortality rates, and so on). Replicating the project would allow us to see whether or not the exposure patterns remains.

More specifically, it would also be useful to further examine the concentration levels of certain metals in urine samples (e.g., arsenic) in order to obtain more accurate data on the level of exposure (given than blood does not necessarily allow for a full analysis of all trace elements).

In our study we did not observe a toxic effect of lead at low concentrations (lower than 10 μg/ dL), which is contrary to most studies dealing with lead toxicity. In fact, we observed a positive effect that is surely an indication of another factor perhaps having a beneficial effect and a greater influence on the child's neuropsychological development. Our general contention is that a component in the child's diet may either be interacting with metals such as lead or compensating for the neurotoxic effects of lead.

Also useful would be to further examine if neuropsychological effects begin to appear at later stages of development. Many of the known negative effects that were not observed in our study may actually be detectable (or more easily detectable) after a certain age (i.e., >12 months). Provided the availability of sufficient resources, it would have been interesting to continue the follow-up throughout later stages of development in order to 1) assess the development during infancy (usually a difficult period to detect possible psychomotor or physical problems) and 2) take additional blood samples in order to see if the levels of exposure change when the children reach the age of walking – and therefore susceptible of being more exposed to environmental contaminants. Blood samples taken from children may have allowed a better assessment of the exposure, particularly when the exposure level tends to increase (i.e., when children begin to crawl they become necessarily more susceptible of being exposed).

Regarding infant growth patterns, important is the fact that a strong association was observed early on between the growth rate of the first six months of life and the BMI at 12 months of age. Further research would be necessary to determine which elements (beyond metals) are associated with growth rate (e.g., endocrine disruptor effect). Equally interesting would be to examine possible associations between growth patterns and chronic illnesses that usually occur later in life such as obesity, cardiovascular diseases, and so forth.

In this study, the effects of lead were contrary to expectancy. Nevertheless, based on the important environmental contamination in Oruro, measures should be taken to reduce exposure, given the known presence of other health issues such as respiratory diseases and immune responses. Also important to consider are the negative effects that may be detected during adulthood.

6.7 Public Health in Oruro: some recommendations

The results of this thesis indicate that an important portion of the adult population of Oruro has not been affected by metal exposure. However, ~2% of the recruited pregnant women were found to have lead concentrations higher that 10 µg/dL. In this city of 220000 inhabitants, this represents a significant number of children born each year with an inacceptable lead exposure. In addition to the above, environmental contamination remains an issue of central importance while there remain critical areas such as that of Vinto still registering high concentration of metals in air, water, and dust.

We observed that 1) mining wastes are located near areas where children play and 2) that there are no systematic measures in place to control the safe disposal of toxic wastes. There are also no awareness programmes in place to protect the environment schemes (**Fig. 7**). Additionally, the actual level of exposure of children remains poorly documented mostly because blood samples have yet to be taken from school age children. Another issue is that many children live in households in the presence of parents that are much more exposed (and that bring into the home their contaminated work clothes and/or materials from work, and so forth).

From the ToxBol project and its subprojects can be drawn a series of recommendations and protective measures limiting the access to places of industrial activity that present higher exposure risks to the most vulnerable portion of the Oruro population: the children. Information and awareness campaigns are also recommended on the appropriate use and disposal of the industrial gear used by many families. Notable improvements have nevertheless been made in the recent years and some forms of measures are now in place (or beginning to): better disposal measures at the San José mine and better control of the water acidity in the Itos areas.

On the topic of child growth, delayed growth patterns remain of central importance with considerable repercussions on infant health. Obesity is a problem in the developed world that is now also beginning to emerge in developing countries such as Bolivia. We recommend the implementation of better monitoring measures in order to detect early on these types of nutritional problems. Amongst such measures are also good awareness campaigns on family food education, especially in hospitals, schools, cultural centres, and so on.

Fig. 7 Children playing near mining wastes ©ToxBol Team.

7. CONCLUSIONS

1. The situation in Bolivia is complex given the coincident occurrence of environmental, cultural, social, political, and sanitary factors.
2. Various sources of contamination exist in Oruro. The main source of contamination is produced by the mining industry to which local populations are directly exposed:
    a. However, in this study the measured biological levels of metals in pregnant women were lower than expected in a mining area. This is contrary to our initial hypothesis anticipating high concentrations of metals in blood.
    b. Only high concentrations of antimony were observed in blood.
3. No neuropsychological development problems/deficiencies were observed from children at 11 and 12 months of age
    a. Although the risk of neurotoxicity associated to certain metals such as lead has been well documented, the results in the present thesis do not support the hypothesis of the effects of lead on development. No toxic effect of metals on BSID scores was found – even at low concentrations (i.e., below established limits of toxicity).
    b. Contrary to expectancy, our results revealed a positive effect of leadon child neuropsychological development at low levels of exposure. We contend

that this ‘positive’ association may be reflective or due to other factors such as diet.

   c. With regards to antimony, the only metal found in high concentrations, no negative effects on health were observed.
   d. Cognitive development also appears not affected by growth patterns.

4. Growth rate is associated with weights at birth and the BMI at 12 months of age. In this Bolivian cohort, children born smaller were more likely to grow/develop faster and attain greater weight and size. Such observations may allow anticipating possible health problems during adulthood (thus emphasising the importance of further follow-up of this birth cohort).
5. This is the largest study to date on the effects of metals on neuropsychological development in a mining area of Bolivia.

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