Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 113–121
Food Chemistry
www.elsevier.com/locate/foodchem

Total and dialyzable levels of manganese from duplicate meals and influence of other nutrients: Estimation of daily dietary intake

Carlos Velasco-Ryenold [a], Miguel Navarro-Alarcón [a,*], Herminia Lopez-G[a] De La Serrana [a], Vidal Perez-Valero [b], Maria C. Lopez-Martinez [a]

[a] Department of Nutrition and Food Science, University of Granada, 18071 Granada, Spain
[b] Hospital of Motril, Motril, Granada, Spain

Received 11 May 2007; received in revised form 8 November 2007; accepted 13 December 2007

Abstract

Both total and dialyzable Mn levels were determined in 108 duplicate meals during 36 consecutive days. Both mineral fractions were measured by a graphite furnace atomic absorption spectrometry (GFAAS) method previously optimized. A total mean Mn fraction of 1.03 ± 0.49 mg was found in the meals. The Mn supplied by the meals is directly and significantly ($p < 0.001$) correlated with macronutrient content (carbohydrates, fibre and protein). The mean Mn fraction dialyzed through the dialysis membrane was 0.23 ± 0.17 mg ($22.0 \pm 8.93\%$ as bioaccessible fraction). The total and dialyzable Mn fractions found for breakfasts were significantly lower ($p < 0.001$). Nevertheless, the Mn bioavailabilities expressed as the percentage of dialyzable element, were not significantly different among the three primary meals (breakfast, lunch and dinner). A significant correlation between the total and the dialyzable fraction of Mn in meals was found ($p < 0.001$, $r = 0.78$, $r^2 = 0.61$). The dialyzed element fractions present in meals were significantly correlated mainly with carbohydrates, protein and several amino acid levels ($p < 0.01$). Foods with higher carbohydrate and therefore energy contents, e.g. cereals, legumes, vegetables and fruits, would be primary sources of bioaccessible Mn in the diet. The bioaccessibility of Mn was only significant influenced by energy, carbohydrates and Se levels present in meals. The mean Mn daily dietary intake (DDI) was 3.05 ± 0.61 mg day^{-1}.

Keywords: Mn; GFAAS; Daily dietary intake; Duplicate diet method; *In vitro* availability

1. Introduction

Mn is an essential element that is a constituent of several enzymes and activates many others (Navarro-Alarcón, Gil Hernandez, & Gil Hernandez, 2005). Specifically, Mn was identified as a constituent of mitochondrial glutamine synthetase, pyruvate carboxylase and mitochondrial superoxide dismutase, a primary enzyme in the anti-oxidative defence system (Mason, 2005). It has been observed that most enzymes activated by Mn are also activated by Mg (Anderson, 2004; Navarro-Alarcón et al., 2005; Nielsen, 2002). Consequently, Mn deficiency in human beings is very rare because of its widespread presence in the human diet. However, when it does occur, Mn deficiency has been related with skeletal abnormalities, ataxia, alterations of reproductive function and lipid and carbohydrate metabolism, osteoporosis, epilepsy, difficulties in wound healing, and impaired growth (Anderson, 2004; Aschner, Erikson, & Dorman, 2005; Goldhaber, 2003; Greger, 1999; Navarro-Alarcón et al., 2005; Nielsen, 2002).

Mn is one of the trace elements with the lowest toxicity when ingested orally. Mn dietary intakes of only 0.8–20.0 mg for 8 weeks did not result in Mn deficiency or toxicity in healthy young women (Finley, Penland, Pettit, & Davis, 2003.). Under certain high-dose exposure conditions, elevations in Mn levels can occur with time. Excessive accumulation can result in adverse neurological

* Corresponding author. Tel.: +34 958 243865; fax: +34 958 249577.
E-mail address: nalarcon@ugr.es (M. Navarro-Alarcón).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.025

effects, headaches, dizziness, abnormal magnetic resonance imaging, and adverse reproductive effects in both animals and humans (Anderson, 2004; Aschner et al., 2005; Barceloux, 1999). Mn has the capacity to be transported across the blood–brain barrier and to be distributed in the central nervous system. The route of exposure influences the Mn disposition. Rodent inhalation studies have reported that Mn deposited within the nose can undergo direct transport along the olfactory nerve to the brain where it can induce neurotoxicity (manganism) that creates a motor dysfunction syndrome recognized as a form of Parkinsonism (Aschner et al., 2005; Barceloux, 1999; Goldhaber, 2003). The excess Mn accumulates in the liver and the encephalon, creating a neurological dysfunction. Toxicity has also been shown in patients receiving total parenteral nutrition, including Mn. Population groups with a potentially greater risk for Mn toxicity due to altered metabolism include: fetuses and neonates (due to their higher capacity to absorb Mn), subjects with diminished hepatic function, elderly people, subjects with suboptimal Mn or Fe intake, or those in a pre-Parkinson state. In human beings, Mn intoxication has been observed in miners and industry workers overexposed to high element levels in the workplace. Inhalation of high Mn amounts creates psychiatric disturbances generally known as Mn madness (Navarro-Alarcón et al., 2005).

The absorption of Mn (1–16%) is affected by dietary factors, such as element concentration, chemical form, source and oxidation state (mainly $Mn^{2+}$and Mn^{3+}), meal composition, lumen intestinal content, and interaction with other nutrients, as well as endogenous physiological factors: status of the intestinal mucosa, nutritional status of individuals, homeostatic mechanisms, carrier proteins, liver dysfunction, age and sex of individuals (Anderson, 2004; Aschner et al., 2005; Ji, Luo, Lu, Liu, & Yu, 2006; Lönnerdal, Jayawickrama, & Lien, 1999; Navarro-Alarcón et al., 2005; Nielsen, 2002). Mn present in meals interacts with other nutrients that influence its net absorption, namely other trace minerals, e.g. phytate, ascorbic acid and some dietary constituents such as proteins (Agte, Jahagirdar, & Chiplonkar, 2005; Aschner et al., 2005; Davidsson, Almgren, Juilerat, & Hurrell, 1995; Davidsson, Almgren, & Hurrell, 1998; Finley et al., 2003; Kilicalp, Dede, Belge, & Tatar, 2005; Lönnerdal, 2002; Navarro-Alarcón et al., 2005; Planells, Sanchez-Morito, Montellano, Aranda, & Llopis, 2000; Sanchez-Morito, Planells, Aranda, & Llopis, 1999; Thompson, Molina, Donaghey, Brain, & Wessling-Resnick, 2006; Windisch, 2002). Specifically, dietary Mn levels determine the element amount absorbed from the gastrointestinal tract and the amount of Mn eliminated in the bile. When dietary Mn levels are high, adaptive changes include reduced gastrointestinal absorption, enhanced liver metabolism and increased biliary and pancreatic excretion of this element (Aschner et al., 2005). Additionally, the net absorption of Mn also seems to be influenced by that of Fe or Co because they all may utilize the same apical and basolateral transporters in Caco-2 cells, as reported by Tallkvist, Bowlus, and Lönnerdal (2000). Therefore, if some of these metals (Fe or Co) are present in high levels, concomitantly with Mn, they can exert an inhibitory effect on the net absorption of the remaining elements (Nielsen, 2002).

In this study, an assessment of daily dietary Mn intake (DDI) was performed on a population group of adults from south eastern Spain using a retrospective method. We conducted a graphite furnace atomic absorption spectrometry analysis (GFAAS) for 108 duplicate meals from the restaurant of the Hospital of Motril (SE Spain) (36 breakfasts, 36 lunches and 36 dinners, corresponding to 36 consecutive days). A previously developed and optimized time–temperature ETAAS programme for Mn measurements was performed. An *in vitro* method that simulates gastrointestinal intestinal conditions by employing a dialysis membrane was used to determine the available Mn fraction and the influence that other existing nutrients might have on that Mn fraction. The analyzed meals included first dish, second dish, bread, dessert and beverage, since all food and beverages consumed at the same meal will be mixed and interact together in the gastrointestinal tract. Additionally, Mn absorption from food depends on the elements and their transformation during food processing and food digestion. Both factors were considered in the duplicate ready-to-eat meals in this study. Additionally, dialysis mineral percentages were calculated as an expression of the Mn bioaccessibility. Furthermore, to assess the magnitude of inadequate intakes of Mn (deficiency or excess and toxicity) we employed the dietary adequate intake (AI) cut off points proposed by the USA Institute of Medicine (2002) (2.3 and 1.8 mg day^{-1} for male and female healthy adults older than 19 years).

2. Materials and methods

2.1. Apparatus

A Perkin–Elmer 1100B double-beam atomic absorption spectrophotometer equipped with a deuterium background corrector (Perkin–Elmer, Norwalk CT, USA) and a Mn hollow cathode lamp were used together with an HGA-700 furnace with pyrolytically coated graphite tubes. The samples were injected manually with a Pipetman micropipeptte. A Stomacher blender, model 400, of which different parts were teflon-coated, was used for sample homogenization. A thermostatic multiplace digestion block (Selecta S.A., Barcelona, Spain) and a Radiometer model 26 pH meter (Radiometer, Copenhagen N.V., Denmark) were used for *in vitro* assays.

2.2. Reagents

A commercially available standard solution of Mn (1000 μg ml^{-1}; Tritisol, Merck, Darmstadt, Germany) was used to prepare calibration graphs. The employment of a chemical modifier was not necessary. Nitric acid

(65% v v^{-1}) and perchloric acid (70% v v^{-1}) were employed to mineralize the diet samples.

Pepsin (Sigma P7000, porcine pancreas, Sigma–Aldrich Chemie Gmbh, Steinheim, Germany), pancreatin, porcine pancreas (Sigma p1500, Sigma Chemical Co., St. Louis, MO) and bile salts (Sigma B8756) were used to simulate gastric and intestinal digestion. A pepsin solution was prepared by dissolving 16 g of pepsin in 100 ml of 0.1 M HCl. Pancreatin–bile extract mixture was prepared by dissolving 4 g of pancreatin and 25 g of bile extract in 1 l of 0.1 M $NaHCO_3$. The dissolutions of 0.5 M NaOH and 6 M HCl were employed to adjust the pH.

A dialysis membrane, Spectra, with molecular mass cut-off (MMCO) of 12–14 kDa (Visking size 3-20/32, Medicell International, London, UK) was used. The dialysis membrane was freed of trace element impurities by boiling in a 2% w v^{-1} $NaHCO_3$ 0.1% w v^{-1} sodium dodecyl sulphate and 0.01 M ethylenediaminetetraacetic acid disodium salt (EDTA) for 30 min, followed by thoroughly washing with double-distilled deionised water, and preserved in 20% ethanol solution.

All solutions were prepared from analytical reagent grade reagents (Merck, Germany, Suprapur). The water employed for preparing the standards for calibration and dilutions was doubly distilled deionised water with a specific resistivity of 18 mΩ cm obtained by filtering distilled water through a Milli-Q purifier (Millipore, Waters, Mildford, MA) immediately before use.

2.3. Duplicate portion sampling of meals and Mn analysis by GFAAS

Duplicate portion samplings of 108 different meals, corresponding to 36 breakfasts, 36 lunches and 36 dinners, were taken over 36 consecutive days, from May to June, 2002, from the restaurant of the Hospital of Motril. The dietician was instructed to collect duplicates of the three meals consumed during each 24-h period in two 2.5 l previously cleaned polyethylene containers. Furthermore, all food and beverage items corresponding to each menu were recorded and weighed on balances available in the hospital. The containers were then stored at −18 °C. Meal samples were transported to the laboratory of the Department of Nutrition and Food Science at the University of Granada. The analyzed meals ($n = 108$) were sliced and mixed in a blender (Stomacher 400), noting the menu weight. Aliquots of about 100 g of the mixed meal were dried at temperatures ranging from 60 to 80 °C. After drying, liquid content was calculated and dried samples were homogenized in previously cleaned Teflon containers. About 400 mg of the pulverized meal sample were placed in a flask and subjected to an acid mineralization in a multiple place digestion block by adding 5 ml of HNO_3 and heating at 60° C for 30 min. Another 1 ml of 4:1 HNO_3–$HClO_4$ (4:1) solution was added and heating continued at 120 °C for 90 min. The digest was then cooled and the resulting solution diluted to 25 ml with doubly distilled deionised water. The analytical solution obtained was proved to be optimal for the Mn GFAAS determination.

The Mn determination was directly performed in acidified samples, which were adequately diluted with 0.2% HNO_3 solution according to element concentrations present in samples. Previous to Mn measurement, the optimisation of the volume of diluted sample was performed. The element was directly determined by the linear calibration method. Samples were manually injected with a micropipette through a graphite tube without L'Vov platform; the volume injected was 20 µl of diluted sample as an analytical solution. Before duplicate meal samples were injected into the tube, they were treated with saturated $(NH_4)_2MoO_4$ to avoid the formation of refractory carbides, and to run under optimized conditions. These were optimized on the basis of time–temperature studies using certified standards (CBR CRM 278R mussel tissue and CBR CRM 185R bovine liver). Absorption was measured as the area under the absorbance peak. Optimum temperatures and times for ashing, charring and atomisation on the analysis in the graphite tube were investigated on the basis of previous assays. Furnace conditions for Mn determination by GFAAS at 279.5 nm are summarised in Table 1. The Mn hollow cathode lamp used at 35 mA and a spectral slit width of 0.2 nm were selected. Argon of 99.999% purity at 300 ml min^{-1} flow was used as an internal gas. The samples were analyzed in triplicate. The same procedure was followed for the blank. Appropriate aqueous calibration graphs were prepared daily in 0.2% HNO_3 medium.

2.4. Duplicate portion sampling of meals and estimation of nutrient intakes by employment of food composition tables

The estimation of contents and corresponding DDIs of 51 nutrients and energy in analyzed meals was done as reported elsewhere (Velasco-Reynold, Navarro-Alarcon, López -G[a] de la Serrana, Perez-Valero, & Lopez-Martinez, 2007).

2.5. Determination of dialyzable Mn fraction and percentage of dialyzed element of duplicate meals by an in vitro method

The simulated gastrointestinal digestion procedure and the *in vitro* absorption estimation were done as previously reported (Garcia, Cabrera, Lorenzo, Lopez, & Sanchez, 2001; Velasco-Reynold et al., in press). The technique

Table 1
Furnace conditions for Mn determination in duplicate meal samples by GFAAS technique

Step	Temperature (°C)	Time (s)	Argon flow (ml min^{-1})
Injection	20	–	300
Ashing	110	40	300
	150	20	300
Charring	1000	20	300
Atomization	1900	6	0
Cleaning	2650	3	300

measures the fraction of a metal which is dialyzed from a sample under simulated gastrointestinal conditions and which, therefore, is available for absorption. Titratable acidity corresponded to the number of equivalents of 0.5 NaOH required to titrate the amount of combined pepsin digest pancreatin–bile extract mixture to pH 7.5. For its determination, a 20 g aliquot of the pepsin digest was used, to which 5.0 ml of the pancreatin–bile extract mixture were added, employing 0.5 M NaOH in the titration.

The determination consists of two sequential processes, a gastric and an intestinal digestion; each one was carried out employing simulated human conditions (enzymes, pH and temperature). In the first stage, a portion of 5 g of homogenized sample was added to 40 ml of doubly distilled deionised water and the pH was adjusted to 2.0 with 6 M HCl. After 15 min, the pH was determined again, once more adjusting to 2.0 if it was necessary. Three milliliters of freshly prepared pepsin solution was added to the sample. Then, doubly distilled deionised water was added until there was 100 g of mixture. Next, the mixture was incubated in a shaking and thermostatic water bath at 37 °C for 2 h.

In the second stage involving extraction with intestinal juices, 20 g samples of pepsin digest were transferred to a 250 ml Erlenmeyer flask. The dialysis membrane, containing 25 ml of doubly distilled deionised water and an amount of $NaHCO_3$ equivalent to the titritable acidity measured previously, was placed in the flask, and incubated in the shaking bath at the same temperature for 30 min. Then, 5 ml of the pancreatin–bile extract mixture were added to each flask and incubated for 2 h. The dialysis membrane was removed and the dialysate was weighed. The dialyzable Mn content was then determined by GFAAS. All digestions were done in triplicate and gastrointestinal blanks were prepared and analyzed in the same procedure.

The Mn bioavailability is expressed as the percentage of dialyzed Mn in relation to the total content in the duplicate meal. Dialysis mineral percentages were calculated as follows: dialysis (%) $= 100 \times D/C$, where D, dialyzed mineral content ($\mu g\ g^{-1}$ sample) and C, total mineral content ($\mu g\ g^{-1}$ sample).

2.6. Statistics

The statistical package *SPSS 12.0 for Windows* programme was employed for interpreting the data. Results are expressed as arithmetic mean and standard deviation. The normal distribution of variables and the homogeneity of variances were checked by the Kolmogorov–Smirnov and Bartlett's tests, respectively. The comparisons were done using the Student's *t*-test when the variable fulfilled parametric conditions and the Kruskall–Wallis test when conditions were non-parametric. Additionally, correlations by the Pearson or Spearman's tests (for parametric and non-parametric conditions, respectively) and linear regression models were also employed.

3. Results and discussion

3.1. Analytical quality assurance

The analytical characteristics found for the developed procedure are included in Table 2. The mean Mn concentrations determined in the BCR CRM 278R and 185R (7.69 ± 0.23 and $11.07 \pm 0.29\ \mu g\ g^{-1}$, respectively) were 8.05 ± 0.51 and $10.5 \pm 0.56\ \mu g\ g^{-1}$, respectively. The relative standard deviation ($n = 7$) ranged from 1.96 to 6.33 (mean value = 4.77) for the four different samples for which between-day variability was determined.

In the present study, a GFAAS method has been developed for measuring total and dialyzed Mn levels in duplicate meals. The results obtained for the standard reference materials show an appropriate accuracy and precision of the programme of time–temperatures developed. The detection limit and sensitivity (Table 2) were suitable for the Mn concentrations found. Additionally, the values obtained for the intermediate reproducibility and percentage of recovery of previously treated samples with Mn show the adequacy of this technique for determining this element in meals to measure the total dietary supply as well as the dialyzable fraction in the meals. Consequently, it could be used to assess the magnitude of inadequate intakes of Mn (deficiency or excess and toxicity).

3.2. Estimation of DDI of Mn

The mean Mn DDI, determined by duplicate diet sampling and analysis by GFAAS for the 36 consecutive days evaluated during the study, was $3.05 \pm 0.61\ mg\ day^{-1}$ (Table 3). This estimated value was 133% and 170% of the AIs (2.3 and 1.8 $mg\ day^{-1}$ for healthy adult men and women aged 19–50 years, respectively; Institute of Medicine, 2002). Only one daily menu (2.14 $mg\ day^{-1}$), supplied element levels lower than the reported AIs. When we compared our results with the AIs for the category of lactating women, we discovered that ≅22% of the daily meals studied had Mn levels lower than the recommended AIs (2.6 mg Mn day^{-1} for 19–50-year old women,

Table 2
Analytical characteristics for the proposed method to determine Mn in duplicate meals by GFAAS

Detection limit ($pg\ ml^{-1}$)	Characteristic mass[a] (pg)	Recovery[b] (%)	Precision, RSD[c] (%)	Blank-to-sample slope ratio
6.0	0.81	100 ± 1.55	4.83 ± 1.99	0.98–1.03

[a] Characteristic mass is ng corresponding to 0.0044 milliabsorbance units.
[b] Mean recovery obtained by analyte recovery assays added to several duplicate meals ($n = 4$).
[c] Mean relative standard deviation obtained by repeated measurements ($n = 7$) in four duplicate meals in order to determine the intermediate reproducibility.

Table 3
Mean intake and DDI of total Mn determined by the technique of duplicate portion sampling of meals (breakfast, lunch and dinner) and analysis by GFAAS

Meal	*n*	Total Mn (mg)	
		Range	Mean ± SD
Breakfast	36	0.08–1.48	0.67 ± 0.49[a]
Lunch	36	0.75–2.37	1.45 ± 0.42
Dinner	36	0.67–1.71	0.98 ± 0.29
24-h meals (DDI)	36	2.12–4.61	3.05 ± 0.61

[a] $p < 0.01$.

Institute of Medicine, 2002). The upper level (UL) for Mn, defined as the maximum level of daily Mn intake that is likely to pose a risk of adverse effects, is reported by the Institute of Medicine (2002) as 10.0 mg. None of the evaluated daily menus supplied levels close to this UL value. In our study, the 24-h daily menus that supplied the highest Mn DDI corresponded to only 40% of the UL. Consequently, no adverse effects related to Mn nutrition (deficiency or toxicity) are present in individuals who eat meals at the Hospital of Motril (SE Spain), namely staff and patients.

The measured values of Mn DDI are within the normal range of mean Mn DDI levels (0.52–10.8 mg day^{-1}; Navarro-Alarcón, Gil Hernandez, & Gil Hernandez, 2005). In mixed western diets, a range of Mn DDI of <1 to >10 mg Mn day^{-1} was reported (Greger, 1999). For the mixed Mediterranean diet evaluated in our study, a daily intake ranging from 2.14 to 4.61 mg Mn day^{-1} was found (Table 3).

Table 5 presents published data on Mn DDIs estimated by several researchers in different countries. We can observe that most of the studies reported daily Mn intakes close to each other that ranged from 2.50 to 6.90 mg day^{-1}. All daily Mn intakes determined in the present study are within in this range (Table 3).

3.3. Total and dialyzable Mn levels in whole meals: correlation with contents of other nutrients

The mean Mn intakes determined by this method for the three groups of daily meals (breakfast, lunch and dinner) are collected in Table 3. The Mn intakes found for breakfasts were significantly lower ($p < 0.001$). Lunch (47.5%) is the meal that contributes most to the 24-h Mn DDI, followed by dinner (32.2%) and finally breakfast (20.2%).

Using the Pearson and Spearman's tests, significant correlations were found between Mn intake levels and the supply of 26 nutrients and energy in the meals analyzed ($p < 0.05$ Table 4). The corresponding correlation coefficients are also shown in this table. The energy contents of meals were also significantly correlated with their corresponding Mn concentrations ($p < 0.001$, $r = 0.59$).

There is a remarkable positive and linear correlation between total Mn levels and energy, carbohydrates, protein and several amino acids supplied by the meals. Our findings establish that Mn supplied by meals is directly related to some macronutrient contents. Consequently, vegetal food, e.g. cereals and by-products, legumes and dry fruits, would be primary sources of Mn in the meals, as previously reported (Leblanc et al., 2005; Navarro-Alarcón et al.,

Table 4
Significant correlation and significance levels among the total and dialyzable Mn levels and the percentage of dialyzed Mn of the analyzed hospital meals ($n = 108$) and other nutrients and energy estimated from the food composition tables compiled in the statistics programme *Dietsource 2.0* (Novartis)

Mn form	Nutrient	*r*	Nutrient	*r*	Nutrient	*r*
Total Mn	Energy	0.53*	Pyridoxine	0.42**	Ile	0.44**
	Carbohydrates	0.68*	Vitamin E	0.35***	Leu	0.45**
	Fibre	0.49**	Folate	0.35***	Lys	0.42**
	Fe	0.58*	Vitamin B_{12}	0.30***	Met	0.42**
	P	0.60*	Thiamine	0.36***	Phe	0.46**
	K	0.44**	Riboflavin	0.47**	Val	0.44**
	Zn	0.39**	Protein	0.52*	Total Cr levels[a]	0.44**
	Mg	0.58*	Trp	0.45**	Total Cu levels[b]	0.39**
	Se	0.38**	Thr	0.44**	Dialyzable Cu fraction[b]	0.65*
Dialyzable Mn	Energy	0.59*	Zn	0.37***	Ile	0.43**
	Carbohydrates	0.76*	K	0.37***	Leu	0.45**
	Fibre	0.34***	Se	0.52*	Lys	0.43**
	P	0.59*	Vitamin E	0.35***	Met	0.43**
	Mg	0.51*	Protein	0.52**	Phe	0.47***
	Ca	0.38**	Trp	0.43**	Val	0.55**
	Fe	0.48*	Thr	0.43**	Total Mn	0.78*
Percentage of dilayzed Mn	Energy	0.345***	Carbohydrates	0.44**	Selenium	0.36***

[a] Total Cr levels measured in duplicate meals by GFAAS (Velasco-Reynold et al., in press).
[b] Total and dialyzable Cu determined in duplicate meals by GFAAS (Velasco-Reynold, Navarro-Alarcon, López-G[a] de la Serrana, Perez-Valero, & Lopez-Martinez, 2007).
* $p \leqslant 0.001$.
** $p < 0.01$.
*** $p < 0.05$.

Table 5
Literature data on daily intakes of Mn estimated by other authors

Estimation method (population group)	Mn DDI (mg day^{-1})	City or region (country)	Reference
Duplicate method performed during 7 consecutive days Healthy adults	6.80–9.22	Thuringia and Brandenburg	Anke, Groppel, Krause, Arnhold, and Langer (1991)
Duplicate diet technique			
Healthy adult women	2.40 ± 0.30	Beltsville (USA)	Anderson, Bryden, and Polansky (1993)
Healthy adult men	4.00 ± 0.40	Beltsville (USA)	Anderson et al. (1993)
Duplicate plate technique			
Healthy adult men	3.30 ± 0.15	Grenoble (France)	Pelus et al. (1994)
USA total diet study			
Healthy children	1.42	USA	Pennington and Schoen (1996)
Healthy women	2.08	USA	Pennington and Schoen (1996)
Duplicate diet technique			
Healthy individuals	4.69	Austria	Wilplinger, Zochling, and Pfannhauser (1999)
Duplicate diets			
Young women	0.70–9.50	USA	Finley (1999)
UK total diet study			
Healthy adult consumers	4.50	United Kingdom	Ysart et al. (1999)
Healthy children	1.34	United Kingdom	Ysart et al. (1999)
Duplicate dietary analysis			
Healthy adult population	2.21	Mumbai (India)	Tripathi, Mahapatra, Raghunath, Sastry, and Krishnamoorthy. (2002)
Duplicate meals from catering establishments and analysis			
Healthy individuals	2.15	France	Noël et al. (2003)
Food and beverage analysis	2.30	Rio de Janeiro (Brazil)	Santos et al. (2004)
Duplicate portion technique			
Healthy women	2.40	Germany	Schafer et al. (2004)
Healthy men	2.70	Germany	Schafer et al. (2004)
Healthy women breast feeding	2.30	Germany	Schafer et al. (2004)
Vegetarian healthy women	5.50	Germany	Schafer et al. (2004)
Healthy women	2.00	Mexico	Schafer et al. (2004)
Healthy men	2.10	Mexico	Schafer et al. (2004)
Food and beverage analysis from French total study			
Children from 3 to 14 yr	1.90	France	Leblanc et al. (2005)
Individuals aged ⩾ 15 yr	2.30	France	Leblanc et al. (2005)
Market basket study			
Healthy adult men and women	3.54	Ibaraki (Japan)	Shiraishi (2005)
Duplicate diet technique			
Healthy children	1.56	Tokyo (Japan)	Aung, Yoshinaga, and Takahashi (2006)
Healthy adults	2.72	Tokyo (Japan)	Aung et al. (2006)

2005; Noël, Leblanc, & Guérin, 2003; Santos, Lauria, & Porto da Silveira, 2004; Shiraishi, 2005). Specifically, seeds, beans and nuts have been reported as major Mn sources in diet. Noël et al. (2003) indicated that vegetables and beverages are also important contributors to Mn intake. Shiraishi (2005) noted that, although the highest Mn concentration was in nuts and seeds, rice was the highest contributor. Consequently, food with high carbohydrate and/or protein content would also be a good source of Mn. Schafer, Anke, Seifert, and Fischer (2004) reported that vegetarian diets supplied a significantly higher Mn intake to daily diet.

Determination of the element fraction that can be absorbed and transformed into a biologically active form in the organism is more informative than just measuring the total mineral content supplied by daily diet. Consequently, the dialyzable Mn fraction from duplicate meals by an *in vitro* method was measured. This method simulates the gastrointestinal conditions, as the elements have to be in a soluble form capable of crossing the intestinal mucosa before they can be absorbed. The mean dialyzable Mn fraction was 0.23 ± 0.17 mg (Table 6) from a total mean element content of 1.03 ± 0.49 mg of Mn meal-1. The corresponding mean percentage of Mn dialyzed from the total element content of the duplicate meals was 22.0 ± 8.93% (Table 6). This mean percentage expresses the element fraction that would be available for absorption by intestinal cells (bioaccessible Mn). Mehra and Baker (2007) found 35.5% of available Mn in 1 l of tea, which is even higher than the mean dialysis Mn percentage found by us in this study. For radish roots, lettuce and spinach leaves, the percentages of soluble Mn in the *in vitro* study were 20.6%, 10.4% and 8.7%, respectively (Intawongse & Dean, 2006). The 20.6% value is close to the mean percentages of dialyzable Mn found for duplicate meals in our study.

Table 6
Dialyzable Mn fraction and percentages of dialyzed Mn determined by the technique of duplicate portion sampling of meals (breakfast, lunch and dinner) and analysis by GFAAS

Meal	*n*	Dialyzable Mn (mg)		Percentage of dialyzed Mn (%)	
		Range	Mean ± SD	Range	Mean ± SD
Breakfast	36	0.01–0.18	0.10 ± 0.07*	2.08–39.9	19.9 ± 12.9
Lunch	36	0.12–0.97	0.38 ± 0.18	16.0–42.3	22.6 ± 10.2
Dinner	36	0.05–0.45	0.21 ± 0.10	6.72–31.3	23.1 ± 5.77
Mean values	108	0.01–0.97	0.23 ± 0.17	2.08–42.3	22.0 ± 8.93

* $p < 0.001$.

In the regression analysis of the results, a significant linear correlation was found between the total and the dialyzable fraction of Mn in the duplicate meals ($p < 0.001$, $r = 0.78$) (Table 4). This finding shows that dialysis ratio enhancements will rise with the total Mn amounts existing in the meal. Nevertheless, the percentage of dialyzed Mn was not significantly correlated with total Mn levels ($p = 0.201$, $r = 0.19$). These findings show that element bioaccesibility is not significantly enhanced with the rise of Mn contents in meals. This result can also be checked by referring to Table 6, where we can see that, although the dialyzable Mn level for breakfasts was significantly lower, the corresponding percentage of element dialyzed is not significantly different from those reported for lunches and dinners ($p > 0.05$). The mean dialyzable Mn fraction determined was 0.23 ± 0.17 mg (Table 6) which represents a 22.0 ± 8.93% of total element present in meals. In the literature, lower absorption values for Mn in human beings were reported (1–16%, Navarro-Alarcón, Gil Hernandez, & Gil Hernandez, 2005; 12.16–14.80%, Agte, Jahagirdar, & Chiplonkar, 2005). The differences observed between these human studies and the *in vitro* study performed by us are related to the endogenous physiological factors of individuals, e.g. the status of their bowel mucosa, nutritional status, homeostatic mechanisms, carrier proteins (Navarro-Alarcón et al., 2005). In the present study, we only determined the dialyzable Mn fraction that forms complexes that were able to dialyze through the dialysis membrane and therefore be absorbed.

Several researchers have noted that the efficiency of the Mn absorption declines with the enhancement of the total intake of Mn (Aschner et al., 2005; Davis, Zech, & Greger, 1993; Finley, 1999; Finley et al., 2003; Malecki, Radzanowski, Radzanowski, Gallear, & Greger, 1996). Specifically, when dietary Mn levels are high, adaptative changes include reduced gastrointestinal absorption of Mn, enhanced Mn liver metabolism and increased biliary and pancreatic excretion of Mn. However, in our study, the percentage of dialyzed Mn was not significantly decreased ($p > 0.05$) with the rise of the element contents in duplicate diets. These differences could be related to the fact that the Mn homeostasis would be regulated mainly by the excretion variable (mainly biliary, and also pancreatic and intestinal excretion) through the digestive tract, a factor that has not been considered in our study. Nevertheless, we have found that the net dialyzable Mn fraction increased significantly with the Mn contents of meals, although not the percentage of dialyzed Mn.

The bioavailability of a single element, and therefore of Mn, may depend on interactions with other components, and both competitive and cooperative interdependence (Navarro & Wood, 2003). In the present study, significant correlations between the dialyzable Mn fraction and the levels of 21 nutrients were found (Table 4). Specifically, the highest correlation coefficients ($r > 0.40$) have been reported for carbohydrates, P, Se, protein, Mg, Fe and several amino acids in decreasing order. However, when considering the dialysis Mn percentages (bioaccessibility of Mn), significant correlations were found only with energy, carbohydrate and selenium levels present in duplicate meals (Table 4). Specifically, the regression analysis showed the existence of significant correlation with carbohydrate content using an exponential model, and with energy levels when using a linear model (Table 4). This finding also reinforces the result previously reported: that foods with high carbohydrate content (cereals, legumes, and some vegetables and fruits) would be primary sources of bioaccessible Mn in the daily diet.

Of all the nutrients, Fe is the one whose interactions with Mn have been the most extensively studied. Several researchers have reported the negative influence that high dietary Fe levels exert on Mn absorption and bioavailability (Aschner et al., 2005; Navarro-Alarcón et al., 2005; Nielsen, 2002; Thompson, Molina, Donaghey, Brain, & Wessling-Resnick, 2006). Animal studies have demonstrated that Fe deficiency enhances Mn absorption across the gastrointestinal tract. Finley, Johnson, and Johnson (1994) noted that men and women differ in their metabolism, and that such differences could be related to Fe. Aschner et al. (2005) indicated that the combination of Fe deficiency and a vegetarian diet created higher than usual Mn levels in vegetarians. This was attributed to the higher Mn content in the diet (cereals, legumes, vegetables), combined with lower competition exerted by the low Fe levels present in these diets (Schafer et al., 2004). Nonetheless, Agte et al. (2005) studied the apparent absorption of Mn from vegetarian meals in ileostomized human volunteers, reporting that the percentage of absorption of Mn was not significantly correlated with either Fe intake or with Mn, Cu, Zn, phytic acid, vitamin C, thiamine, riboflavin and β-carotene. Similarly, in our study, dialysis Mn percentages were not significantly correlated with Fe contents of duplicate meals (Table 4).

Phytate is another nutrient whose interaction with Mn absorption has also been extensively studied. Contradictory results have been reported. Some authors indicate that phytic acid has an inhibitory effect on Mn absorption (Davidsson, Almgren, Juilerat, & Hurrell, 1995; Lönnerdal, 2002). Other researchers did not find any significant influence of

dietary phytate levels on the percentage of Mn absorption (Agte et al., 2005; Davidsson, Cederblad, Lonnerdal, & Sandstrom, 1991; Lönnerdal et al., 1999). In our study, we found that carbohydrate contents of duplicate meals were significantly correlated with dialysis Mn percentages. Therefore, foods with high carbohydrate content, e.g. cereals and legumes, which also have high phytic levels, are the main sources of bioaccessible Mn in duplicate diets studied. Indirectly, we also found that the presence of phytates in studied meals did not have any effect on Mn absorption. This finding could also be related to the fact that analyzed meals were ready-to-eat meals. Therefore, food processing and preparation would produce hydrolysis of phytates by enzymatic (phytase activation by thermal treatment, fermentation processes, baking) or non-enzymatic (thermal processing) means (Lönnerdal, 2002).

Finally, a significant correlation using a potential model in the regression analysis done between the Se contents and dialysis Mn percentages was noted (Table 4, $p = 0.014$). An explanation of this particular interaction has not been found.

During food processing and cooking of ready-to-eat meals, transformations of soluble species of Mn have been reported (e.g. boiling of peas) (Koplik et al., 2004; Koplik, Mestek, Kominkovam, Borkova, & Sucahnek, 2004). Specifically, the boiling markedly diminished the Mn present in blanched green peas (Koplik & Kominkovam et al., 2004) and made this element almost completely insoluble (Koplik & Mestek et al., 2004). In our study, the evaluation of the Mn intake and the dialyzable Mn fraction of duplicate ready-to-eat meals also allowed us to consider the influence that technological processing and cooking procedures had on the level of bioaccessible Mn measured.

Despite everything reported in the present study, future research is required to further define Mn bioavailability from diet. Specifically, human *in vivo* studies are needed. *In vitro* studies of bioavailability can be useful for evaluating the percentage of Mn that could be transformed into absorbable forms in the digestive tract (bioaccessible Mn) and the influence exerted by other nutrients in the diet, by food processing and by cooking. But, the procedures do not exactly reproduce actual physiological conditions and therefore do not allow for a comprehensive evaluation. Consequently, *in vivo* studies should be combined with *in vitro* studies to achieve a better understanding of the bioavailability process in the human organism.

Acknowledgments

The authors would like to thank Mr. J. Robert Mason for his help in editing the manuscript in English.

References

Agte, V., Jahagirdar, M., & Chiplonkar, S. (2005). Apparent absorption of eight micronutrients and phytic acid from vegetarian meals in ileostomized human volunteers. *Nutrition, 21*, 481–489.

Anderson, R. A., Bryden, N. A., & Polansky, M. M. (1993). Dietary intake of calcium, chromium, copper, iron, magnesium, manganese, and zinc: Duplicate plate values corrected using derived nutrient intake. *Journal of the American Dietetic Association, 93*, 462–466.

Anderson, JJB. (2004). Minerals. In L. K. Mahan & S. Escott-Stump (Eds.), *Food, nutrition, & diet therapy* (11th ed.). Philadelphia: Saunders.

Anke, M., Groppel, B., Krause, U., Arnhold, W., & Langer, M. (1991). Trace-element intake (zinc, manganese, copper, molybdenum, iodine and nickel) of humans in Thuringia and Brandenburg of the Federal-Republic-of-Germany. *Journal of Trace Elements and Electrolytes in Health and Disease, 5*, 69–74.

Aschner, M., Erikson, K. M., & Dorman, D. C. (2005). Manganese dosimetry: Species differences and implications for neurotoxicity. *Critical Reviews in Toxicology, 35*, 1–32.

Aung, N. N., Yoshinaga, J., & Takahashi, J. I. (2006). Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo metropolitan area, Japan. *Food Additives and Contaminants, 23*, 883–894.

Barceloux, D. G. (1999). Manganese. *Journal of Toxicology–Clinical Toxicology, 37*, 293–307.

Davidsson, L., Cederblad, A., Lonnerdal, B., & Sandstrom, B. (1991). The effect of individual dietary-components on manganese absorption in humans. *American Journal of Clinical Nutrition, 54*, 1065–1070.

Davidsson, L., Almgren, A., Juilerat, M. A., & Hurrell, R. F. (1995). Manganese absorption in humans – the effect of phytic acid and ascorbic-acid in soy formula. *American Journal of Clinical Nutrition, 62*, 984–987.

Davidsson, L., Almgren, A., & Hurrell, R. F. (1998). Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant does not influence absorption and urinary excretion of manganese in healthy adults. *Journal of Nutrition, 128*, 1139–1143.

Davis, C. D., Zech, L., & Greger, J. L. (1993). Manganese metabolism in rats: An improved methodology for assessing gut endogenous losses. *Proceedings of the Society and Experimental Biology and Medicine, 202*, 103–108.

Finley, J. W., Johnson, P. E., & Johnson, L. K. (1994). Sex affects manganese absorption and retention by humans from a diet adequate in manganese. *American Journal of Clinical Nutrition, 60*, 949–955.

Finley, J. W. (1999). Manganese absorption and retention by young women is associated with serum ferritin concentration. *American Journal of Clinical Nutrition, 70*, 37–43.

Finley, J. W., Penland, J. G., Pettit, R. E., & Davis, C. D. (2003). Dietary manganese intake and type of lipid do not affect clinical or neuropsychological measures in healthy young women. *The Journal of Nutrition, 133*, 2849–2856.

Garcia, E., Cabrera, C., Lorenzo, M. L., Lopez, M. C., & Sanchez, J. (2001). Estimation of chromium bioavailability from diet by an in vitro method. *Food Additives and Contaminants, 18*, 601–606.

Goldhaber, S. B. (2003). Trace element risk assessment: Essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology, 38*, 232–242.

Greger, J. L. (1999). Nutrition versus toxicology of manganese in humans: Evaluation of potential biomarkers. *Neurotoxicolgy, 20*, 205–212.

Intawongse, M., & Dean, J. R. (2006). Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. *Food Additives and Contaminants, 23*, 36–48.

Institute of Medicine (IOM). (2002). Food and Nutrition Board. *Dietary references intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. Washington, DC: The National Academic Press.

Ji, F., Luo, X. G., Lu, L., Liu, B., & Yu, S. X. (2006). Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broilers' intestine. *Poultry Sciences, 85*, 1217–1225.

Kilicalp, D., Dede, S., Belge, F., & Tatar, M. (2005). Effect of protein deficiency on macroelement and trace element levels of weanling rats' small intestine and liver tissues. *Biological Trace Element Research, 107*, 255–261.

Koplik, R., Kominkovam, J., Borkova, M., Mestek, O., Kvasnicka, F., & Sucahnek, M. (2004a). Effect of technological processing and maturity stage of seeds on the content and speciation of phosphorus and trace elements in peas. *Food Chemistry, 87*, 423–432.

Koplik, R., Mestek, O., Kominkovam, J., Borkova, M., & Sucahnek, M. (2004b). Effect of cooking on phosphorus and trace elements species in peas. *Food Chemistry, 85*, 31–39.

Leblanc, J. C., Guérin, T, Noël, L, Calamassi-Tran, G., Volatier, J. L., & Veger, P. (2005). Dietary exposure estimates of 18 elements from the 1st French total diet study. *Food Additives and Contaminants, 22*, 624–641.

Lönnerdal, B., Jayawickrama, L., & Lien, E. L. (1999). Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. *American Journal of Clinical Nutrition, 69*, 490–496.

Lönnerdal, B. (2002). Phytic acid-trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology, 37*, 749–758.

Malecki, E. A., Radzanowski, G. M., Radzanowski, T. J., Gallear, D. D., & Greger, J. I. (1996). Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat. *Journal of Nutrition, 126*, 489–498.

Mason, P. (2005). In P. Mason (Ed.), *Suplementos dieteticos*. Barcelona: Pharma Editores.

Mehra, A., & Baker, C. L. (2007). Leaching and bioavailability of aluminium, copper and manganese from tea (*Camelia sinensis*). *Food Chemistry, 100*, 1456–1463.

Navarro, M., & Wood, R. J. (2003). Plasma changes in micronutrients following a multivitamin and mineral supplement in healthy adults. *Journal of the American College of Nutrition, 22*, 124–129.

Navarro Alarcón, M., Gil Hernandez, F., & Gil Hernandez, A. (2005). Selenio, manganeso, cromo, molibdeno, yodo y otros oligoelementos minoritarios. In A. Gil-Hernández (Ed.). *Tratado de nutrición* (Vol. II). Madrid: Acción Médica.

Nielsen, F. H. (2002). Minerales ultratraza. In M. E. Shils, J. A. Olson, M. Shike, & A. C. Ross (Eds.), *Nutricion en salud y enfermedad*. Madrid: McGRaw-Hill Interamericana.

Noël, L., Leblanc, J. C., & Guérin, T. (2003). Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: Estimation of daily dietary intake. *Food Additives and Contaminants, 20*, 44–56.

Pelus, E., Arnaud, J., Ducros, V., Faure, H., Favier, A., & Roussel, A. M. (1994). Trace element (Cu, Zn, Fe, Mn, Se) intakes of a group of French men using the duplicate diet technique. *International Journal of Food Science and Nutrition, 45*, 63–70.

Pennington, J. A. T., & Schoen, S. A. (1996). Total diet study: Estimated dietary intakes of nutritional elements 1982–1991. *International Journal for Vitamin and Nutritional Research, 66*, 350–362.

Planells, E., Sanchez-Morito, N., Montellano, M. A., Aranda, P., & Llopis, J. (2000). Effect of magnesium deficiency on enterocyte Ca, Fe, Cu, Zn, Mn and Se content. *Journal oh Physiology and Biochemistry, 56*, 217.

Sanchez-Morito, N., Planells, E., Aranda, P., & Llopis, J. (1999). Magnesium–manganese interactions caused by magnesium deficiency in rats. *Journal of the American College of Nutrition, 18*, 475–480.

Santos, E. E., Lauria, D. C., & Porto da Silveira, C. L. (2004). Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. *Science of the Total Environment, 327*, 69–79.

Schafer, U., Anke, M., Seifert, M., & Fischer, A. B. (2004). Influences on the manganese intake, excretion and balance of adults, and on the manganese concentrations of the consumed food determined by means of the duplicate portion technique. *Trace Elements and Electrolytes, 21*, 68–77.

Shiraishi, K. (2005). Dietary intakes of 18 elements and ^{40}K in 18 food categories by Japanese subjects. *Journal of Radioanalytical and Nuclear Chemistry, 206*, 61–69.

Tallkvist, J., Bowlus, C. L., & Lönnerdal, B. (2000). Functional and molecular responses of human intestinal Caco-2 cells to iron treatment. *American Journal of Clinical Nutrition, 72*, 770–775.

Thompson, K., Molina, R., Donaghey, T., Brain, J. D., & Wessling-Resnick, M. (2006). The influence of high iron diet on rat lung manganese absorption. *Toxicology and Applied Pharmacology, 210*, 17–23.

Tripathi, R. M., Mahapatra, S., Raghunath, R., Sastry, V. N., & Krishnamoorthy (2002). Daily intake of manganese by the adult population of Mumbai. *Science of the Total Environment, 250*, 43–50.

Velasco-Reynold, C., Navarro-Alarcon, M., López -G[a] de la Serrana, H., Perez-Valero, V., & Lopez-Martinez, M. C. (in press). Determination of daily dietary intake of chromium by duplicate diet sampling: In vitro availability study. *Food Additives and Contaminants*.

Velasco-Reynold, C., Navarro-Alarcon, M., López-G[a] de la Serrana, H., Perez-Valero, V., & Lopez-Martinez, M. C. (2007). Analysis of total and dialyzable copper levels in duplicate meals by ETAAS: Daily intake. *European Food Research and Technology*. doi:10.1007/s00217007-0730-9.

Wilplinger, M., Zochling, S., & Pfannhauser, W. (1999). An analysis of the manganese supply in Austria on the basis of a selected diet. *Zeitschrift Fur lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology, 208*, 251–253.

Windisch, W. (2002). Interaction of chemical species with biological regulation of the metabolism of essential trace elements. *Analytical and Bioanalytical Chemistry, 372*, 421–425.

Ysart, G., Miller, P., Crews, H., Robb, P., Baxter, M., De L'Argy, C., et al. (1999). Dietary exposure estimates of 30 elements from the UK total diet study. *Food Additives and Contaminants, 16*, 391–403.