Determinants of serum manganese levels in an Italian population

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Abstract. Manganese (Mn) is both essential and toxic for humans, mainly depending on the total levels and its species. Main sources of exposure include food and air pollution, particularly motorized traffic. We sought to determine the potential influence of these sources on serum total levels of Mn and Mn species. We selected a random sample of municipality residents from an Italian urban municipality, from whom we collected detailed personal information, dietary habits and a blood sample for serum Mn determination. We also assessed outdoor air Mn exposure, by modeling levels of particulate matter $\leq 10\,\mu m$ (PM_{10}) from motorized traffic at the residence of geocoded subjects. Serum Mn species generally showed higher levels in males and positive correlation with age, while no such differences were found according to smoking habits or use of dietary supplements. Among nutrients, only iron intake showed a relation with Mn [an inverse correlation with Mn-ferritin (Mn-Fer) and a direct one with inorganic-Mn (Inorg-Mn)]. Meat consumption directly correlated and fish and seafood inversely correlated with total Mn, Mn-transferrin (Mn-Tf) and Mn-citrate (Mn-Cit). Fruits and vegetables, including legumes and nuts, generally showed a positive correlation with all Mn species, especially Mn-Cit, and an inverse one with Inorg-Mn. Odds ratios (ORs) of having serum Mn levels above median value increased with increasing PM_{10} tertiles, with an OR for highest-to-lowest tertile of 7.40 (1.36-40.25) in multivariate analysis. Analyses for Mn species did not highlight a clear comparable pattern. In conclusion, our results seem to demonstrate that PM_{10} exposure positively influences total Mn serum levels, while single Mn species show conflicting results.

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Introduction

Manganese (Mn) is both an essential and a toxic trace element, mainly depending on its levels in human tissues and its speciation. As a component of enzymes such as superoxide dismutase (SOD), Mn is involved in oxidative stress response and it is also important for the maintenance of a normal bone structure (1). On the other hand, elevated Mn levels may induce neurotoxic effects, including a Parkison-like disease called manganism (2). Mn is a natural component of the environment and one of the main exposure routes is through the diet. Mn is primarily absorbed in the gastrointestinal tract, followed by the lung (3). The main dietary sources are cereals and tubers, fruits and vegetables, followed by meat, fish and seafood (4). Other foods with high Mn content include nuts, dried fruits and seeds, chocolate and tea leaves (3,5). Moreover, intestinal absorption (generally <10% of the ingested Mn) is influenced by iron storage status and intake (6), calcium or phosphorus intake (7), and by the use of dietary supplements (8), including soy formula (9). Besides dietary intake, Mn exposure may arise from its release into the air, soil and water from industries manufacturing products containing Mn such as pesticides and Mn alloys, from mining activities, and from automobile exhausts, leading to environmental contamination of potential public health concern (10,11). Among the toxic metals associated with fine particulate matter (PM) in the ambient

air (12), there are Mn residues depending on the season and on the sampling site, residential, industrial or rural (13). Finally, tobacco smoke, including second-hand smoke, may expose to Mn at levels higher than those not exposed (14), although not all results are consistent (15).

The attempt to identify reliable biomarkers of Mn exposure yielded conflicting results, since whole blood levels seem to be very variable and of limited value as biomarker of intake (7). Serum and plasma levels could be more sensitive to variations of dietary intake and have half-life ranging between 13-37 days, thus being short-term indicators of exposure (3). A more recent and accurate approach to address the issue of Mn exposure includes analysis of the different Mn species, namely Mn-ferritin (Mn-Fer), Mn-transferrin (Mn-Tf), Mn-citrate (Mn-Cit) and inorganic-Mn (Inorg-Mn) (10,16).

In this cross-sectional study we aimed to assess the influence on serum total levels of Mn and its species of foods known as major contributors of Mn intake, personal life style and habits, and air pollution, and to investigate the relation between serum Mn and other dietary elements such as iron, calcium and phosphorus.

Materials and methods

Study participants. The methodology for the recruitment of the study population was previously described (17,18). Briefly, by accessing the databases of the Modena Municipality General Registry Office and following the approval of the local Ethics Committee, we recruited a random sample of 50 subjects residing in Modena, a municipality located in the Emilia-Romagna region (~180,000 inhabitants). To do that, we randomly selected a list of eligible subjects from each gender- and age-specific subgroup of the Modena adult population, using the 'sample' routine of the Stata-11 statistical software (Stata Corp., College Station, TX, USA). We contacted these subjects by phone asking for their participation in the study. The 34% of subjects who accepted to participate were invited in the morning to the Modena Health Unit for collection of a fasting blood sample after they gave their written consent.

Laboratory analyses. Blood samples were collected in a plastic tube, immediately centrifuged at 1,000 x g for 10 min and stored as serum aliquots of 1 ml at -15˚C until use. We determined serum Mn concentrations at the laboratory of the Research Center for Environmental Health (Research Unit Analytical BioGeoChemistry, Neuherberg, Germany), where a 1 ml aliquot for each study subject was transferred frozen in dry ice, using the previously described methodology (19,20).

Total Mn determination: briefly, we slowly thawed the samples in a refrigerator at 4˚C, vortexed and subsequently diluted 500 μl aliquots of samples 1:10 with Milli-Q water (Millipore, Bedford, MA, USA), containing ^{103}Rh as internal standard. The final ^{103}Rh concentration in the diluted serum samples was 1 μg/l. An ELEMENT 2 [Thermo Fisher Scientific, Bremen, Germany] inductively coupled plasma-sector field mass spectrometry (ICP-SFMS) instrument was employed for ^{55}Mn determination in medium resolution mode. Sample introduction was carried out using a peristaltic pump connected to a SeaSpray™ Nebulizer with a cyclon spray chamber (Perkin-Elmer Inc., Waltham, MA, USA). The RF power was set to 1,200 W, the plasma gas was 15 l/min Ar, whereas the nebuliser gas was ~0.9 l/min Ar after daily optimization.

Mn species determination: Mn speciation was performed as previously described (16) using size-exclusion chromatography (SEC) coupled to ICP-dynamic rection cell (DRC)-MS. In short terms, SEC (mass vs. retention time calibrated) was performed with a Knauer 1100 Smartline inert series gradient HPLC system with a 100 μl injection loop (Knauer, Berlin, Germany) and further to two serially installed SEC columns separation ranges ca. 200-10 kDa and 2-0.1 kDa. This column combination provided separation of various Mn-proteins from each other and from Mn-Cit as well as the latter clearly from Inorg-Mn. Tris-HAc (10 mM, pH 7.4) + 250 mM NH_4Ac was used as the eluent at a flow-rate of 0.75 ml/min. The column effluent was directly provided to ICP-DRC-MS. A PerkinElmer NexION ICP-MS (Sciex, Toronto, ON, Canada) with dynamic reaction cell capability was employed for on-line determination of ^{55}Mn in the graphic mode. For SEC coupling the column effluent was passing the UV detector and then directed to a Meinhard® nebulizer (Meinhard Co., Golden, CO, USA) (which was mounted to a cyclone spray chamber) using a PEEK transfer tube (ID 100 μm; Perkin-Elmer Inc.). The RF power was set to 1,250 W, the plasma gas was 15 l/min Ar. The nebulizer gas was optimized and finally set to 0.98 ml/min Ar. The dwell time was 500 msec. The dynamic reaction cell (DRC) was operated using NH_3 as DRC gas, finally at a flow rate of 0.58 ml/min. The DRC band pass (q) was set to 0.45 LOD for Mn-peaks in SEC-ICP-DRC-MS were calculated as 3 σ-criterion and found between 28-35 ng/l, thus, uniformly set to 35 ng/l.

Exposure assessment. We collected from study participants general information including smoking habits, education, occupational history and the use of dietary supplements during the last year. We also assessed their dietary habits through a semi-quantitative food frequency questionnaire used for the Central-Northern Italian population within the EPIC study (21). This questionnaire assessed the frequency and amount of consumption of 188 food items over the previous year, and allowed to estimate frequency and quantity of consumption of the individual foodstuff and related intake of nutrients and contaminants through an ad hoc software (22,23). With regard to the intake of such nutrients, we selected only those previously reported to influence Mn content, i.e., iron, calcium and phosphorus (6,7). Finally, foodstuff giving the major contribution of Mn intake were selected according to the Mn EFSA scientific opinion and the Italian Total Diet Study (4,5). The food categories included were cereals, meat, fish and seafood, dairy products, chocolate, fruits, vegetables, legumes, nuts and tea consumption.

We also considered the participants exposure to traffic contaminants as a proxy of outdoor Mn exposure, by estimating the concentration of particulate matter <10 μm (PM_{10}) at their residences as described elsewhere (18,24,25). Previous studies showed that motor vehicle emissions are important sources of ambient Mn (26,27). We implemented this approach since traffic was expected to be by far the main source of exposure for this population, also for the absence of major industrial emissions in the study area. To do that, we took into account the road distribution and the location of the municipal solid waste incinerator and its modeled emissions (28). Briefly,

we used Google Earth to geocode the home address of all study subjects and we modeled the average ambient air PM_{10} concentration at these locations for 2006, using the CAlifornia LINE version 4 (CALINE4 air quality dispersion model; CALTRANS 1989, California Department of Transportation) air quality dispersion model for roads and other linear sources (29). The 2006 assessment carried out in Modena (24) was generated by a model including: i) demographic and occupational information for all residents of the provinces; ii) detailed personal mobility information collected by the National Institute of Statistics 2001 Census; and iii) validated through ad hoc surveys and automatic vehicle counters. The model yielded a matrix of vehicle movements for each road, based on daily movements estimated for the residents of Modena considering their age, gender, family structure and occupation (30). This model was validated in the study area by comparing measured and modeled PM_{10} levels in the air monitoring stations (24). Limited changes occurred in the municipal area with respect to circulating vehicles (from 117,310-115,887) and to the census of adult population (from 152,372-155,998), since the year of exposure assessment (2006) until the beginning of the study (2011), according to the data released by the Modena municipality (www.comune.modena.it/serviziostatistica/pubblicazioni/annuari/annuario2012/incidenti2012/inci_tav2012.shtml).

Data analysis. We calculated median and interquartile range (IQR) for total Mn and for all Mn species. Since we found a subject showing an extremely high level of total Mn (22.6 μg/l), we considered this sample as an outlier and removed it from data analyses. Pearson's coefficients and 95% confidence intervals (CI) were calculated between Mn species and continuous variables (age, BMI, energy and food and nutrients intake, estimates of PM_{10}). Influence of several dietary items and nutrients were tested also using bivariate and multivariate regression models. In order to reduce the influence of measurement errors that are frequently produced by food frequency questionnaires, we adjusted the estimates of dietary nutrients, e.g., iron intake, for total energy intake, using Willett's residual method (31). In order to test the effect of outdoor environmental Mn levels on the serum levels of the different Mn species, we performed bivariate and multivariate logistic regression analysis using as endpoint the odds ratio (OR) of being above the median value of the Mn species.

When Mn species had values below the limit of detection (0.04 μg/l), they were set to 0.02 (=LOD/2). We carried out sensitivity analyses with different methods, i.e., by replacing LOD/2 with zero or by dividing it by $\sqrt{2}$, and/or the use of log-transformed values, and finally with winsorized values by setting data exceeding the 5th/95th percentile to the 5th/95th percentile. Dealing with censored data in some Mn-species, we tested also the tobit regression ('tobit' routine in STATA-14), which is specifically designed to estimate linear relations between variables when there is either left or right censoring in the dependent variable (32,33).

Results

Table I summarizes the main characteristic of the study population. Age ranged between 35-70 years with mean ± SD value of 52.3 (±10.4), with males and females equally represented. Eight subjects reported consumption of dietary supplements containing a mean value of 1.25 mg/day of Mn (in the form of Mn sulphate monohydrate) at the time of the sampling. Distribution of total Mn serum levels and specific species are presented in Table II. Overall, total Mn ranged from 0.72-8.18 μg/l, with median value of 2.32 (IQR, 1.81-3.06). Total Mn levels and Mn-Tf show slightly higher levels in males and in people aged ≥50 years, while no relevant difference was detected according to BMI categories or smoking habits independently from supplement use, whereas Mn-Fer, Mn-Cit and Inorg-Mn showed little variation among the categories.

Mn total level positively correlated with individual Mn species except for Inorg-Mn, which demonstrated a negative correlation with Total Mn and with the other species. Moreover, Mn-Cit shows a positive correlation with both Mn-Fer and Mn-Tf, which in turn directly correlated with

Table I. Characteristics of study population.

Study population	n (%)
All subjects	50 (100)
Gender	
Males	26 (52)
Females	24 (48)
Age (years)	
35-49	22 (44)
50-70	28 (56)
BMI	
<18.5	1 (2)
18.5-24.9	21 (42)
>25	28 (56)
Education	
Elementary school	3 (6)
Middle school	10 (20)
High school	23 (46)
College or higher	14 (28)
Occupation	
Agriculture	0 (0)
Industry	6 (12)
Services[a]	27 (53)
Housewife	2 (4)
Retired	11 (21)
Other	5 (10)
Smoking habits	
Never smokes	25 (50)
Former smokers	16 (32)
Current smokers	9 (18)
Mn supplement users	
Not users	42 (84)
Users	8 (16)

[a]Health, education and business.

Table II. Distribution with median (50th) and interquartile range (IQR) of total serum Mn and its species in overall population and selected subgroups (values in μg/l).

	Total Mn		Mn-Fer		Mn-Tf		Mn-Cit		Inorg-Mn	
Study subjects (N)	50th	IQR	50th	IQR	50th	IQR	50th	IQR	50th	IQR
All participants	2.36	(1.81-3.06)	0.33	(0.13-0.55)	0.76	(0.18-1.24)	0.55	(0.24-0.84)	0.20	(0.02-0.62)
Gender										
Males (26)	2.47	(2.06-3.06)	0.28	(0.15-0.37)	0.84	(0.21-1.32)	0.55	(0.27-0.83)	0.16	(0.02-0.59)
Females (24)	2.07	(1.65-3.39)	0.40	(0.11-1.12)	0.55	(0.11-1.11)	0.54	(0.14-0.88)	0.24	(0.10-0.75)
Age (years)										
<50 (22)	2.27	(1.70-2.68)	0.29	(0.15-0.43)	0.43	(0.17-1.45)	0.50	(0.09-1.12)	0.19	(0.15-0.54)
≥50 (28)	2.42	(1.97-3.80)	0.35	(0.12-0.56)	0.87	(0.19-1.23)	0.56	(0.27-0.78)	0.21	(0.02-1.10)
BMI										
<25 (22)	2.13	(1.60-2.68)	0.27	(0.11-0.55)	0.64	(0.18-1.09)	0.54	(0.12-0.75)	0.37	(0.05-0.62)
≥25 (28)	2.42	(1.98-3.80)	0.35	(0.14-0.56)	0.91	(0.17-1.63)	0.56	(0.24-1.12)	0.17	(0.02-0.64)
Smoking habits										
Non-smokers (25)	2.32	(2.05-3.18)	0.35	(0.12-0.56)	0.76	(0.26-1.45)	0.53	(0.24-1.30)	0.21	(0.02-0.64)
Ex-smokers (16)	2.29	(1.71-3.03)	0.37	(0.11-0.51)	0.24	(0.09-1.13)	0.56	(0.17-0.89)	0.47	(0.02-1.08)
Smokers (9)	2.10	(1.78-2.63)	0.23	(0.22-0.35)	0.91	(0.18-1.24)	0.57	(0.26-0.71)	0.16	(0.02-0.29)
Mn supplement users										
No (42)	2.29	(1.93-3.18)	0.32	(0.14-0.56)	0.68	(0.17-1.28)	0.56	(0.24-0.84)	0.21	(0.02-0.64)
Yes (8)	2.39	(1.73-2.45)	0.35	(0.11-0.55)	0.82	(0.24-0.96)	0.46	(0.06-0.93)	0.16	(0.02-0.58)

IQR, interquartile range; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

Table III. Pearson's correlation coefficients (r) and 95% confidence intervals (CI) between total serum Mn and its species.

	Mn-Fer	Mn-Tf	Mn-Cit	Inorg-Mn
Mn-Fer	1			
Mn-Tf	0.222 (-0.063 to 0.473) P=0.126	1		
Mn-Cit	0.204 (-0.082 to 0.459) P=0.160	0.259 (-0.024 to 0.504) P=0.072	1	
Inorg-Mn	-0.193 (-0.449 to 0.094) P=0.185	-0.332 (-0.561 to -0.056) P=0.020	-0.145 (-0.409 to 0.142) P=0.314	1
Total Mn	0.462 (0.208 to 0.658) P<0.001	0.676 (0.487 to 0.804) P<0.001	0.694 (0.512 to 0.816) P<0.001	0.091 (-0.195 to 0.363) P=0.534

CI, confidence intervals; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

each other (Table III, Fig. 1). Sensitivity analyses, using a different substitution method for values below the LOD, or log or winsorized transformation did not alter the results (data not shown).

Correlations of Mn species with individual characteristics and dietary factors are reported in Table IV. Generally, age demonstrated a strong positive relation with all but one Mn species, while no association emerged for BMI. Total energy intake showed a strong correlation only with Inorg-Mn, while among nutrients, iron intake showed an inverse relation with Mn-Fer and a positive one with Inorg-Mn. Phosphate was slightly inversely associated with all Mn-species except Mn-Tf, while calcium did not show such strong correlation. Finally, Table V showed Pearson's correlation coefficients of Mn with

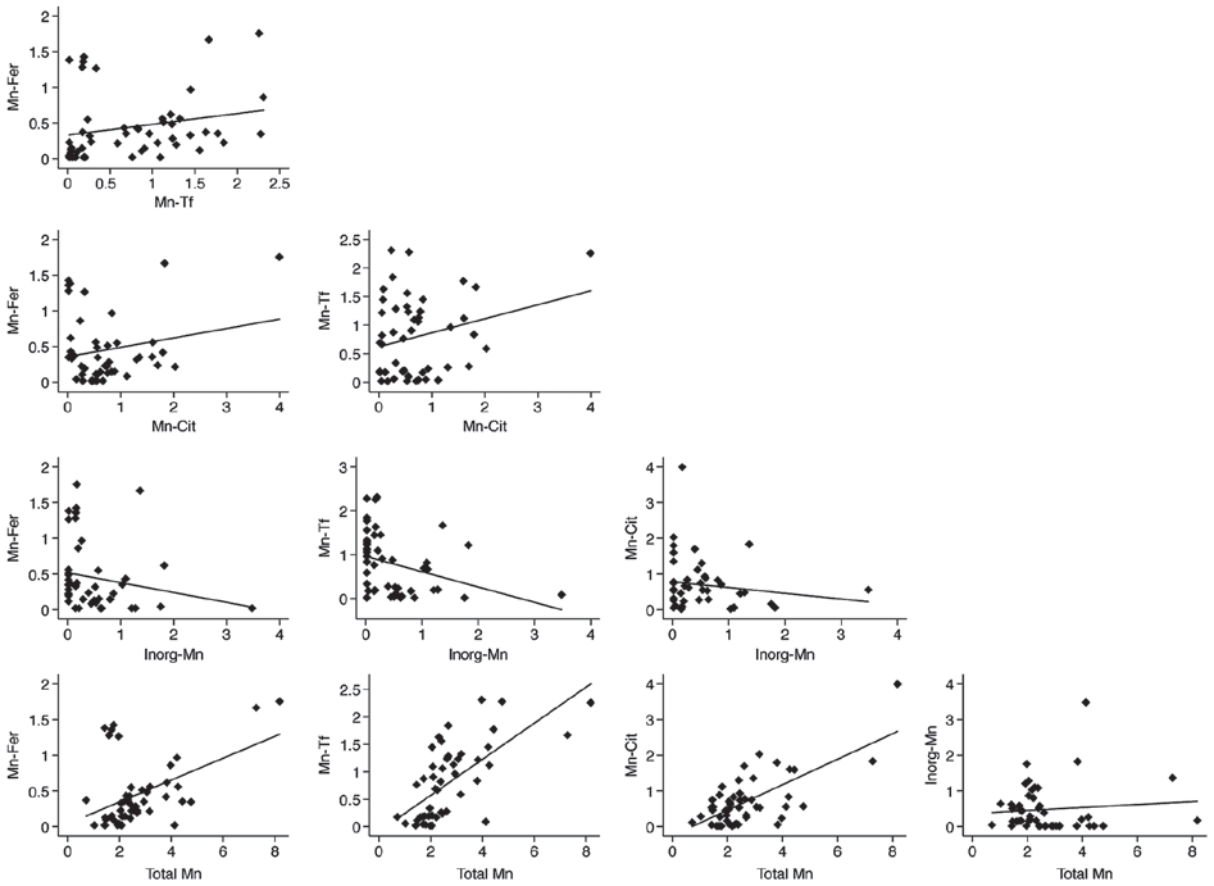

Figure 1. Scatter plots between total serum Mn and its species (μg/l) with linear regression fitted values (solid line). Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

the main food items. Generally, cereals and dairy products intake did not correlate with Mn serum levels. Meat consumption demonstrated an inverse relation with Mn-Fer levels, while a positive one emerged for Inorg-Mn. Also fish and seafood showed an inverse relation with total Mn, mainly for Mn-Cit and Mn-Tf. Fruit intake demonstrated a positive correlation with all Mn species, except with Inorg-Mn. Similar results were found for vegetable intake, showing a positive relation with Mn-Tf and Mn-Cit and an inverse one with Inorg-Mn. Pulse and nut intake positively correlated with quite all Mn-species, mainly with Mn-Cit. Finally chocolate and tea consumption demonstrated little correlation with Mn-species. In general, this was true both for linear and tobit regression analyses, and in both bivariate and multivariate analyses, adjusted for age, gender and total energy intake (data not shown).

OR of having serum levels of Mn species above the median value for increasing PM_{10} tertile are shown in Table VI, Fig. 2. For total Mn, OR increased with increasing PM_{10} tertiles. When Mn species were considered, Mn-Fer and Inorg-Mn showed an increased OR in the intermediate category, but a decreased risk in the highest category, while Mn-Tf and Mn-Cit showed a decreased OR in both intermediate and highest categories.

Discussion

An adequate assessment of Mn exposure is an important public health issue, for which evidence is still rather limited (34-36). Serum total Mn distribution in our random sample of Modena municipality is similar or slightly higher than those reported in previous Italian (37-40) and European adult populations (41-48), as we found a median/mean value of 2.32/2.68 μg/l, while other studies reported median/mean values ranging from 0.18 to 3.30 μg/l. These results also allow to rule out any contamination of our samples during their collection and management, as frequently occurred when using serum specimens (49,50), also taking into account the results of a recent study on healthy subjects (51).

Interestingly, similarly to our previous findings (16), we observed a switch in Mn-carrier from Mn-Tf to Mn-Cit at increasing concentration of Total Mn (data not shown). Moreover, Mn-Tf demonstrated a stronger correlation for values below the cut point of 4 μg/l, whereas above this value it no longer increased, while Mn-Cit showed an opposite behavior. In accordance with previous studies, the present results show no significant gender-related difference in serum Mn levels, despite a slightly higher concentration in males. Similarly to other studies, no relevant differences in Mn levels were detected according to age (41,43), BMI (52) or smoking habits of study participants (41,46,53). Conversely, the use of Mn-containing supplements seems not to affect the Mn serum levels, differently from what was expected (8).

Our study subjects were fasting because the blood samples were taken in the morning, however, to our knowledge, no author has described either any influence of an omitted meal on Mn serum concentration or a circadian variation for the element. In contrast to the high variation demonstrated by

Table IV. Pearson's correlation coefficients (r) and 95% confidence intervals (CI) between Mn serum species and characteristic and nutrients intake of study subjects.

	r	95% CI	P-value
Age			
Total Mn	0.307	(0.028 to 0.541)	0.032
Mn-Fer	0.235	(-0.049 to 0.485)	0.126
Mn-Tf	0.194	(-0.092 to 0.451)	0.181
Mn-Cit	0.146	(-0.141 to 0.410)	0.319
Inorg-Mn	0.097	(-0.189 to 0.368)	0.508
Total energy intake			
Total Mn	0.038	(-0.246 to 0.315)	0.798
Mn-Fer	-0.179	(-0.438 to 0.107)	0.217
Mn-Tf	-0.170	(-0.431 to 0.116)	0.242
Mn-Cit	0.072	(-0.213 to 0.347)	0.621
Inorg-Mn	0.376	(0.106 to 0.595)	0.008
Calcium intake			
Total Mn	-0.104	(-0.375 to 0.182)	0.476
Mn-Fer	-0.112	(-0.381 to 0.175)	0.443
Mn-Tf	0.164	(-0.123 to 0.425)	0.262
Mn-Cit	-0.121	(-0.390 to 0.165)	0.404
Inorg-Mn	-0.156	(-0.419 to 0.131)	0.285
BMI			
Total Mn	0.029	(-0.254 to 0.308)	0.843
Mn-Fer	0.026	(-0.258 to 0.304)	0.862
Mn-Tf	-0.041	(-0.318 to 0.243)	0.783
Mn-Cit	0.110	(-0.176 to 0.380)	0.451
Inorg-Mn	0.035	(-0.249 to 0.313)	0.811
Iron intake			
Total Mn	0.047	(-0.238 to 0.324)	0.751
Mn-Fer	-0.188	(-0.445 to 0.099)	0.196
Mn-Tf	-0.046	(-0.323 to 0.239)	0.756
Mn-Cit	0.001	(-0.281 to 0.282)	0.997
Inorg-Mn	0.255	(-0.028 to 0.501)	0.077
Phosphate intake			
Total Mn	-0.203	(-0.458 to 0.083)	0.162
Mn-Fer	-0.230	(-0.480 to 0.055)	0.112
Mn-Tf	0.002	(-0.279 to 0.283)	0.988
Mn-Cit	-0.199	(-0.455 to 0.087)	0.171
Inorg-Mn	-0.017	(-0.297 to 0.265)	0.906

CI, confidence intervals; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

Mn whole blood concentrations that seemed to be extremely variable which may preclude it as a viable status indicator (7), serum Mn concentration appeared to be somewhat sensitive to large variations in Mn intake and response to dietary intake (8). Moreover, among the several biomarkers investigated in human studies, only hair (54,55) and plasma or serum were representative of Mn average exposure, with corresponding half-life between 13-37 days of serum Mn levels (3).

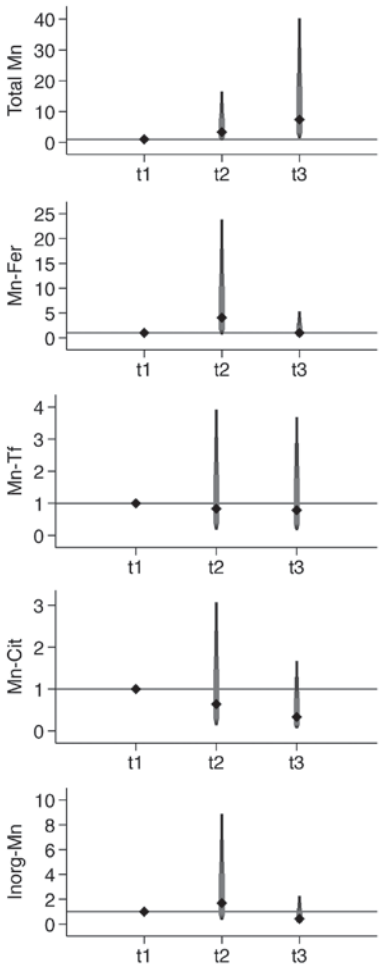

Figure 2. ORs with 95% CI for Mn species above median with increasing tertiles of PM_{10}. ORs for model adjusted for age, gender, total energy and iron intake, smoking habits and cadmium serum levels is reported. ORs, odds ratios; CI confidence intervals; PM_{10}, particulate matter ≤10 μm; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

The regulation of Mn absorption is very efficient and appears to be one of the adaptive responses to dietary Mn intake, and such regulation allows Mn homeostasis to be maintained over a wide range of intakes (7). The main food contributors (>5% of the total intake) to Mn intake in European population and in particular in Northern Italy, are cereal-based products, vegetables, fruits, fruit products and beverages (coffee, tea, alcoholic beverages) (4,5). In our population we did not clearly identify a positive relation between Mn levels and cereal consumption, while vegetable intake, especially legumes, and partially dry fruits (nuts), showed a positive relation. Interestingly, we observed an inverse relation of Mn serum levels with meat and fish and seafood consumption, possibly reflecting the influence of iron intake. Consistently, when we performed a regression model between meat intake and Mn levels, the inclusion of iron intake in the model toned down the observed inverse relation (data not shown). Also, serum ferritin has been noted to be inversely associated with blood Mn concentration (56). Finally, in out study tea and chocolate consumption was not associated with the serum Mn levels, although about one third of the study participants reported no tea consumption, so weakening our possibility to find any association.

Table V. Pearson's coefficients coefficients (r) and 95% confidence intervals (CI) between total serum Mn and its species with food intake of study subjects.

	r	95% CI	P-value
Cereal			
Total Mn	0.122	(-0.165 to 0.390)	0.404
Mn-Fer	0.088	(-0.198 to 0.360)	0.547
Mn-Tf	0.038	(-0.246 to 0.316)	0.795
Mn-Cit	0.077	(-0.209 to 0.350)	0.600
Inorg-Mn	0.050	(-0.235 to 0.327)	0.733
Meat			
Total Mn	0.082	(-0.204 to 0.355)	0.574
Mn-Fer	-0.238	(-0.487 to 0.046)	0.099
Mn-Tf	-0.064	(-0.339 to 0.222)	0.665
Mn-Cit	0.059	(-0.226 to 0.335)	0.687
Inorg-Mn	0.306	(0.027 to 0.541)	0.032
Fish and seafood			
Total Mn	-0.217	(-0.470 to 0.068)	0.134
Mn-Fer	0.059	(-0.226 to 0.334)	0.688
Mn-Tf	-0.197	(-0.453 to 0.090)	0.176
Mn-Cit	-0.207	(-0.461 to 0.079)	0.154
Inorg-Mn	-0.036	(-0.314 to 0.248)	0.806
Milk and dairy products			
Total Mn	-0.087	(-0.359 to 0.199)	0.552
Mn-Fer	-0.071	(-0.345 to 0.215)	0.629
Mn-Tf	-0.075	(-0.348 to 0.211)	0.611
Mn-Cit	-0.040	(-0.317 to 0.244)	0.786
Inorg-Mn	-0.011	(-0.291 to 0.271)	0.943
Chocolate			
Total Mn	-0.110	(-0.380 to 0.176)	0.451
Mn-Fer	-0.161	(-0.423 to 0.126)	0.270
Mn-Tf	0.015	(-0.267 to 0.295)	0.919
Mn-Cit	-0.194	(-0.267 to 0.295)	0.182
Inorg-Mn	0.082	(-0.204 to 0.355)	0.574
Fruit			
Total Mn	0.139	(-0.148 to 0.404)	0.341
Mn-Fer	0.134	(-0.153 to 0.400)	0.361
Mn-Tf	0.020	(-0.263 to 0.299)	0.892
Mn-Cit	0.148	(-0.139 to 0.412)	0.311
Inorg-Mn	-0.023	(-0.302 to 0.260)	0.875
Vegetables			
Total Mn	0.342	(0.067 to 0.568)	0.016
Mn-Fer	0.157	(-0.130 to 0.420)	0.282
Mn-Tf	0.246	(-0.038 to 0.493)	0.089
Mn-Cit	0.388	(0.120 to 0.603)	0.006
Inorg-Mn	-0.192	(-0.449 to 0.095)	0.187
Legumes			
Total Mn	0.316	(0.038 to 0.548)	0.027
Mn-Fer	0.175	(-0.112 to 0.434)	0.230
Mn-Tf	0.120	(-0.167 to 0.388)	0.412
Mn-Cit	0.302	(0.022 to 0.537)	0.035
Inorg-Mn	0.182	(-0.105 to 0.441)	0.211
Nuts			
Total Mn	0.140	(-0.147 to 0.405)	0.337
Mn-Fer	0.158	(-0.129 to 0.420)	0.279
Mn-Tf	0.099	(-0.188 to 0.370)	0.499
Mn-Cit	0.252	(-0.031 to 0.498)	0.081
Inorg-Mn	-0.144	(-0.409 to 0.143)	0.324
Tea			
Total Mn	-0.045	(-0.322 to 0.239)	0.760
Mn-Fer	0.049	(-0.235 to 0.326)	0.737
Mn-Tf	0.039	(-0.245 to 0.317)	0.791
Mn-Cit	-0.074	(-0.348 to 0.212)	0.613
Inorg-Mn	-0.108	(-0.378 to 0.178)	0.458

CI, confidence intervals; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

The assessment of air-Mn in order to evaluate the influence of air pollution on human Mn exposure and the related health implications is of relevant and growing interest. Previous Italian studies, which assessed levels of PM_{10} air-Mn using a 24-h personal sampling, reported deficits in olfactory and motor neuron function in the higher exposed group of both elderly (57) and adolescents (58), as well an increased risk of asthma only in the youngest population (35). More recently, in the absence of facility emissions data for individual study subjects, a US study used the long-term air measurements available from local air monitoring stations in order to derive receptor-specific concentrations of respirable air Mn through total suspended particulates (TSP), PM_{10} and $PM_{2.5}$ (59).

Our results indicate that increasing tertiles of PM_{10} exposure are likely to be associated with Mn serum levels above the median, but only when total Mn is considered. Similarly, a study carried out in Canada showed that geographic regions with higher levels of airborne Mn contributed positively to total Mn blood levels (60) and a recent study carried out in welders showed that plasma Mn correlates with air-Mn during occupational exposure (61). However, in the present study the relation between increasing PM_{10} tertiles and serum Mn was less evident when Mn species were considered, especially for Mn-Cit and Mn-Tf which demonstrated an inverse trend with respect to total Mn, while Mn-Fer and Inorg-Mn analyses yielded some evidence of a direct relation but no clear dose-response association. A possible explanation of these conflicting results could be associated to the fact that total Mn is not simply the sum of considered Mn carriers, but it includes additional Mn chemical forms. Actually, overall Mn in serum could be bound to α-2-macroglobulin and also to albumin fraction, thus other non-measured Mn species could be influenced by airborne-Mn and may explain the positive association highlighted only for total Mn.

A strength of our study for Mn exposure assessment was the analysis of various Mn species in serum (16). The switch from the overall content of a trace element to the single

Table VI. Odds ratios (ORs) and 95% confidence intervals (CI) for having Mn species above the median value and increasing tertiles of estimated PM_{10}. Median estemated PM_{10} values ($\mu g/m^3$) for each tertile and median value ($\mu g/l$) for each Mn species is reported for each tertile.

	PM_{10} tertiles (median)			
	I (11.06)	II (14.22)	III (18.37)	P trend[e]
Total Mn				
Median	2.05	2.37	2.46	
Crude model	1.00	3.09 (0.73-12.98)	5.28 (1.20-23.32)	0.105
Adjusted model[a]	1.00	2.78 (0.60-12.90)	5.43 (1.15-25.91)	0.124
Adjusted model[b]	1.00	2.91 (0.62-13.76)	5.47 (1.15-25.91)	0.118
Adjusted model[c]	1.00	3.46 (0.69-17.26)	7.54 (1.37-41.57)	0.085
Adjusted model[d]	1.00	3.35 (0.68-16.53)	7.40 (1.36-40.25)	0.094
Mn-Fer				
Median	0.23	0.40	0.25	
Crude model	1.00	1.87 (0.47-7.53)	0.87 (0.22-3.45)	0.101
Adjusted model[a]	1.00	3.07 (0.61-15.48)	0.80 (0.17-3.83)	0.200
Adjusted model[b]	1.00	3.21 (0.63-16.37)	0.80 (0.16-3.88)	0.203
Adjusted model[c]	1.00	3.76 (0.70-20.15)	0.97 (0.19-5.04)	0.262
Adjusted model[d]	1.00	4.06 (0.69-23.82)	1.01 (0.19-5.35)	0.268
Mn-Tf				
Median	0.76	0.79	0.75	
Crude model	1.00	0.89 (0.23-3.49)	0.89 (0.23-3.49)	0.996
Adjusted model[a]	1.00	0.88 (0.20-4.00)	0.86 (0.20-3.74)	0.713
Adjusted model[b]	1.00	0.96 (0.20-4.54)	0.85 (0.19-3.82)	0.636
Adjusted model[c]	1.00	0.90 (0.18-4.42)	0.76 (0.16-3.74)	0.704
Adjusted model[d]	1.00	0.83 (0.18-3.92)	0.79 (0.17-3.68)	0.754
Mn-Cit				
Median	0.71	0.54	0.49	
Crude model	1.00	0.70 (0.18-2.77)	0.54 (0.14-2.17)	0.117
Adjusted model[a]	1.00	0.81 (0.19-3.50)	0.52 (0.12-2.19)	0.099
Adjusted model[b]	1.00	0.72 (0.16-3.26)	0.51 (0.12-2.21)	0.081
Adjusted model[c]	1.00	0.59 (0.13-2.79)	0.35 (0.07-1.73)	0.030
Adjusted model[d]	1.00	0.64 (0.13-3.07)	0.33 (0.07-1.67)	0.022
Inorg-Mn				
Median	0.45	0.23	0.08	
Crude model	1.00	0.90 (0.23-3.58)	0.42 (0.10-1.70)	0.312
Adjusted model[a]	1.00	1.43 (0.30-6.76)	0.34 (0.07-1.64)	0.240
Adjusted model[b]	1.00	1.34 (0.27-6.53)	0.33 (0.07-1.60)	0.207
Adjusted model[c]	1.00	1.66 (0.32-8.68)	0.46 (0.09-2.42)	0.400
Adjusted model[d]	1.00	1.69 (0.32-8.88)	0.42 (0.08-2.29)	0.339

[a]Adjusted for gender, age, total energy intake and iron intake; [b]Adjusted for gender, age, total energy intake, iron intake and use of supplement containing Mn; [c]Adjusted for gender age, total energy intake, iron intake, use of supplement containing Mn and smoking habits; [d]Adjusted for gender, age, total energy intake, iron intake, smoking habits and cadmium serum levels; [e]P trend calculated using continuous values. OR, odds ratios; CI, confidence intervals; PM_{10}, particulate matter ≤10 μm; Mn-Fer, Mn-ferritin; Mn-Tf, Mn-transferrin; Mn-Cit, Mn-citrate; Inorg-Mn, inorganic-Mn.

speciation analysis is of growing interest in medicine, due to the different, and in some cases opposite effects that could be yielded on target tissues by different species, as previously demonstrated for other trace elements, i.e., aluminium, lead and selenium (17,62-64) and recently for Mn (65,66). Finally, the reliability of results did not appear to be affected by the values below the LOD, as shown by the sensitivity analyses.

This study has some limitations. First, dietary information was collected with self-administered questionnaires that although validated for the Northern Italian population (67), may still carry some inaccuracies and induce some exposure

misclassification. Secondly, our sample size was small, due to difficulties in recruiting 'healthy' volunteers from the general population despite our efforts, thus, reducing the statistical power of the study, especially in subgroup analyses, as reflected by the generally wide CI of the point estimates. However, despite the limited sample size, the selection of study participants is unlikely to be biased as extraction was randomly performed and characteristics of included subjects appeared to be similar to those of the general Italian population (68,69). Finally, we relied on PM assessment in outdoor air and not directly on airborne or deposited Mn levels. However, in a study carried out in Greece, the analysis of size distribution and composition of airborne particulate matter and associated Mn in the roadside environment showed that ~1/3 of Mn content is detected in coarse (i.e., PM_{10}) particles (70). Furthermore, a similar study in the Paris region, demonstrated a higher contribution of Mn in the PM_{10} fraction, due to road dust resuspension, break and tire wear, and road wear abrasion (26).

In conclusion, our study is one of the few to assess the relation between total Mn levels and its species in serum, and the influence of different sources of exposure. In particular, the inverse correlation is of interest between meat intake and serum Mn concentrations, mainly Mn-Fer, probably due to the associated changes in iron intake. Another interesting finding was the positive correlation between vegetable and fruit intake and Mn levels, especially Mn-Cit. Finally, results suggest that PM_{10} exposure increases total Mn serum levels, while its relation with the single Mn species was inconsistent.

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