BioMetals **17:** 389–397, 2004.

Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain

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Received 12 May 2003; Accepted 6 November 2003; Published online: April 2004

Key words: toxic and trace metals, metal interactions, cattle, ICP-AES

Abstract

Since the toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic or essential metals, studies addressing the chemical interactions between trace elements are increasingly important. In this study correlations between the main toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements were evaluated in the tissues (liver, kidney and muscle) of 120 cattle from NW Spain, using Spearman rank correlation analysis based on analytical data obtained by ICP-AES. Although accumulation of toxic elements in cattle in this study is very low and trace essential metals are generally within the adequate ranges, there were significant associations between toxic and essential metals. Cd was positively correlated with most of the essential metals in the kidney, and with Ca, Co and Zn in the liver. Pb was significantly correlated with Co and Cu in the liver. A large number of significant associations between essential metals were found in the different tissues, these correlations being very strong between Ca, Cu, Fe, Mn, Mo and Zn in the kidney. Co was moderately correlated with most of the essential metals in the liver. In general, interactions between trace elements in this study were similar to those found in polluted areas or in experimental studies in animals receiving diets containing high levels of toxic metals or inadequate levels of nutritional essential elements. These interactions probably indicate that mineral balance in the body is regulated by important homeostatic mechanisms in which toxic elements compete with the essential metals, even at low levels of metal exposure. The knowledge of these correlations may be essential to understand the kinetic interactions of metals and their implications in the trace metal metabolism.

Introduction

Human activities have been contaminating the environment with toxic metal and metalloid compounds. The toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic and essential metals, although relatively high concentrations can also occur naturally (Goyer 1995; WHO 1996). This is not surprising in that some toxic and nutritionally essential metals share common chemical properties (Hill & Matrone 1970). Essential trace metals may modify health risks from exposure to non-essential toxic metals. A well known example of this is that pre-treatment with Zn or the simultaneous administration of Zn with Cd reduce Cd toxicity (Webb 1979). On the other hand, the ingestion of a diet that is deficient in a particular essential element can enhance the accumulation and toxicity of some toxic metals.

Ca, Fe and Zn deficiency, for instance, enhance susceptibility to Cd and Pb toxicity (WHO 1996; Goyer 1997). Finally, it has been demonstrated that toxic metals can disrupt trace element metabolism. Cd toxicity affects Ca metabolism either by direct toxicity to bone or indirectly from renal toxicity (Webb 1979; Goyer 1997). With little exceptions, the molecular mechanisms of most of these metal interactions are not understood. The necessity to elucidate these chemical interactions is well recognised (Rahil-Khazen *et al.* 2002); this is because increasing evidence supports an important role for metals in neurobiology and many other diseases (WHO 1996; Patriarca *et al.* 1998).

Chemical interactions between toxic and essential trace elements have been investigated in animals exposed to relatively high levels of contaminants (Koh & Judson 1986; Spierenburg *et al.* 1988) or in experimental studies (Reddy *et al.* 1987; Groten *et al.* 1991). However, in spite of it is well documented that interactions between metals can occurs at levels of exposure well below those at which toxicity may be detected (Goyer 1997; López Alonso *et al.* 2002a), there is little information on the interactions between metals at the low level of metal exposure that usually occurs in the nature; when it exits, is generally limited to a few number of trace elements, mainly copper, zinc and iron. Knowledge about the quantitative relationships between trace elements in the body at low levels of exposure could be essential to increase our understanding of the pathological mechanisms of metal exposure and to evaluate the importance of the essential metal status in the toxic metal accumulation in a specific area or population group.

Due to the importance of liver and kidneys in the metabolism of trace elements and the vulnerability of these organs to metal accumulation and toxicity, the metabolism and the interactions between metals should be necessarily studied in these tissues (Rahil-Khazen *et al.* 2002). Blood, and other non lethal samples as urine, can at the most give a rough indication of the total amount of a trace metal in the body but tell nothing about the distribution and interaction of the metals in the different tissues. The difficulties of metal interaction studies on humans and most animal species are to get a significant and comparable number of samples. Animals in livestock production could be good candidates to this type of studies because a sufficient number of well documented samples can be collected at slaughterhouses. Domestic animals reared on grass or locally grown fodder have also proved to be good biomonitor species for metal contamination (López Alonso *et al.* 2002b). In addition, domestic animals have the advantage that they are an importance source of food for humans and so provide a direct measure of pollutant transfer to humans.

In a previous study we have evaluated the concentrations and interactions between some toxic and essential metals in cattle in the rural region of Galicia (NW Spain) (López Alonso *et al.* 2000, 2002a). In spite of the low level of pollution in NW Spain there were significant interactions between toxic and essential metals. That study was however limited to the nutritional essential metals Cu and Zn. The aim of the present study is to evaluate the concentrations and interactions between the toxic (As, Cd, Pb, Hg) and the main trace essential elements (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn) in cattle in NW Spain.

Material and methods

Tissue samples

Samples were collected from cows raised in the Leon Province (latitude 42–43° N, longitude 4° 50′–6° 35′ W), NW Spain. Cattle production in the most important form of agriculture in this region and comprises approximately 25% of the bovine production for the whole country (MAPA 1996). The cattle are predominantly fed on locally grown fodder and are the primary livestock species exposed to metal contamination in this region.

From October 2000 to July 2001, samples from 120 animals were collected at the slaughterhouses of the Leon Province. Information on the age, precise origin, and husbandry history of the animals was obtained from the farm documentation. Samples were only taken from healthy animals. The age of the animals ranged from 3 to 16 years.

Samples of at least 200 g were taken from the *lobus caudatus* of the liver, the cranial half of the left kidney (including both cortex and medulla) and diaphragm. The liver and kidneys were examined because their importance in the metabolism and accumulation of metals; muscle was also analysed because its relevance as human foodstuffs. All samples were packed in plastic bags and immediately transported to the laboratory and stored at −18 °C until analysed.

Chemical analysis

For the liver and the muscle, approximately 2 g subsamples were excised from semi-thawed tissues and

Table 1. Detection limits (μg/l) and results of analysis of the certified reference material (Pig Kidney CRM 186) expressed as mg/kg

Element	Detection limit	Certified Reference Material (Pig Kidney CRM 186)*	
		certified levels (mean±95%CI)	Analysed levels (mean±95%CI)
As	17	0.063 ± 0.009	< ld
Ca	0.6	(295)	271 ± 14
Cd	1.4	2.71 ± 0.15	2.47 ± 0.10
Co	2.1	–	0.287 ± 0.032
Cr	3.0	(58–142)	198 ± 0.043
Cu	5.3	31.9 ± 0.4	29.6 ± 0.3
Fe	4.6	299 ± 10	284 ± 10
Hg	9.1	1.97 ± 0.04	1.76 ± 0.05
Mn	1.4	8.5 ± 0.3	7.94 ± 0.29
Mo	3.1	–	3.54 ± 0.10
Ni	7.2	(0.420)	0.478 ± 0.051
Pb	1.78	0.306 ± 0.011	0.329 ± 0.053
Se	16	10.3 ± 0.5	11.0 ± 0.5
Zn	1.8	128 ± 3	124 ± 3

*In parantheses indicative values; ld: limit of detection.

digested in 5 ml of concentrated nitric acid (Suprapur grade) and 2 ml 30% w/v hydrogen peroxide in a microwave digestion system (Milestone, Ethos Plus). Because of the different metal content in the cortex and medulla, the kidney samples were homogenised previously to digestion to ensure the subsample analysed was representative of the whole organ. Digested samples were transferred to polypropylene sample tubes and diluted to 25 ml with ultrapure water. Metal concentrations in the digest were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer Optima 4300 DV).

An analytical quality control programme was employed during the study. Blank absorbance values were monitored throughout the survey and were subtracted from the readings before the results were calculated. The limit of detection in the acid digest was set at three times the standard deviation of the reagent blanks (Table 1). The limits of quantification, expressed as a concentration in the tissue, were calculated on the basis of the mean sample weight and volume analysed.

Analytical recoveries were determined from a certified reference material (Pig kidney CRM 186, BCR Reference Materials) analysed together with the samples. The results are given in Table 1 and show acceptable agreement between the found and certified values for Cd, Cu, Fe, Hg, Mn, Pb, Se and Zn. Indicative values only were given for Ca, Cr and Ni; there was, however, good agreement between our results and those indicated for the CRM. No information was given regarding Co and Mo in the CRM and analytical recoveries were determined using spiked samples at a level that gave absorbance values that were generally 2–10 times greater than the normal levels in the various tissues ($n = 10$), recoveries being between 91 and 97% respectively. To evaluate the precision of the analytical method and the overall method, 12 absorbance readings from the same digest and single absorbance readings from 12 digest of the same sample respectively were recorded. The relative standard deviation (RSD) of these values were 0.63–7.91% for the analytical method and 3.02–11.3% for the overall procedure.

Data analysis

All statistical analyses were run using the SPSS for Windows (v. 10.0) program. To calculate the mean metal concentrations in the different tissues nondetectable concentrations were assigned a value of half the quantification limit. For the toxic metals a high proportion of samples had nondetected values (especially in the muscle) and datasets were not all normally distributed, even after log-transformation. Therefore, the significance of correlations between the levels of the trace and toxic elements within the same and between different tissues were calculated using Spearman rank correlation analysis (R_s) and the average values for the data given as geometric means. Correlations between pairs of metals were highly influenced by the samples that had undetectable metal residues and were excluded from the analysis. Only results with a significance of $p \leq 0.01$ are reported.

Results

Trace metal concentrations in the liver, kidney and muscle of cattle in this study are presented in Table 2. In relation to the toxic elements, all samples were below the limit of quantification for As and Hg. Cd accumulated mainly in the kidney and in a lesser extend in the liver, whereas in the muscle Cd residues were very low, most of samples (78.7%) having Cd residues below the quantification limit. For Pb, the highest residues were found in the liver and slightly lower Pb concentrations were found in the kidney; in

Table 2. Concentrations of the toxic and essential trace elements in the liver, kidney and muscle in cattle in NW Spain. All samples had As, Ni and Hg concentrations below the limit of quantification. (ND: non-detected. To calculate the mean metal concentrations ND values were assigned a value of half of the quantification limit)

	Cd (μg/kg)	Pb (μg/kg)	Ca (mg/kg)	Co (μg/kg)	Cr (μg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Mo (mg/kg)	Se (mg/kg)	Zn (mg/kg)
Liver											
N (<ld)	121	121 (57)	120	120	120	120	120	120	120	120 (57)	120
Geometric mean	59.6	28.0	47.3	95.0	243	40.2	70.3	2.37	1.07	0.219	49.4
Median	54.7	24.8	46.1	95.4	189	43.9	66.2	2.38	1.10	0.229	44.5
Minimum	13.0	ND	29.9	43.7	52.6	2.35	37.0	1.27	0.260	ND	31.4
Maximum	564	320	436.7	153.6	4641	287	223	3.92	1.456	1.221	148
Kidney											
N (<ld)	117	117 (67)	117	117	117(49)	117	117	117	117	117 (5)	117
Geometric mean	318	20.2	76.9	38.9	54.0	3.05	51.3	0.694	0.322	1.028	15.1
Median	320	ND	77.3	38.7	44.3	3.19	55.0	0.725	0.357	1.243	15.9
Minimum	29.8	ND	30.3	25.1	ND	1.84	18.9	0.308	0.073	ND	8.00
Maximum	3393	462	216.7	59.4	1583	4.83	112	1.19	0.655	2.266	31.3
Muscle											
N (<ld)	122 (96)	122 (92)	122	122 (90)	122(5)	122	122	122	122	122 (122)	122
Geometric mean	8.51	14.5	47.2	15.5	75.9	1.66	38.7	0.191	0.0870	ND	50.4
Median	ND	ND	44.9	ND	54.8	1.63	38.9	0.194	0.0854	ND	50.4
Minimum	ND	ND	26.5	ND	ND	1.14	30.2	0.122	0.0513	ND	38.7
Maximum	189	594	129.8	73.1	5657	13.8	60.8	0.312	0.1669	ND	62.5

ND: non detected.

both tissues Pb residues were very low and around half of the samples had undetectable Pb levels, this percentage being higher (75 %) in the muscle.

For most of the essential trace elements the liver was the organ that shown the highest concentrations, followed by the kidney, whereas in the muscle metal concentrations were generally low (Table 2). The kidney was the tissue that showed the highest concentrations of Ca, nearly two times than those in the liver and muscle, and Se, this last element showing concentrations below the limit of quantification in all the muscles analysed. The muscle was the most important tissue for Zn accumulation and had Zn concentrations that were similar to those in the liver. All samples analysed had undetectable Ni concentrations.

Within tissue correlations

Table 3 presents the significant correlation coefficients between pairs of trace elements in the tissues analysed. The p-values varied between 0.01 and 0.0005 with 65% of the p-values <0.0005.

When analysing the correlations between toxic and essential metals it was observed than in the kidney Cd was positively correlated with most of the essential metals, the most significant being between Cd-Ca, Cd-Mn and Cd-Zn. Cd was also correlated with Ca, Co and Zn in the liver but not in the muscle. For Pb only significant correlations with the essential metals Co and Cu were found in the liver.

Most of the significant associations between essential metals were found in the kidney (Table 3), these correlations being very strong between Cu, Fe, Mn, Mo and Zn ($R_{117} > 0.642$ and $p < 0.0005$ in all the cases). Calcium was also highly correlated with all these metals ($R_{117} > 0.469$ and $p < 0.0005$ in all the cases) and in a lesser extend with Se. In the liver, there was a number of moderately significant association between essential metals, especially between Co and most of the other essential metals; in this tissue, Cu was positively associated with Se and Cr with Fe. In the muscle there was a number of positively significant correlations between Ca, Cu, Fe, Mn, Mo and Zn although the significance of these associations was quite low in most cases.

Between tissue correlations

Significant correlations between pairs of metals in the different tissues are presented in Table 4. Most of these

Table 3. Spearman rank correlations between concentrations of different toxic and trace elements in cattle in NW Spain. Observations below the limit of quantification were excluded from the analysis and the number of samples (n) is given in brackets. Unless otherwise stated significance level is $p < 0.0005$. The dotted lines separate correlations between toxic-essential and essential-essential elements, respectively

Kidney		Liver		Muscle	
Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr
Cd-Ca (117)	0.501	Cd-Ca (120)	0.256[b]		
		Cd-Co (120)	0.227[a]		
Cd-Cu (117)	0.349				
Cd-Fe (117)	0.243[a]				
Cd-Mn (117)	0.515				
Cd-Mo (117)	0.300[c]				
Cd-Zn (117)	0.449	Cd-Zn (120)	0.391		
		Pb-Co (64)	0.360[b]		
		Pb-Cu (64)	0.679		
Ca-Cu (117)	0.533				
Ca-Fe (117)	0.480			Ca-Fe (122)	0.240[a]
Ca-Mn (117)	0.575				
Ca-Mo (117)	0.469			Ca-Mo (122)	0.314
Ca-Se (117)	0.248[a]				
Ca-Zn (117)	0.547	Ca-Zn (120)	0.410		
		Co-Cu (120)	0.274[b]		
		Co-Fe (120)	0.343		
		Co-Mn (120)	0.416		
Co-Mo (117)	0.263[b]	Co-Mo (120)	0.462		
Co-Zn (117)	0.247[a]	Co-Zn (120)	0.269[b]		
Cu-Fe (117)	0.653			Cu-Fe (122)	0.295[c]
Cu-Mn (117)	0.789			Cu-Mn (122)	0.576
Cu-Mo (117)	0.774				
		Cu-Se (63)	0.381[b]		
		Cr-Fe (120)	0.370		
Cu-Zn (117)	0.814				
Fe-Mn (117)	0.642			Fe-Mn (122)	0.352
Fe-Mo (117)	0.666			Fe-Mo (122)	0.217
Fe-Zn (117)	0.674	Fe-Zn (120)	0.272[b]		
Mn-Mo (117)	0.668	Mn-Mo (120)	0.310[c]		
Mn-Zn (117)	0.692				
Mo-Zn (117)	0.781			Mo-Zn (122)	0.224[a]

[a]$p \leq 0.01$, [b]$p \leq 0.005$, [c]$p \leq 0.001$.

correlations were found between the liver and kidney, the most important organs in the metal accumulation in cattle in this study. There were significant associations between hepatic and renal concentrations of Cd, Co and Se. All the mixed correlations between toxic and essential metals were also found in the liver versus kidney. Mixed correlations between essential metals were found in the liver versus kidney but also in the liver versus muscle; most of these associations (7 out of 9) being negative.

Within age correlations

Toxic metal accumulation and essential trace metal status in animal tissues can be significantly affected by age. When interactions between metals that significantly vary with age are evaluated in animals of different ages, such correlations are likely to be spurious.

Table 4. Spearman rank correlations between concentrations of different toxic and trace elements in cattle in NW Spain in the different tissues analysed. Observations below the limit of quantification were excluded from the analysis and the number of samples (n) is given in brackets. Unless otherwise stated significance level is $p < 0.0005$. The dotted lines separate correlations between metals in different tissues, mixed correlations between toxic and essential and mixed correlations between essential elements, respectively.

Liver-Kidney		Liver-Muscle		Kidney-Muscle	
Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr
Cd-Cd (117)	0.627				
Co-Co (117)	0.362				
Se-Se (63)	0.366				
Ca-Cd (117)	0.245[a]				
Cd-Co (117)	0.298[c]				
Mo-Cd (117)	−0.326				
Se-Cd (63)	−0.261[b]				
Cd-Zn (117)	0.240[a]				
Pb-Co (64)	0.313[a]				
Co-Ca (117)	−0.242[a]				
Mo-Ca (117)	−0.265[b]				
		Co-Mn (117)	−0.221[a]	Co-Zn (117)	0.255[b]
Cu-Se (112)	0.346	Cu-Mn (117)	−0.301[c]		
Fe-Mn (117)	−0.239[a]	Fe-Cu (117)	−0.242[a]		
Mo-Zn (117)	−0.232[a]				

[a]$p \leq 0.01$, [b]$p \leq 0.005$, [c]$p \leq 0.001$

For this reason, to determine the possible influence of age on the metal interaction between metals, variations in the metal concentrations in cattle tissues with age were investigated by correlation analysis.

As expected, Cd significantly accumulated with age in the kidney in cattle in this study ($R_{117} = 0.343$, $p < 0.0005$), however no relationship between age and concentrations of any of the other toxic and essential trace metals analysed in this study were observed in the liver, kidney or muscle.

Discussion

The levels of the toxic metals measured in cattle from NW Spain in this study were very low. As and Hg residues were undetectable in all the samples analysed. Cd and specially Pb residues were also low and similar to those found in tissues of cattle from relatively unpolluted rural areas in other countries (López Alonso *et al.* 2000). None sample analysed achieved toxic metal residues associated with toxicity in animals (see Puls 1994).

In relation to the essential elements, levels of Ca, Co, Cr, Cu, Fe, Mo, Se and Zn measured in cattle in this study were within the adequate ranges for cattle tissues (Puls 1994) and in general agree well with those reported in other studies (Ellen *et al.* 1989; Jorhem *et al.* 1989; Falandysz 1993; López Alonso *et al.* 2000). Mn concentrations in this study were generally below the adequate range in cattle tissues (liver: 2.5-6, kidney: 1.2–2 mg/kg fresh weight; Puls 1994); most of the liver samples were within the marginal range (1.5–3 mg/kg) whereas 81% of kidneys were below this range (0.96–1.20 mg/kg). When compared with other studies in the literature, cattle from NW Spain contained less of Mn in their kidneys and in a lesser extend in their livers, but for muscle the concentrations were comparable (Ellen *et al.* 1989; Jorhem *et al.* 1989; Falandysz 1993). Ni concentrations were undetectable in all the samples analysed in cattle in this study. These results are in agreement with Ellen *et al.* (1989) and Jorhem *et al.* (1989) who indicate that Ni levels are generally below the quantification limits and suggest that some published data for Ni in biological materials might be erroneously high due to contamination or analytical errors.

Despite the generally low level of pollution in NW Spain and the adequate range of essential metals, there

were significant correlations between toxic and essential trace metals in cattle in this study. As indicated by Rahil-Khazen *et al.* (2002) in a recent study on metal interactions in human tissues, from the present descriptive correlation data it cannot be concluded about kinetic interactions between metals. Correlations are not proof of causation and significantly associations between metals in this study do not necessarily indicate that toxic metal accumulation in cattle tissues can affect essential element homeostasis or even that the level of essential metals can determine the amount of toxic metals accumulation within the body. Besides, the interactive mechanisms at the molecular level may be complicated and more than two metals can be involved. However, solid knowledge about the distribution of trace elements and their mutual correlations in animals exposed to low environmental pollution are consider to be of fundamental importance in our effort to understand more of the complex interrelationships in trace elements metabolism.

The highest number of significant correlations between toxic and essential trace metals, and also between pairs of essential elements, in cattle in this study were found in the kidney and in a lesser extend in the liver. This may reflect a main role of both organs, especially the kidney, in trace element metabolism (Taylor 1996; Rahil-Khazen *et al.* 2002).

Cd was positively associated with most of the essential metals analysed in the kidney, the organ that accumulates the highest concentrations of Cd following low level chronic exposure (García Fernández *et al.* 1996), and to a lesser extend in the liver. Interactions between Cd, Zn and Cu have been widely reported in mammals from contaminated areas and in experimental studies (Webb 1979; Nicholson *et al.* 1984; Spierenburg *et al.* 1988) and are a consequence of the shared ability of these metals to induce metallothionein (MT) synthesis and compete for the cation-binding thiol sides of MT (Webb 1979). Interactions between Cd, Cu and Zn have been also described at low level of Cd exposure both in humans (Rahil-Khazen *et al.* 2002) or animals (Szefer *et al.* 1994; López Alonso *et al.* 2002a) that could suggest that Cd-mediated effects on trace element metabolism occur even at low levels of environmental exposure.

Cd was also positively correlated with Ca both in the liver and kidney in this study. Cd toxicity in the body is largely the consequence of the interactions with Ca, particularly in the bone (Cd interferes with calcification, decalcification and bone remodelling) and in the kidney (where Cd interferes with Ca and vitamin D metabolism) (WHO 1992; Goyer 1997). Ca concentrations in the tissues are not frequently measured in metal interaction studies so we do not have references to compare if at low levels of exposure, Cd can affect Ca metabolism. However, interactions between Cd and Ca in this study are consistent with Cd effects at high levels of exposure, suggesting that Cd-Ca metabolic interactions could happen at low levels of Cd exposure.

Cd also interacts with Fe, Mn and Mo metabolism. It has been shown both in experimental animals and in humans that Cd absorption from the intestinal tract is inversely related to blood ferritin levels (Goyer 1997). Moreover, the addition of Fe was effective in preventing signs of Cd toxicity in rats and pigs (Pond & Walker 1973; Groten *et al.* 1991). A Cd-Mn association has been described in sheep exposed to high dietary Cd (Doyle & Pfander 1975) and in humans exposed to low environmental Cd (Rahil-Khazen *et al.* 2002). Increased dietary levels of Mo reduce Cd accumulation in sheep (Smith & White 1997). The mechanisms behind Cd, Fe, Mn and Mo interactions in the tissues are still not understood. Recent investigations suggest that on the basis of these relationships could be common transporters that control the metal uptake and trafficking (Garrick *et al.* 2003). One of the best studied is the transporter DMT1 (divalent metal transporter 1) that clearly plays a major part in Fe and Cu metabolism, although emerging evidence suggest that it has a lesser role in other divalent metals transport, as Mn, Cd, Ni, Co or Pb (Gunshin *et al.* 1997; Garrick *et al.* 2003). DMT1 levels are very high in the kidney, that could indicate a relevant role in the homeostasis of some essential metals (Garrick *et al.* 2003). The existence of DMT1 and other transporters could explain not only the interactions of Cd with some essential elements in this study, but also the strong correlations between some essential metals (Cu, Zn, Fe, Mn and Mo) in the kidney. Significant antagonisms between most of these nutritional essential metals have been largely demonstrated in experimental studies in animals exposed both at high or deficient mineral intakes (NRC 1980; Mertz 1986; Puls 1994; WHO 1996). Strong correlations between essential metals in the kidney have been also described in human tissues with a correct mineral status (Tanaka *et al.* 1987; Rahil-Khazen *et al.* 2002). The molecular basis for these relationships are far from completely known, and when evaluating them, it must be considered that they can be very complex, involving more than two metals.

Cd and Co are positively associated in the liver in this study. Besides, Co is positively correlated with Pb and with most of the essential elements. Associations between Co and other trace elements in the liver have been shown in previous studies (Rahil-Khazen *et al.* 2002) and an antagonism between Co, Mn and Zn have been also suggested (NRC 1980; Mertz 1986; WHO 1995; Puls 1994). However, as far we are aware, the mechanisms of these relationships are not known.

Interactions between Pb and other toxic and essential metals have been described in the literature. The best defined are those between Pb and the essential metals Ca and Fe (NRC 1980; Mertz 1986; Goyer 1997); deficient levels of both essential metals in the diet considerably increase Pb absorption, which results in a higher Pb body burden. On the contrary, increased Ca and Fe reduce Pb toxicity. However, Pb-Ca-Fe interactions have not been described in animals or humans with Ca and Fe levels within the adequate ranges and exposed to low environmental Pb (Szefer *et al.* 1994; Rahil-Khazen *et al.* 2002) as in this study. Other interactions worse defined are those between Pb-Zn and Pb-Cu (Bebe & Panemangalore 1996; Goyer 1997). Experimental studies have shown that Zn excretion is elevated in Pb-exposed animals and Zn-deficiency enhances Pb absorption (Goyer 1997), although the mechanisms for these interactions are not understood. In animals and humans exposed to relatively low levels of Pb, Pb-Zn are positively correlated in the liver and kidney in some studies (López Alonso *et al.* 2002a; Rahil-Khazen *et al.* 2002) although not in other (Norheim *et al.* 1992; Szefer *et al.* 1994) as in this study. Pb exposed animals have a significant decrease in hepatic Cu (Dhawan *et al.* 1995). Whether Pb-Cu interaction is at the site of absorption of Cu from the gastrointestinal tract or at some other metabolic or transport site has not been determined. Negative associations between Pb and Cu in the liver have also been described in animals exposed to low environmental Pb (Spierenburg *et al.* 1988; Miranda 1999), although, both metals were not statistically associated in other studies (Rahil-Khazen *et al.* 2002; López Alonso *et al.* 2002a). On the contrary Pb-Cu are strongly positively correlated in cattle in this study. With the actual knowledge we cannot explain this interaction. It is possible that Pb-Cu, and also other interactions, depend on the relative concentrations of these metals in the tissues (Pb residues are very low in cattle in this study) but could also be related to a common source of both metals in the diet.

Another interesting correlation is that between Se and Cu in cattle in this study. Experimental studies in ruminants have demonstrated that after injection of Se, Cu concentration in the liver increased in animals receiving both normal (Hussein *et al.* 1985) or Cu-deficient diets (Thomson & Lawson 1970). Research efforts on Cu-Se interactions have not been revealing and the mechanism of this chemical interaction remains unknown (WHO 1998). One explanation of this phenomenon could be the inhibition of MT synthesis after the Se treatment (Chmielnicka *et al.* 1983), that results in a lowered Cu-binding capacity of the liver cytosol, a Cu saturation of the nuclear fraction and a lowered Cu-excretion into the bilis, which finally results in a higher Cu accumulation in the hepatocyte and a higher susceptibility to chronic copper toxicity.

Another fact that points out the importance of the liver and kidney in the trace elements metabolism is that most of the associations between pairs of metals within the different tissues occurs in the liver versus kidney. As shown here, and in many other studies (Spierenburg *et al.* 1988; Szefer *et al.* 1994; García Fernández *et al.* 1996; Rahil-Khazen *et al.* 2002) several toxic and essential metals are correlated between the two organs. All the mixed correlations between toxic and essential metals in cattle in this study also happen in the liver versus kidney. Of particular interest is that most of the mixed correlations between essential elements in liver versus kidney and liver versus muscle are negative. Similar results have been described by Rahil-Khazen *et al.* (2002) in normal human tissues, although the real significance of these associations are unknown.

Conclusion

Although exposure and accumulation of toxic elements in cattle in this study is very low and trace essential metals are within the adequate ranges, there were significant associations between toxic and essential metals. This is similar to those found in polluted areas or in experimental studies in animals receiving diets containing high levels of toxic metal or inadequate levels of nutritional essential elements. These interactions probably indicate that mineral balance in the body is regulated by important homeostatic mechanisms, especially in the kidney, in which toxic elements compete with the essential metals, even at low levels of metal exposure. The molecular basis for most of these interactions are far from completely known.

The knowledge of these correlations may be essential to understand the kinetic interactions of metals and their implications in the trace metal metabolism

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