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Hypertension

Ambulatory Blood Pressure in Relation to Plasma and Urinary Manganese

Running Title: Blood Pressure in Relation to Manganese

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Abstract—The association of blood pressure (BP) with manganese, an essential trace element required for human health, remains poorly studied. In 734 randomly recruited Swiss participants (mean age, 47.5 years; 51.4% women), we related ambulatory BP to two biomarkers, plasma manganese (pMn) and the urinary manganese excretion (uMn). To allow for diurnal variation, we assessed BP and uMn over 24-h and during wakefulness and sleep, using split urine samples. 24-h, day- and night-time systolic/diastolic BPs averaged 119.8/78.1, 123.8/81.2 and 107.0/68.3 mm Hg; the corresponding medians uMn were 199.5, 83.0, 51.5 μmol and median pMn 0.52 μg/L. In analyses dichotomized by the median of the biomarkers, greater pMn was associated with higher 24-h systolic/diastolic BP (+4.1/+2.3 mm Hg; $P \leq 0.0003$), greater daytime uMn with lower daytime BP (-3.5/-1.9 mm Hg; $P \leq 0.0067$) and greater nighttime uMn with higher nighttime BP (+2.9/+1.2 mm Hg; $P \leq 0.046$). In multivariable-adjusted analyses, significance ($P \leq 0.030$) was retained for the positive association of 24-h and daytime diastolic BP with pMn and for systolic BP in relation to uMn at night. The association sizes for a twofold increment in the biomarkers amounting to 0.77 mm Hg (95% confidence interval [CI], 0.08–1.47 mm Hg), 0.97 (CI, 0.20–1.76) and 1.33 (CI, 0.20–2.50 mm Hg), respectively. In conclusion, there were positive associations between diastolic BP and pMn over 24 h and during daytime and between systolic BP and uMn at night.

Key Words: Blood pressure ■ environmental exposure ■ hypertension ■ manganese ■ population science.

Introduction

Manganese is an essential trace element required for human health.[1-4] It acts as a cofactor in the active centers of various enzymes, is required for maintaining the integrity of the nervous and immune system,[1,2,4] regulation of mitochondrial[2] and endothelial[1] function, protection against oxidative stress,[2] and the transmembrane transport of various divalent cations via, among others, the zinc ion transmembrane transporter (ZIP8),[4,5] which is encoded by the *SLC39A8* gene.[6] The phenotypes associated with the *rs13107325 SLC39A8* missense variant include blood pressure,[7] blood manganese levels,[8] high-density lipoprotein (HDL) cholesterol[9] and Crohn's disease.[10] A manganese intake slightly above the dietary requirement induces a pro-inflammatory status.[11]

There is an abundant literature on manganese as neurotoxic agent,[3,12] whereas the potential association of cardiovascular function[1] or blood pressure[13-17] with biomarkers of manganese exposure remains poorly studied. Moreover, the scarce reports focusing on blood pressure produced contradictory results,[13-17] three studies[13,14,17] reporting an inverse association of blood pressure with manganese, whereas others reported positive associations.[15,16] All previous studies[13-17] ignored the diurnal profile of blood pressure[18] and urinary manganese excretion[19] and applied conventional approaches to blood pressure measurement, whereas current guidelines recommend the use of 24-h ambulatory monitoring as the gold standard for assessing the blood pressure level and its variation through the day.[20] To close this knowledge gap, we analyzed the database of the Swiss Kidney Project on Genes in Hypertension (SKIPOGH). We related the ambulatory blood pressure to

the manganese concentration in plasma and the urinary manganese excretion. To allow for diurnal variation in blood pressure[18] and the urinary manganese excretion,[19] we assessed these associations not only over 24 hours, but also during wakefulness and sleep, using split urine samples and a diary method.

Methods

Study Population

The data that support the findings of this study are available from the corresponding author upon reasonable request. SKIPOGH is a multicenter family-based study exploring the genetic determinants of blood pressure and kidney function in the general adult population of Switzerland.[21] SKIPOGH was nested within the European Project on Genes in Hypertension and conducted according to the same standardized protocol.[21,22] Inclusion criteria included: (i) minimum age of 18 years; (ii) European ancestry, defined as having both parents and all grandparents born in a restricted list of countries; (iii) at least one, and preferably three, first-degree family members also willing to take part.[21,22] All participants provided written informed consent. The study was approved by Ethics Committee of the Universities of Bern, Geneva, and Lausanne.

Of 1029 participants with available ambulatory blood pressure recordings, we excluded 295, because the number blood pressure readings during daytime and nighttime was less than 10 or 5 (n=20), their urine collection was either incomplete or over-collected according to previously published sex-specific criteria[21] (n=9), the urine sample collected during daytime (n=83) or nighttime (n=98) was too much

degraded to allow measurement of manganese, or because plasma manganese was unavailable (n=85). Thus, the number of participants statistically analyzed totaled 734.

Blood Pressure Measurement

Office blood pressure was measured with an oscillometric sphygmomanometer (A&D UM-101, A&D Company Ltd., Tokyo, Japan), using the appropriate cuff size after the participants had rested for 10 minutes in the seated position. Five consecutive readings were obtained. To reduce the white-coat effect, the first reading was discarded and the mean of the remaining four readings was used for analysis. For ambulatory blood pressure monitoring, Diasys Integra (Novacor, Rueil-Malmaison, France) portable monitors[23] were programmed to obtain blood pressure readings every 15 minutes during the day (7 AM until 10 PM) and every 30 minutes during the night (from 10 PM until 7 AM). The same SAS macro processed all ambulatory recordings. Reading were excluded, if systolic blood pressure was higher than 280 mm Hg, if diastolic blood pressure was higher than 200 mm Hg or lower than 40 mm Hg, or if heart rate was faster than 200 beats per minute or lower than 40 beats per minute. We determined the awake and asleep periods from the participants' diary cards. They were asked to record the time when they got up in the morning and went to bed at night. In the context of this article, daytime and nighttime therefore refer to the awake and asleep periods of the day according to participants' diary. Hypertension was the use of antihypertensive drugs or systolic/diastolic blood pressure levels equal to or exceeding 140/90 mm Hg for office blood pressure and

130/80 mm Hg for the 24-h blood pressure.[20]

Biochemical Measurements

Venous blood samples, drawn after an overnight fast in the morning, were analyzed for the serum levels of creatinine, total and high-density lipoprotein (HDL) cholesterol, liver enzymes, including γ-glutamyltransferase as index of alcohol intake, iron and transferrin and for plasma glucose, using automated methods in certified laboratories. Serum creatinine was measured by Jaffe's method[24] with modifications described elsewhere and with the application of isotope-dilution mass spectrometry for calibration.[25] The glomerular filtration rate was estimated from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[26] The iron saturation of transferrin was computed using a published formula.[27] Diabetes mellitus was a fasting plasma glucose 7.0 mmol/L or higher or use of antidiabetic agents.[28] Participants were also requested to collect a precisely timed 24-h urine sample in separate containers for daytime and nighttime use. All urine produced during wakefulness was passed into the daytime bottle, including the last urine on retiring. Urine passed during the night and upon awakening was placed in the nighttime container. To avoid external contamination, participants were asked to keep the containers closed and to void directly into the wide-neck recipients.

Manganese levels in plasma and urine were measured by inductively coupled plasma mass spectrometry (ICP-MS; 7700 Series; Agilent, Palo Alto, CA), as described elsewhere.[29] Prior to analysis, samples (200 µL) were diluted with 1.8 mL of HNO3 1% solution, containing 10 ng/mL Rh and 10 ng/mL Indium as internal

standards. In addition, as part of the internal quality control program, each batch of study samples was processed with blank controls and standard reference materials. The detection limit of manganese in plasma and urine was 0.123 µg/L. All participants had a plasma manganese concentration above the detection limit. The laboratory also participated in external quality control. In participants with urinary (10.8%) biomarker values below the detection threshold, we set the manganese concentration at half the detection limit.

Statistical Analysis

For database management and statistical analysis, we used SAS software version 9.4 (SAS Institute Inc., Cary, NC). Significance was a two-tailed α-level of 0.05 or less. Means and proportions were compared by a large sample z-test or ANOVA and by Fisher's exact test, respectively. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by the computation of the coefficient of skewness, namely the third moment about the mean divided by the cube of the standard deviation. We logarithmically transformed the distributions of plasma manganese, γ-glutamyltransferase, and the urinary manganese excretion. We performed the Spearman rank correlations to assess the associations of plasma manganese with 24-hour, day and night urinary manganese excretions.

In exploratory analyses, we first examined blood pressure and covariables of potential interest by quantiles of the manganese distributions. Next, we constructed multivariable-adjusted linear or logistic regression models for blood pressure as continuous or categorical outcome variable. All models accounted for clustering of

the observations within families as random effect. The covariables accounted for were center, sex, age, age^2, body mass index, heart rate, plasma glucose, eGFR, smoking, γ-glutamyltransferase (as index of alcohol intake) and antihypertensive drug treatment. Models including urinary manganese were also adjusted for the duration of the urine collection and additionally, to account for pressure natriuresis,[30] for the urinary sodium and potassium excretion. We assessed whether sex modified the effect of manganese on BP by including an interaction variable (sex and plasma or urinary manganese excretion) in the models. Because the gastrointestinal absorption is higher in individuals with low iron stores,[31] in sensitivity analyses, we additionally adjusted for the iron saturation status available in 681 (92.8%) participants. We also ran sensitivity analyses in people not on antihypertensive drug treatment and in all participants with a logarithmic transformation of the ambulatory blood pressure values.

Results

Characteristics of Participants

The study population consisted of 39 singletons and 695 related individuals. All 734 participants were white Europeans. On questioning, none of the participants reported occupational exposure. Table 1 lists the characteristic of the participants by period of day and the medians of the distributions of the exposure biomarkers.

The median plasma concentration of manganese (Figure 1) was 0.52 μg/L (interquartile rang [IQR], 0.32 – 0.82); the median manganese excretion was 199.5 μmol (IQR, 35.1–869.0), 83.0 μmol (IQR, 5.6–602.6) and 51.5 μmol (IQR, 0.20 –

387.3) over 24 h and during daytime and nighttime, respectively. The Spearman rank correlations of plasma manganese with 24-hour, daytime and nighttime urinary manganese excretion were -0.074 (P=0.045), -0.15 (P<0.0001) and -0.056 (P=0.13).

Unadjusted Analyses

As shown in Table 2, participants with higher plasma manganese (median, 0.650 *vs.* 0.418 µg/L) had higher 24-h (+4.1/+2.3 mm Hg), daytime (+4.4/+2.8 mm Hg) and nighttime (+4.8/+5.5 mm Hg) systolic/diastolic blood pressure. Participants with higher daytime manganese excretion (median, 375.8 *vs.* 26.6 µmol) had lower 24-h systolic blood pressure (-3.1 mm Hg), lower systolic/diastolic blood pressure during daytime (-3.5/-1.9 mm Hg) and lower nighttime systolic blood pressure (-2.9 mm Hg). Furthermore, participants with high nighttime urinary manganese excretion (median, 156.7 *vs.* 16.7 µmol) had higher 24-h (+2.5 mm Hg) and daytime (+2.3 mm Hg) systolic blood pressure and higher nighttime systolic/diastolic pressure (+2.9/+1.2 mm Hg). The prevalence of ambulatory hypertension was similar in the high and low halves of the biomarker distributions with exception of the nighttime manganese excretion (21.1% *vs.* 26.3%; P=0.0050).

Multivariable-Adjusted Analyses

Figure 2 shows the associations of 24 h systolic (A,C) and diastolic (B,D) blood pressures with plasma manganese (A,B) and the 24 h urinary manganese excretion (C,D) accounting for center, sex and age. In Table 3, significance ($P \leq 0.030$) was retained for 24-h and daytime diastolic pressure in relation to plasma manganese and for nighttime systolic pressure in relation to the urinary manganese excretion

during sleep. Association sizes for a twofold increment of the biomarker amounted to 0.77 mm Hg (95% confidence interval [CI], 0.08 to 1.47 mm Hg), 0.97 mm Hg (CI, 0.20 to 1.76 mm Hg) and 1.33 (CI, 0.20 to 2.50 mm Hg), respectively. None of the interaction terms between the sex and the manganese biomarkers was significant ($P \geq 0.17$). In categorical analyses with whole-day hypertension as outcome, none of the associations with the biomarkers reached significance ($P \geq 0.78$).

Sensitivity Analyses

Iron saturation of transferrin was available in 681 participants (92.8%). Sensitivity analyses in which models were additionally adjusted for iron saturation produced results directionally similar as in the main analysis (Table 3), although with lower significance, because the analysis included fewer participants (Table S1 available in the online only Data Supplement). This was also the case when patients on antihypertensive drug treatment were excluded (Table S2). In a sensitivity analysis from which we excluded approximately 50% of the daytime readings (Table S3), the median between-reading intervals were similar during daytime and nighttime (30 minutes). Results were confirmatory (Table S3).

Discussion

The key findings of our current study were that diastolic blood pressure was positively associated with plasma manganese over 24 h and during daytime and that at nighttime systolic blood pressure was positively correlated with the urinary manganese excretion. Ambulatory hypertension was more prevalent in participants

with a higher than median urinary manganese excretion at night (21.1% *vs.* 26.3%). As reported in the online only Data Supplement, there was consistency in sensitivity analyses accounting for the influence of the body iron stores on the gastrointestinal absorption of manganese (Table S1)[31] and by limiting the analyses to participants not on antihypertensive drug treatment (Table S2).

The scarce literature on the association of blood pressure and manganese is contradictory,[13-17] some reports describing an inverse association[13,14,17] and others a positive relation.[15,16] In multivariable-adjusted analyses of 639 older men (mean age, 72 years) enrolled in the Normative Aging Study,[13] systolic/diastolic blood pressure was 1.09/0.63 mm Hg (CI, 0.10 to 2.08/0.09 to 1.15 mm Hg) lower per interquartile range increase in toenail manganese. Similarly, among 3853 people (mean age, 37.3 years; 50.0% women) enrolled in the National Health Examination Survey (2011–12 and 2013–14 cycles),[17] a 1 μg/L (0.018 μmol/L) increment in urinary manganese in a spot urine sample was independently associated (P<0.0001) with a 1.31/1.22 mm Hg (CI, 0.97 to 1.65/0.83 to 1.62) lower systolic/diastolic blood pressure.[17] In contrast, in a Brazilian study of 947 randomly recruited adults aged 40 years or older (55.5% women),[16] the participants were clustered into five groups based on sex, blood manganese and the use of antihypertensive drugs. Among 133 women not on antihypertensive medications, mean values were 54.3 years for age, 14.7 μg/L (0.268 μmol/L) for whole-blood manganese and 145.6 mm Hg and 89.7 mm Hg for systolic and diastolic blood pressure. The diastolic blood pressure levels were significantly higher than in the four other clusters.[16]

Discrepancies in the literature can be explained by the kinetics of ingested or

inhaled manganese and the biological medium in which the metal is measured. Only 3 to 5 percent of ingested manganese is absorbed. Rich dietary sources of manganese include whole grains, nuts, leafy vegetables, and teas. The absorption and excretion of manganese are under tight homeostatic control.[32] Excess circulating manganese is rapidly removed by the liver and excreted via the bile. In healthy adults on a normal diet and not occupationally exposed, most of the manganese content of whole blood is in the cellular compartment with plasma manganese accounting for less than 5% of the circulating metal.[1] However, manganese in plasma reflects the diffusible fraction of the biomarker in the blood and is the most readily available and biologically active fraction of manganese in the blood pool.[3,4] Manganese in blood reflects recent exposure over 2 to 3 months, but is not a reliable index of the total body burden. Inhaled fine particulate matter that is contaminated by manganese penetrates the air-blood barrier at the lung alveoli,[33] bypasses the liver and is directly taken up by the heart and by the brain via the olfactory nerve, which lacks a blood-brain barrier. Sources of environmental manganese contamination include processing of manganese, welding, steel and dry-cell battery manufacture, use of manganese salts in chemical industries, and addition of organic manganese compounds, such as methylcyclopentadienyl manganese tricarbonyl, for instance as antiknock agent in gasoline.[3] The re-uptake of manganese proximal renal tubules in the kidney is regulated by cation transporters.[34] The low correlation coefficients between manganese in plasma and urine in our present study are in keeping with these kinetics.

In our current study, a twofold higher plasma manganese concentration was

associated with a 0.77 mm Hg and 0.97 mm Hg higher diastolic blood pressure over the whole day and during daytime, respectively, and a twofold higher nighttime manganese excretion with a 1.33 mm Hg higher systolic blood pressure during sleep. At first sight, these association sizes might seem small, but as shown in Figure 1, the range of plasma and 24-h urinary manganese encompassed a 10-fold and 100-fold increase, respectively. In animal experiments, variation in circulating manganese affected vascular sensitivity to α_1-adrenergic receptor stimulation, thereby influencing signaling pathways and the contractility of vascular smooth muscle cells.[35,36] This mechanism might underlie the association of plasma manganese with diastolic blood pressure, which reflects peripheral arterial resistance (Table 3). The association between nighttime systolic blood pressure and the manganese excretion during sleep was positive (Table 3). In salt-sensitive and hypertensive patients the urinary excretion of sodium shifts from daytime to nighttime,[37-39] a phenomenon usually referred to as pressure natriuresis.[30] This supports the hypothesis of pressure natriuresis as a possible explanation why the association between diastolic blood pressure and plasma manganese was positive during daytime, but not at night, and *vice versa* for systolic blood pressure in relation to urinary manganese.

Strong points of our current study are that we measured blood pressure using ambulatory monitoring and that we allowed for diurnal variation in blood pressure[18] and the urinary excretion of manganese,[19] using a diary approach and split urine samples. Notwithstanding these strong points, our current study must also be interpreted within the context of its limitations. First, the cross-sectional design precluded making causal inferences. However, all[13,14,16,17] but one[15] of the

reviewed studies on the association between blood pressure and manganese had a cross-sectional design. Second, the main elimination route of manganese is not via the kidney, but via the liver, where manganese is excreted in bile. Participants in the top 5 percent of the daytime and nighttime distributions of the manganese excretion all had normal liver function, as exemplified by the liver enzymes alanine transaminase, aspartate transaminase and γ-glutamyltransferase, did not report occupational exposure, and were not residing close to a known industrial or natural source of manganese. The official Swiss food composition tables (www.valeursnutritives.ch/en/downloads) do not list manganese, but leafy vegetables, a main source of manganese, are available throughout the whole year to Swiss consumers (www.sge-ssn.ch/media/tableau_des_legumes.pdf). Third, of 1029 with an ambulatory blood pressure recording, we had to exclude 295 (28.7%), because of missing or unreliable measurements. However, participants analyzed and not analyzed had similar characteristics ($P \geq 0.10$), including 24-h, daytime and nighttime blood pressures, the manganese biomarkers, sex distribution, body mass index and heart rate. Finally, the current study cannot exclude the possibility that the plasma and urine manganese concentrations do not mirror body manganese status but are proxies for an unmeasured entity which shows positive associations with segments of 24-h ABPM.

Perspectives

In our population study, associations of systolic and diastolic blood pressure with manganese varied according to whether the metal was measured in plasma or urine.

Further research should clarify the mechanisms underlying the association of blood pressure with manganese in plasma and urine. Even though worldwide environmental and occupational regulations are increasingly limiting the tolerable limits of exposure to manganese, there remains room for further experimental and hemodynamic research to clarify the molecular and pathophysiological relations underlying the association between blood pressure and manganese exposure. From a public health viewpoint, blood pressure is the predominant modifiable cardiovascular risk factor. Nutritional intake of vegetables in an amount recommended by national Swiss guidelines (www.fao.org/nutrition/education/food-dietary-guidelines/regions/countries/switzerland/en), should ensure that the dietary requirements of the essential micronutrient manganese are met without adverse health effects affecting liver, kidneys, the central nervous and the heart, organs in which manganese is concentrated.[3,4]

Author Contributions

M Bochud conceived and coordinated the Swiss Kidney Project on Genes in Hypertension (SKIPOGH). D Petrović and M Bochud constructed the SKIPOGH database. C Carmeli and M Bochud provided statistical advice. B Ponte, M Pruijm, D Ackermann, G Ehret, I Guessous, A Pechère-Bertschi, B Vogt, PY Martin collected clinical data. S Lenglet, M Augsburger, A Thomas did the manganese measurement. M Burnier provided insight on the pathogenetic role of manganese in hypertension. ZY Zhang did the statistical analyses and wrote the first draft of the manuscript. All

authors interpreted the results, commented on successive drafts of the manuscript, and approved the final version.

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Disclosure

None.

Novelty and Significance

What is new?

The association of blood pressure (BP) with Mn (Mn) remains poorly studied. To our knowledge, this is the first study relating ambulatory BP to plasma Mn (pMn) and the urinary Mn (uMn) excretion. To allow for diurnal variation in BP and uMn, we assessed these associations over 24 h and during daytime and nighttime in 734 randomly recruited Swiss participants. In multivariable-adjusted analyses, association sizes were expressed for a twofold increase in the pMn and uMn.

What is relevant?

- 24-H systolic/diastolic BP averaged 123.7/73.7 mm Hg, pMn 0.52 μg/L and 24-h uMn 199.5 μg/L.
- pMn was associated with a 0.77/0.97 mm Hg higher 24-h/daytime diastolic BP.
- At night, uMn was associated with a 1.33 mm Hg higher systolic BP.

Summary

The associations between diastolic BP and pMn are positive over 24 h and during the day and positive between systolic BP and uMn at night. Increased peripheral resistance might explain the positive associations of diastolic BP and pMn during the day and the positive association of systolic BP with uMn at night.

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Legend to Figures

Figure 1. Logarithmically transformed distributions of plasma manganese (left) and 24–h urinary manganese excretion (right). S and K are the coefficients of skewness and kurtosis. The blue and red lines represent the normal and kernel density distributions. The P–values are for departure of the actually observed distribution from normality according to Shapiro-Wilk's statistics.

Figure 2. Associations of 24 h systolic (A,C) and diastolic (B,D) blood pressures with plasma manganese (A,B) and the 24 h urinary manganese excretion (C,D) accounting for center, sex and age. The dots plotted over the regression line and its 95% confidence interval represent the means of blood pressure and the biomarker in each fifth of the distribution of the biomarker. Vertical bars denote the SE of blood pressure.

Table 1. Characteristics of participants by time of day and medians of the plasma and urinary biomarkers

Characteristic	All	Whole day Plasma Mn (μg/L)		Daytime Mn excretion (μmol)		Nighttime Mn excretion (μmol)	
Limits		<0.524	≥0.524	<115	≥115	<73.3	≥73.3
All participants in category	734	367	367	367	367	367	367
Women	377 (51.4)	185 (50.4)	192 (52.3)	179 (48.8)	198 (54.0)	188 (51.2)	189 (51.5)
Smokers	170 (23.2)	85 (23.2)	85 (23.2)	91 (24.8)	79 (21.5)	103 (28.1)	67 (18.3)†
Drinking	454 (61.9)	227 (61.9)	227 (61.9)	234 (63.8)	220 (60.0)	222 (60.5)	232 (63.2)
Diabetes mellitus	25 (3.4)	12 (3.3)	13 (3.6)	14 (3.9)	11 (3.0)	9 (2.5)	16 (4.4)
Mean of characteristic							
Age, years	47.5±17.6	48.4±17.6	46.7±17.5	46.3±17.9	48.7±17.2	46.0±17.4	49.0±17.7*
Body mass index, kg/m^2	25.0±4.4	25.1±4.6	24.8±4.3	24.7±4.3	25.2±4.6	24.6±4.6	25.3±4.3*
Total cholesterol, mmol/L	5.05±1.00	4.98±0.96	5.12±1.04	5.03±1.03	5.07±0.98	5.08±1.01	5.03±1.00
HDL cholesterol, mmol/L	1.51±0.41	1.46±0.40	1.57±0.42‡	1.52±0.41	1.51±0.42	1.54±0.42	1.49±0.40
Plasma glucose, mmol/L	5.17±0.69	5.23±0.68	5.11±0.70*	5.04±0.69	5.30±0.67§	5.13±0.66	5.21±0.72
Serum creatinine, μmol/L	73.5±13.7	73.2±13.9	85.2±14.7	74.7±13.5	72.3±13.7*	74.2±13.4	72.8±13.9
eGFR, min/L/1.73 m^2	96.5±17.6	96.5±17.7	96.6±17.4	96.8±17.9	96.3±17.2	96.7±17.3	96.4±17.8
γ-Glutamyltransferase (units/l)	17.1 (12.0-28.0)	14.4 (7.9-26.9)	20.3 (14.0-29.0)§	19.9 (14.0-31.0)	14.6 (8.0-26.0)§	17.2 (12.0-28.0)	17.1 (12.0-28.0)
Transferrin saturation (%)	13.5 (7.4-22.4)	11.7 (6.7-17.7)	15.6 (8.2-28.9)§	13.4 (7.3-22.5)	13.6 (7.5-22.2)	13.7 (7.6-22.4)	13.4 (7.3-22.2)
Sodium excretion (mmol)	143.8±60.8	142.6±63.2	145.0±58.4	47.9±19.8	50.0±19.6	37.2±21.4	51.4±29.5§
Potassium excretion (mmol)	64.3±22.7	65.3±23.3	63.3±22.0	47.9±19.8	50.0±19.6	13.9±6.9	16.8±9.5§
Creatinine excretion (mmol)	12.8±4.1	13.1±4.3	12.6±3.9	8.56±4.83	8.39±4.66	12.6±6.9	9.34±5.37§

Abbreviations: Mn, manganese; HDL, high-density lipoprotein cholesterol; eGFR, glomerular filtration rate estimated from serum creatinine. Diabetes mellitus was a fasting plasma glucose level of ≥ 7.0 mmol/L or use of antidiabetic agents. Values are number of participants (%), arithmetic means (±SD) or median (interquartile range). Significance of the between-group differences: * $P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$; § $P < 0.0001$.

Table 2. Blood pressure by time of day and medians of the plasma and urinary biomarkers

Characteristic	Whole day Plasma Mn (μg/L)		Daytime Mn excretion (μmol)		Nighttime Mn excretion (μmol)	
Limits	<0.524	≥0.524	<115	≥115	<73.3	≥73.3
All participants in category	367	367	367	367	367	367
Biomarkers of exposure						
Plasma Mn, μg/L	0.42 (0.38-0.48)	0.65 (0.56-0.70)§	0.54 (0.45-0.63)	0.51 (0.42-0.62)‡	0.53 (0.44-0.63)	0.51 (0.44-0.62)
Urinary Mn excretion, μmol	210.3 (120.6-380.2)	189.3 (102.6-370.7)	26.3 (9.9-71.8)	261.8 (168.7-338.8)§	16.8 (19.7-53.1)	157.4 (96.8-203.4)§
Duration of urine collection, minutes	1424 ± 100	1426 ± 105	929 ± 114.1	937 ± 107	490 ± 89	496 ± 79
Blood pressure, mm Hg						
Systolic	117.8 ± 12.9	121.9 ± 14.5§	125.5 ± 15.3	122.0 ± 13.7†	105.6 ± 13.9	108.5 ± 14.1†
Diastolic	77.0 ± 8.0	79.3 ± 9.2‡	82.2 ± 10.2	80.3 ± 9.1†	67.7 ± 8.1	68.9 ± 8.2*
Heart rate, beats per minute	77.3 ± 8.7	76.7 ± 9.1	80.4 ± 9.6	81.4 ± 9.8	64.9 ± 9.5	64.9 ± 9.3

Abbreviations: Mn, manganese. Values are median (interquartile range) for the Mn biomarkers and arithmetic mean (±SD for the duration of the urine collection, blood pressure and heart rate). Significance of the between-group differences: * $P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$; § $P < 0.0001$.

Table 3. Multivariable-adjusted associations of blood pressure with the exposure biomarkers in 734 participants

Characteristic	Biomarker	Estimate (95% confidence interval)	*P*
Whole day			
SBP, mm Hg	plasma manganese	0.67 (-0.50 to 1.83)	0.27
	manganese excretion	0.80 (-0.37 to 1.96)	0.18
DBP, mm Hg	plasma manganese	0.77 (0.08 to 1.47)	0.030
	manganese excretion	0.47 (-0.23 to 1.17)	0.20
Daytime			
SBP, mm Hg	plasma manganese	0.77 (-0.47 to 2.00)	0.23
	manganese excretion	-0.27 (-1.53 to 1.03)	0.69
DBP, mm Hg	plasma manganese	0.97 (0.20 to 1.76)	0.013
	manganese excretion	-0.10 (-0.90 to 0.73)	0.84
Nighttime			
SBP, mm Hg	plasma manganese	0.77 (-0.47 to 2.00)	0.22
	manganese excretion	1.33 (0.20 to 2.50)	0.023
DBP, mm Hg	plasma manganese	0.47 (-0.27 to 1.17)	0.22
	manganese excretion	0.43 (-0.23 to 1.13)	0.20

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. All models accounted for family cluster and included as covariables center, sex, age, age2, body mass index, heart rate, plasma glucose, γ-glutamyltransferase, the estimated glomerular filtration rate, smoking and antihypertensive drug treatment. Models including urinary manganese were additionally adjusted for the duration of the urine collection and sodium and potassium excretion. Association sizes express the difference in blood pressure for a twofold increase in the biomarker.

HYPERTENSION

Ambulatory Blood Pressure in Relation to Plasma and Urinary Manganese

This appendix formed part of the original submission and has been peer reviewed.

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Table S1. Models additionally adjusted for iron saturation in 681 participants

Characteristic	Biomarker	Estimate (95% confidence interval)	*P*
Whole day			
SBP, mm Hg	plasma manganese	1.33 (-6.65 to 9.30)	0.74
	manganese excretion	1.74 (-0.56 to 4.04)	0.14
DBP, mm Hg	plasma manganese	3.23 (-1.54 to 8.01)	0.18
	manganese excretion	0.47 (-0.90 to 1.85)	0.50
Daytime			
SBP, mm Hg	plasma manganese	1.78 (-6.63 to 10.2)	0.68
	manganese excretion	-0.42 (-2.23 to 1.40)	0.65
DBP, mm Hg	plasma manganese	3.97 (-1.28 to 9.22)	0.14
	manganese excretion	-0.35 (-1.48 to 0.77)	0.54
Nighttime			
SBP, mm Hg	plasma manganese	1.13 (-7.19 to 9.45)	0.79
	manganese excretion	1.72 (0.44 to 3.00)	0.0086
DBP, mm Hg	plasma manganese	2.00 (-2.90 to 6.91)	0.42
	manganese excretion	0.46 (-0.31 to 1.23)	0.24

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. All models accounted for family cluster and included as covariables center, sex, age, age^2, body mass index, heart rate, plasma glucose, γ-glutamyltransferase, the estimated glomerular filtration rate, smoking and antihypertensive drug treatment, and iron saturation. Models including urinary manganese were additionally adjusted for the duration of the urine collection and sodium and potassium excretion. Association sizes express the difference in blood pressure for a twofold increase in the biomarker.

Table S2. Models relating blood pressure to urinary manganese in 589 participants not on antihypertensive drug treatment

Characteristic	Biomarker	Estimate (95% confidence interval)	*P*
Whole day			
SBP, mm Hg	plasma manganese	2.05 (-5.18 to 9.95)	0.61
	24-h manganese excretion	0.51 (-1.93 to 2.95)	0.68
DBP, mm Hg	plasma manganese	2.46 (-2.30 to 7.11)	0.32
	24-h manganese excretion	0.03 (-1.42 to 1.49)	0.96
Daytime			
SBP, mm Hg	plasma manganese	2.58 (-5.81 to 11.0)	0.55
	manganese excretion	-1.51 (-3.43 to 0.41)	0.12
DBP, mm Hg	plasma manganese	2.62 (-2.55 to 7.80)	0.32
	manganese excretion	-0.98 (-2.16 to 0.20)	0.10
Nighttime			
SBP, mm Hg	plasma manganese	2.52 (-5.72 to 10.8)	0.55
	manganese excretion	1.14 (-0.22 to 2.50)	0.099
DBP, mm Hg	plasma manganese	2.75 (-2.26 to 7.76)	0.28
	manganese excretion	0.65 (-0.17 to 1.48)	0.12

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. All models accounted for family cluster and included as covariables center, sex, age, age^2, body mass index, heart rate, plasma glucose, γ-glutamyltransferase, the estimated glomerular filtration rate, smoking and antihypertensive drug treatment. Models including urinary manganese were additionally adjusted for the duration of the urine collection and sodium and potassium excretion. Association sizes express the difference in blood pressure for a twofold increase in the biomarker.

Table S3. Multivariable-adjusted associations of blood pressure with the exposure biomarkers in 734 participants, excluding about 50% of the daytime readings

Characteristic	Biomarker	Estimate (95% confidence interval)	*P*
Whole day			
SBP, mm Hg	plasma manganese	0.73 (-1.52 to 2.98)	0.53
	manganese excretion	0.52 (-0.14 to 1.18)	0.12
DBP, mm Hg	plasma manganese	1.36 (-0.02 to 2.75)	0.053
	manganese excretion	0.24 (-0.16 to 0.65)	0.24
Daytime			
SBP, mm Hg	plasma manganese	0.91 (-1.48 to 3.30)	0.46
	manganese excretion	-0.20 (-0.74 to 0.33)	0.45
DBP, mm Hg	plasma manganese	1.55 (0.01 to 3.10)	0.048
	manganese excretion	-0.06 (-0.41 to 0.28)	0.71

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. All models accounted for family cluster and included as covariables center, sex, age, age^2, body mass index, heart rate, plasma glucose, γ-glutamyltransferase, the estimated glomerular filtration rate, smoking and antihypertensive drug treatment. Models including urinary manganese were additionally adjusted for the duration of the urine collection and sodium and potassium excretion. Association sizes express the difference in blood pressure for a twofold increase in the biomarker.