

Dyslipidemia and tobacco smoking synergistically increase serum manganese

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Abstract. Manganese is a trace metal involved in both physiology and toxicity. The association between manganese and dyslipidemia has been scarcely revised, and results from studies in both animals and humans are inconsistent. The aim of this study was to evaluate the association between serum manganese levels and dyslipidemia, considering some manganese sources and factors that could affect its concentration, especially tobacco smoking. Serum manganese concentration in 63 volunteers was determined and their smoking habits were recorded. Dietary manganese, iron, fat and alcohol consumption was also estimated by a food-frequency questionnaire. A bivariate analysis was carried out to identify those factors affecting manganese concentration. Only dyslipidemia and smoking resulted statistically significant and thus were considered for the subsequent two-way analysis of variance, to test a possible interaction between dyslipidemia and smoking. Marginal means for serum manganese were as follows: 8.32 ± 2.14 nmol/L for nonsmokers without dyslipidemia, 9.21 ± 2.22 nmol/L for smokers without dyslipidemia, 10.21 ± 2.53 nmol/L for nonsmokers with dyslipidemia, and 14.21 ± 3.44 nmol/L for smokers with dyslipidemia. Dyslipidemia and tobacco smoking were synergistically associated with increased serum manganese. To maintain adequate manganese levels in the organism, other factors in addition to its dietary intake should be considered, for instance, lipid status and smoking habits, particularly in those conditions in which manganese accumulation is an issue.

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La dislipidemia y el tabaquismo aumentan de manera sinérgica el manganeso sérico.

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Palabras clave: dislipidemia; manganeso; tabaquismo.

Resumen. El manganeso es un metal traza esencial involucrado tanto en procesos fisiológicos como en toxicidad. La asociación entre el manganeso y las dislipidemias se ha estudiado poco, y los resultados de estudios en animales y en humanos son inconsistentes. El objetivo de este trabajo fue evaluar la asociación entre el manganeso sérico y las dislipidemias, considerando algunas fuentes de manganeso y factores que pudieran afectar su concentración, especialmente el tabaquismo. Se determinaron las concentraciones séricas de manganeso de 63 voluntarios y se registraron sus hábitos de consumo de tabaco. Se estimó la ingesta de manganeso, hierro, grasa y alcohol mediante un cuestionario de frecuencia de consumo. Se realizó un análisis bivariado para identificar los factores que afectaron las concentraciones de manganeso; únicamente las dislipidemias y el tabaquismo resultaron estadísticamente significativos y se consideraron en el subsecuente análisis de varianza de dos vías, para examinar una posible interacción entre las dislipidemias y el tabaquismo. Las medias marginales para el manganeso sérico fueron: $8,32 \pm 2,14$ nmol/L para no fumadores sin dislipidemia, $9,21 \pm 2,22$ nmol/L para fumadores sin dislipidemia, $10,21 \pm 2,53$ nmol/L para no fumadores con dislipidemia, y $14,21 \pm 3,44$ nmol/L para fumadores con dislipidemia. Las dislipidemias y el tabaquismo se asociaron sinérgicamente con el aumento del manganeso sérico. Para mantener niveles adecuados de manganeso en el organismo, se deben tomar en cuenta factores adicionales a su consumo dietético, como el estatus lipídico y el tabaquismo, particularmente en condiciones en las que la acumulación de manganeso sea un problema.

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INTRODUCTION

The status of several trace metals such as copper, iron and manganese has been suggested to be associated with dyslipidemia (1); however, most of the data to this respect come from studies in animals (1–4). Among trace metals, manganese has received little attention, and the results regarding its association with dyslipidemia are contradictory (5, 6); even the results about modification of manganese levels by the type and quantity of fat consumed are different between animals and humans. In humans, satu-

rated fat increased manganese balance and its retention (7), while in rats, unsaturated fat increased its absorption (8).

Dyslipidemia-manganese association can also be seen in the opposite direction, that is, manganese could affect lipid levels; for instance, studies in animals show that manganese deficiency can decrease high density lipoprotein-cholesterol (HDL-C) (9) and increase total cholesterol and total lipids(5); while others have reported mixed results for the effects of manganese supplementation, where it can induce hypercholesterolemia (6) and hypertriglyceride-

mia (10) or reduce total cholesterol in diabetic rats (11).

The adequate daily intake of manganese is 1.8 and 2.3 mg (32.7 and 41.9 μmol) for women and men, respectively (12). Dietary manganese sources include whole grains, cereals, brown rice, pineapples, nuts, black tea and spinach (13). In addition, it can be found in other sources such as tobacco (2821 – 7281 $\mu\text{mol/g}$) (14), water and air (15).

Manganese homeostasis is of great importance to the organism because it is an essential mineral for the activity of several enzymes such as pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate (16); arginase, which converts arginine to urea (17); and manganese superoxide dismutase (Mn-SOD), critical for antioxidant defense (18). Manganese also activates enzymes related to fatty acid metabolism and protein synthesis (19). Therefore, important deviations in manganese concentration in the organism can contribute to a number of complications such as cardiovascular and neurological (20); hence the relevance of maintaining adequate levels of this metal in the organism and of knowing the factors associated with alterations in its homeostasis.

We hypothesized that higher serum manganese levels would be present in people with dyslipidemia compared with those without dyslipidemia and that such levels would be affected by manganese sources. The main objective of this work, was to study the relationship between dyslipidemia and serum manganese levels, taking into account other factors that could modify manganese concentration, particularly tobacco smoking.

PATIENTS AND METHODS

Volunteers

Sixty-three participants enrolled in the Tlalpan2020 cohort were included in this study.

Tlalpan2020 is an ongoing cohort study, whose main objective is to estimate the incidence of hypertension in the Mexico City population, in a period of ten years with bi-annual clinical evaluations. All participants are clinically healthy people aged between 20 and 50 years. Exclusion criteria for the cohort are hypertension, diabetes, cardiovascular or renal diseases, thyroid diseases and pregnancy. In addition to the criteria for the cohort, participants selected for this study were not taking lipid-lowering medication. The Tlalpan2020 study follows the principles of the declaration of Helsinki and was approved by The Research Ethics Committee of the National Institute of Cardiology ‘Ignacio Chávez’ (approval no. 13-802). All participants provided their written informed consent to be part of this cohort.

Dietary intake and smoking habits recording

Dietary manganese and fat consumption were estimated through the semiquantitative instrument ‘system of evaluation of nutrition habits and nutrient consumption’ (SNUT), previously validated for the Mexican population by the National Institute of Public Health of Mexico (21, 22). It consists of 116 questions about the frequency of consumption of standard portions of foods and beverages over the year prior to the interview.

During the interview, the smoking habits of participants were recorded. They were asked about the consumption of cigarette, pipe or cigar and if they had consumed more than 100 units in their entire life, as well as if they were current smokers. They were classified as smokers, according to the criteria of the Centers for Disease Control and Prevention of the United States (23), if they have consumed more than 100 cigarettes and were current smokers at the moment of the interview.

Biochemical determinations and dyslipidemia classification

Venous blood samples were drawn after a 12-h fasting period. Total, low density lipoprotein-cholesterol (LDL-C), and HDL-C, as well as triglycerides were determined with commercial kits in a COBAS 6000 analyzer (Roche, Indianapolis, USA). Participants were classified as having or not having dyslipidemia according to the Adult Treatment Panel III criteria (24): cholesterol >5.2 mmol/L; triglycerides >1.7 mmol/L; HDL-C <1.3 mmol/L for women, or <1.0 mmol/L for men; and/or LDL-C >3.4 mmol/L.

Serum manganese determination

Serum manganese levels were quantified in an atomic absorption spectrophotometer Analyst 600 (Perkin Elmer, Shelton, USA) with a manganese hollow cathode lamp. Serum samples were diluted with a matrix modifier composed of 0.01% diammonium hydrogen phosphate $[(\text{NH}_4)_2\text{HPO}_4]$ in 0.05% polyoxyethyleneoctyl phenyl ether (Triton™ X-100), under the following analytical conditions: Zeeman background correction, 279.5 nm wavelength, 100 °C 30s for drying, 1000 °C 30 s for charring, 2400 °C 3s for atomization, and 2600 °C 4s for cleaning. Results were interpolated in the corresponding calibration curve constructed with a commercial standard (Perkin Elmer GFAA N9300244). Quality control was assured by measuring a biological matrix-based external standard (bovine liver, National Institute of Standard Technology 1577b); in every analytical session, the resulting manganese concentration was 95-105% from that of the certificate of analysis (25). All samples were analyzed in duplicate and in a blind fashion.

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). Initially, bivariate analyzes (Stu-

dent's t test or Pearson correlation coefficient, as required) were performed in order to identify the relationship between serum manganese and other biochemical parameters, as well as dietary intake and demographic data. Due to dyslipidemia and tobacco smoking were the only factors associated with higher manganese levels in the bivariate analysis, they were considered as main factors for a two-way analysis of variance (ANOVA), to explore a possible interaction between them. The factorial design resulted in four groups: nonsmokers-without dyslipidemia (NS-ND) (n=12), nonsmokers with dyslipidemia (NS-D) (n=29), smokers without dyslipidemia (S-ND) (n=7), and smokers with dyslipidemia (S-D) (n=15). Later, we included in the model other variables potentially affecting manganese concentration, such as sex, age, serum iron, and the consumption of manganese, iron, alcohol and different types of fat. It was determined the best fitting by means of the adjusted R^2 , regardless of the p-value for those variables. In addition, one-way ANOVA or Kruskal-Wallis test were used to compare continuous variables between the groups resulting from the factorial design, while Fisher's exact test was selected for categorical variables. Statistical significance was considered when $p < 0.05$. Statistical analysis and graphs were made by employing SPSS v. 22.

RESULTS

Serum manganese

The overall mean \pm SD for serum manganese was 10.69 ± 3.36 nmol/L. The initial bivariate analysis yielded only tobacco smoking and dyslipidemia as significant modifying factors for serum manganese levels. Forty-four participants (70%) had some type of dyslipidemia and displayed higher serum manganese compared with people without dyslipidemia (11.58 ± 3.42 vs. 8.65 ± 2.15 nmol/L). Moreover, 22 partici-

pants (35%) were smokers and had higher serum manganese than nonsmokers (12.62 ± 3.87 vs. 9.66 ± 2.55 nmol/L). No differences by sex were found (10.62 ± 3.44 and 10.84 ± 3.28 nmol/L for women and men, respectively).

Demographic characteristics and biochemical parameters

Demographic characteristics and biochemical parameters were compared between the groups obtained from the factorial design (see Statistical analysis section). Sex and age distributions were similar between the four groups. Statistical differences were identified regarding some biochemical parameters; importantly, serum manganese was higher in smokers with dyslipidemia compared to the other three groups. A trend to higher serum iron was observed in smokers with dyslipidemia but without statistical significance. Also, no significant differences were observed for total cholesterol; however, nonsmokers without dyslipidemia and smokers without dyslipidemia had the highest HDL-C and the lowest LDL-C and triglycerides concentration (Table I). In addition, mean HDL-C was 1.33 ± 0.31 mmol/L for all women and 1.18 ± 0.38 for all men.

Dietary intake

Manganese consumption was higher than recommended in all groups (mean consumption 304 to 389 μ mol/day) and a trend to an inverse relationship between serum manganese and manganese consumption was observed. No significant differences between groups for the intake of any type of fat, manganese, iron or alcohol were found (Table II).

Serum manganese levels (factorial analysis)

Both dyslipidemia and smoking by themselves slightly increased manganese levels; however, when the interaction between both factors was tested, a synergistic interaction was obser-

ved ($p=0.047$) (Fig. 1). Marginal means \pm SD for serum manganese levels were: 8.32 ± 2.14 nmol/L for nonsmokers without dyslipidemia, 9.21 ± 2.22 nmol/L for smokers without dyslipidemia, 10.21 ± 2.53 nmol/L for nonsmokers with dyslipidemia, and 14.21 ± 3.44 nmol/L for smokers with dyslipidemia (Fig. 1). Adjusted R^2 for the factorial model, without considering any other factor in addition to dyslipidemia and smoking, was 0.363. When other factors were included, the best fitting for the model was that considering sex ($p=0.065$) and alcohol consumption ($p=0.089$), obtaining an adjusted $R^2 = 0.404$; other factors tested in the model resulted in lower R^2 values. The interaction between smoking and dyslipidemia was preserved ($p=0.021$) and marginal means changed only slightly: 8.25 ± 2.64 nmol/L for nonsmokers without dyslipidemia, 9.09 ± 2.63 nmol/L for smokers without dyslipidemia, 10.12 ± 2.62 nmol/L for nonsmokers with dyslipidemia, and 14.50 ± 2.71 nmol/L for smokers with dyslipidemia.

DISCUSSION

In this work, it was found that serum manganese and dyslipidemia are associated, but tobacco smoking also plays an important role in this association, as shown by the synergistic effect between smoking and dyslipidemia on serum manganese, regardless of other factors potentially modifying manganese levels, such as the consumption of manganese, iron or alcohol, serum iron or the amount of different types of fat ingested.

Manganese is a trace metal, whose entry into the body is subjected to the hepatic first-pass effect, before it reaches the systemic circulation; consequently, only 2-5% of ingested manganese is absorbed into the body (26). It has been seen that manganese consumption in a wide range (0.8-20 mg/day) does not result in deficiency or toxicity, because of the ability of the organism to

TABLE I
DEMOGRAPHIC AND BIOCHEMICAL PARAMETERS OF PARTICIPANTS

Variable	Nonsmokers		Smokers	
	Without dyslipidemia	With dyslipidemia	Without dyslipidemia	With dyslipidemia
	NS-ND group	NS-D group	S-ND group	S-D group
Sex (%women)	83	76	57	40
Age (years)	32.3 ± 9.9	39.8 ± 8.1	33.7 ± 9.3	37.1 ± 7.6
Serum manganese (nmol/L)	8.32±2.14 ^	10.21±2.53^	9.21±2.22^	14.21±3.44*§ #
Serum iron (µmol/L)	18.76±9.89	18.98±9.37	19.87±4.12	22.21±6.45
HDL-cholesterol (mmol/L)	1.59±0.29^§	1.19±0.31*	1.42±0.13^	1.14±0.33#*
LDL-cholesterol (mmol/L)	2.69±0.42^	3.41±0.89	2.65±0.43^	3.70±0.82#*
Triglycerides (mmol/L)	1.01±0.33§^	2.04±1.35#*	0.99±0.25§^	1.98±0.98#*

Data are expressed as the mean ± standard deviation. *p<0.05, different from NS-ND group; § p<0.05, different from NS-D group; # p<0.05, different from S-ND group, ^ p<0.05; different from S-D group

TABLE II
DIETARY CONSUMPTION DATA OF PARTICIPANTS

Variable	Nonsmokers		Smokers	
	Without dyslipidemia	With dyslipidemia	Without dyslipidemia	With dyslipidemia
	NS-ND group	NS-D group	S-ND group	S-D group
Manganese (mmol/day)	0.39±0.29	0.30±0.25	0.34±0.19	0.33±0.27
Iron (mmol/day)	0.22±0.06	0.23±0.06	0.23±0.04	0.23±0.06
Heme iron (mg/day)	1.03±0.93	0.94±0.38	0.99±0.35	0.89±0.34
Saturated fat (g/day)	28.67±13.31	25.75±7.91	27.97±6.06	29.42±14.67
Monounsaturated fat (g/day)	39.20±17.34	34.25±10.11	35.68±5.94	40.17±21.18
Polyunsaturated fat (g/day)	19.17±5.59	20.33±6.56	26.91±10.42	20.70±7.88
Alcohol (mmol/day)	13.68±15.41	23.88±57.96	48.84±35.38	34.51±49.27

Consumption data are expressed as the mean ± standard deviation. No significant differences between groups were found.

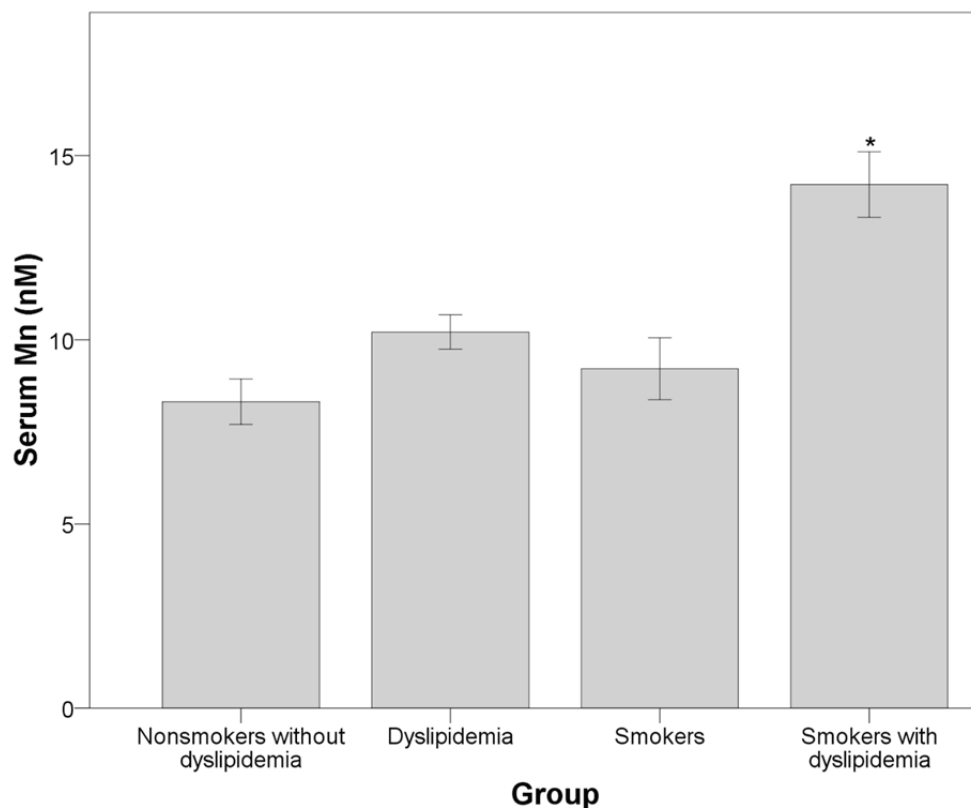


Fig. 1. Serum manganese levels. Participants that only had dyslipidemia or only smoked tobacco, displayed a modest increase in serum manganese, while a synergistic effect was observed in those that smoked tobacco and had dyslipidemia. Bars represent the mean \pm standard error of the mean. Two-way ANOVA, * $p < 0.05$ for the interaction between dyslipidemia and tobacco smoking.

change the extent of absorption and elimination of this mineral (27). In fact, we found a mean manganese consumption higher than the daily adequate intake, while serum levels were within normal values (7.28-15.47 nmol/L or 40-85 ng/dL, according to the Agency of Toxic Substances & Disease Registry (28)) in 92% of participants. Thus, manganese levels in the organism may depend, to a greater extent, on other factors in addition to those related to its intake.

We measured manganese in serum instead of whole blood because of the availability of the samples in optimal conditions for the analysis. In fact, Ottaway and Halls (29) mentioned that

measurement of serum manganese is adequate when samples are not contaminated, that is, when in a normal population mean serum manganese is between 9.83 and 11.47 nmol/L; the overall mean for our sample was 10.69 nmol/L.

Iron (30) and alcohol (31) consumption have been reported to affect manganese homeostasis, mainly at the absorption level. Iron competes with manganese by transport mechanisms; therefore, iron deficiency can lead to increased manganese levels (30). Additionally, experiments in animals showed that alcohol could increase manganese absorption through the gut (31). To this respect, there was a trend in

our data to higher manganese levels as the mean consumption of alcohol by group increased, except by the group of smokers without dyslipidemia, which broke away from such trend; however, no significant differences were detected between groups regarding iron or alcohol consumption.

The relationship between serum manganese and dyslipidemia was of particular interest to us. The type of fat ingested is a potential modifier of manganese concentration, although results are contradictory: in rats, unsaturated fat consumption resulted in increased manganese levels (8), while in humans, such effect was observed with the consumption of saturated fat (7), which is associated with the presence of dyslipidemia (32). In the bivariate analysis, that is when we compared people with and without dyslipidemia, without taking into account any other factor, the first had higher manganese levels. Likewise, smokers had higher serum manganese than nonsmokers. Then, in order to analyze the effect of those factors separately and altogether, we used a factorial analysis. First, we found that those individuals only having dyslipidemia or only being smokers without alterations in their lipid status had slightly higher serum manganese compared to those in which both conditions were absent.

Regarding saturated fat, we did not find any relationship between its consumption and serum manganese or dyslipidemia. To this respect, it should be pointed out that we did not control the diet of the participants, which is a limitation of our study; the dietary consumption data were based on their self-report, probably leading to a low precision in the estimation of dietary intake; nevertheless, we were able to evaluate the lipid profile, which can be, at least partially influenced by the type of fat consumed (32). A study by Finley et al. (7), in women consuming a controlled diet, showed that the type of fat ingested affects manganese retention, being higher in

the group that consumed cocoa butter compared with the group consuming corn oil (the first has a higher saturated fat content) (7).

Similarly, tobacco is a recognized source of metals, manganese among them (33). The slight but non-significant increase in serum manganese (11%) that smokers without dyslipidemia displayed compared with nonsmokers without dyslipidemia is in agreement with a previous study that did not find any association between smoking and serum manganese levels (34). A different picture was noted when the interaction between smoking and dyslipidemia was studied; we observed a synergistic effect between both factors, with a 71% increase in serum manganese in the group of smokers with dyslipidemia compared with the group of nonsmokers without dyslipidemia.

A possible explanation for the synergistic effect observed could be as follows: manganese in tobacco smoke would enter the organism by inhalation and it would be absorbed from the olfactory mucosa and lungs, avoiding the hepatic first-pass effect (35). Smokers without dyslipidemia could eliminate the manganese introduced into their body by excretion mechanisms, mainly in the bile, while manganese elimination would be retarded, by the effect of fat, as observed in the experiments of Finley et al. (7), in smokers with dyslipidemia. The exact mechanisms by which dyslipidemia or saturated fat intake can increase manganese retention remains to be elucidated; however, potential alterations in Mn transport as a consequence of dyslipidemia could represent a plausible explanation for the findings of this study, as observed in mice exposed to a high fat diet which displayed increased expression of the divalent metal transporter 1 (DMT-1) in the duodenum (36).

On the other hand, when alcohol consumption and sex were included as covariates in the statistical model, p-values of 0.089 and 0.065 were obtained, suggesting a possible influence

of alcohol and sex on serum manganese in our sample, but without affecting the interaction between smoking and dyslipidemia.

One limitation of our study is the apparently small sample size; however, the 2x2 factorial design used here has the advantage of providing information about two factors separately and together with a reduced sample size, as every subject is set in one of the levels (yes or no) of every factor (smoking and dyslipidemia)(37).

Even when we evaluated serum manganese with respect to lipid status, dyslipidemia-manganese relationship has been studied more in the other direction; for instance, low manganese intake has been associated to a better lipid profile in healthy adults (38) while workers exposed to manganese in ferromanganese industry had increased serum triglycerides (39); studies in animals have reported that manganese supplementation induces hypercholesterolemia and hypertriglyceridemia (6, 10). Taking into account all these works and ours, we consider that it would be interesting to study the interplay between manganese, dyslipidemia and tobacco smoking and its relevance for diseases associated with high manganese levels.

Serum manganese in our sample was within normal values; however, the interaction we found could be relevant in cases where manganese can accumulate; for instance, in diseases involving biliary obstruction, such as primary biliary cirrhosis and cholestatic liver disease, in which dyslipidemia may also be present (40, 41). Since Mn is considered as an essential part of the redox defense, mainly because of its role as a cofactor of Mn-SOD (42), it will also be interesting to test the effects of this interaction on oxidative stress in future studies.

A limitation of this study and a common problem found when Mn is studied in humans is the difficulty accessing its main site of accumulation, the mitochondria (41), because they would have to be obtained from Mn target organs, such

as the central nervous system, liver, pancreas, kidney, testes/ovaries or heart (28), while blood is the most accessible sample from humans.

It is worth mentioning that manganese has been identified as a potential factor contributing to hypertension (43) and that both dyslipidemia (44) and smoking (45) represent risk factors for hypertension and cardiovascular disease. Thus, interactions in this regard also deserve to be analyzed in more detail.

To our knowledge, this is the first time that a synergistic effect between dyslipidemia and smoking on serum manganese concentration is reported. From our results, we can suggest that when trying to maintain adequate manganese levels, other factors, in addition to dietary sources, should be taken into account; for instance, lipid status, smoking habits and probably other life style factors.

A wider study of the metal profile of people with dyslipidemia, considering life style factors, would contribute to comprehend the dynamics of metal homeostasis in dyslipidemia.

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REFERENCIAS

1. Charradi K, Elkahoui S, Limam F,

- Aouani E.** High-fat diet induced an oxidative stress in white adipose tissue and disturbed plasma transition metals in rat: prevention by grape seed and skin extract. *J Physiol Sci* 2013;63:445-455.
2. **Song M, Schuschke DA, Zhou Z, Chen T, Pierce WM Jr, Wang R, Johnson WT, McClain CJ.** High fructose feeding induces copper deficiency in Sprague-Dawley rats: a novel mechanism for obesity related fatty liver. *J Hepatol* 2012;56:433-440.
 3. **Bae YJ, Choi MK, Kim MH.** Manganese supplementation reduces the blood cholesterol levels in Ca-deficient ovariectomized rats. *Biol Trace Elem Res* 2011;141:224-231.
 4. **Bonomo L de F, Silva M, Oliveira R de P, Silva ME, Pedrosa ML.** Iron overload potentiates diet-induced hypercholesterolemia and reduces liver PPAR- α expression in hamsters. *J Biochem Mol Toxicol* 2012;26:224-229.
 5. **Basumallik AK.** Blood cholesterol profile in guinea pigs: effect of ascorbic acid ingestion on hypercholesterolemia induced by manganese deficiency or overdosage of an anti-thyroid agent in diet. *Pathophysiology* 1995;2:227-233.
 6. **Sentürk UK, Oner G.** The effect of manganese-induced hypercholesterolemia on learning in rats. *Biol Trace Elem Res* 1996;51:249-257.
 7. **Finley JW, Penland JG, Pettit RE, Davis CD.** Dietary manganese intake and type of lipid do not affect clinical or neuropsychological measures in healthy young women. *J Nutr* 2003;133:2849-2856.
 8. **Finley JW, Davis CD.** Manganese absorption and retention in rats is affected by the type of dietary fat. *Biol Trace Elem Res* 2001;82:143-158.
 9. **Klimis-Tavantzis DJ, Leach RMJr, Kris-Etherton PM.** The effect of dietary manganese deficiency on cholesterol and lipid metabolism in the Wistar rat and in the genetically hypercholesterolemic RICO rat. *J Nutr* 1983;113:328-336.
 10. **Jenkins KJ, Kramer JKG.** Effect of excess dietary manganese on lipid composition of calf blood plasma, heart, and liver. *J Dairy Sci* 1991;74:3944-3948.
 11. **Burlet E, Jain SK.** Manganese supplementation reduces high glucose-induced monocyte adhesion to endothelial cells and endothelial dysfunction in Zucker diabetic fatty rats. *J Biol Chem* 2013;288:6409-6416.
 12. **Health and Medicine Division, The National Academies of Sciences, Engineering and Medicine.** Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.[Internet]. 2001 [cited 2016 Oct 13]. Available from: <http://www.nationalacademies.org/hmd/Reports/2001/Dietary-Reference-Intakes-for-Vitamin-A-Vitamin-K-Arsenic-Boron-Chromium-Copper-Iodine-Iron-Manganese-Molybdenum-Nickel-Silicon-Vanadium-and-Zinc.aspx>.
 13. **Costa LG, Aschner M.** Manganese in health and disease. Cambridge: The Royal Society of Chemistry; 2015.
 14. **Bernhard D, Rossmann A, Wick G.** Metals in cigarette smoke. *IUBMB Life* 2005;57:805-809.
 15. **Chen P, Culbreth M, Aschner M.** Exposure, epidemiology, and mechanism of the environmental toxicant manganese. *Environ Sci Pollut Res Int* 2016;23:13802-13810.
 16. **Baly DL, Keen CL, Hurley LS.** Pyruvate carboxylase and phosphoenolpyruvate-carboxykinase activity in developing rats: effect of manganese deficiency. *J Nutr*

- 1985;115:872-879.
17. **Kocyigit A, Zeyrek D, Keles H, Koylu A.** Relationship among manganese, arginase, and nitric oxide in childhood asthma. *Biol Trace Elem Res* 2004;102:11-18.
 18. **Candas D, Li JJ.** MnSOD in oxidative stress response-potential regulation via mitochondrial protein influx. *Antioxid Redox Signal* 2014;20:1599-1617.
 19. **Klimis-Tavantzis DJ.** Manganese in health and disease. Boca Raton (FL): CRC Press; 1994.
 20. **Jiang Y, Zheng W.** Cardiovascular toxicities upon manganese exposure. *Cardiovasc Toxicol* 2005;5:345-354.
 21. **Hernández-Avila, JE, González-Avilés, L, Rosales-Mendoza, E.** Manual de usuario. SNUT Sistema de Evaluación de Hábitos Nutricionales y Consumo de Nutrientes. México: Instituto Nacional de Salud Pública; 2003.
 22. **Hernández-Avila M, Romieu I, Parra S, Hernández-Avila J, Madrigal H, Willett W.** Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. *Salud Publica Mex* 1998;40:133-140.
 23. **Centers for Disease Control and Prevention (CDC).** State-specific second-hand smoke exposure and current cigarette smoking among adults - United States, 2008. *MMWR Morb Mortal Wkly Rep* 2009;58:1232-1235.
 24. **National Cholesterol Education Program, National Heart, Lung, and Blood Institute, National Institutes of Health.** Detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III), NIH. [Internet]. 2001 [cited 2016 Oct 13]. Available from: <https://www.nhlbi.nih.gov/files/docs/guidelines/atp3xsum.pdf>. Accessed 13 Sep 2016.
 25. **Montes S, Riojas-Rodríguez H, Sabido-Pedraza E, Ríos C.** Biomarkers of manganese exposure in a population living close to a mine and mineral processing plant in Mexico. *Environ Res* 2008;106:89-95.
 26. **Andersen ME, Gearhart JM, Clewell HJ 3rd.** Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. *Neurotoxicology* 1999;20:161-171.
 27. **Greger JL, Davis CD, Suttie JW, Lyle BJ.** Intake, serum concentrations, and urinary excretion of manganese by adult males. *Am J Clin Nutr* 1990;51:457-461.
 28. **Agency of Toxic Substances & Disease Registry ATSDR.** Toxicological profile: manganese. [Internet]. 2012 [cited 2016 Oct 13]. Available from: <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=102&tid=23>. Accessed 12 Sep 2016.
 29. **Ottaway JM, Halls DJ.** Determination of manganese in biological materials. *Pure Appl Chem* 1986;58:1307-1316.
 30. **Farina M, Avila DS, da Rocha JBT, Aschner M.** Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury. *Neurochem Int* 2013;62:575-594.
 31. **Schafer DF, Stephenson DV, Barak AJ, Sorrell MF.** Effects of ethanol on the transport of manganese by small intestine of the rat. *J Nutr* 1974;104:101-104.
 32. **Riccardi G, Vaccaro O, Costabile G, Rivellesse AA.** How well can we control dyslipidemias through lifestyle modifications? *Curr Cardiol Rep* 2016;18:66.
 33. **Yaprak E, Yolcubal I, Sinanoğlu A, Doğrul-Demiray A, Guzeldemir-Akçakanat E, Marakoğlu I.** High levels of heavy metal accumulation in dental calculus of smokers: a pilot inductively coupled plasma mass spectrometry study. *J Periodontal Res* 2016; 52: 83-88.
 34. **Díaz C, López F, Henríquez P, Rodríguez**

- guez E, Serra-MaJem L. Serum manganese concentrations in a representative sample of the Canarian population. *Biol Trace Elem Res* 2001;80:43-51.
35. Bertinchamps AJ, Miller ST, Cotzias GC. Interdependence of routes excreting manganese. *Am J Physiol* 1966;211:217-224.
36. Yamano N, Ikeda Y, Sakama M, Izawa-Ishizawa Y, Kihira Y, Ishizawa K, Miyamoto L, Tomita S, Tsuchiya K, Tamaki T. A long-term high-fat diet changes iron distribution in the body, increasing iron accumulation specifically in the mouse spleen. *J Nutr Sci Vitaminol (Tokyo)* 2015;61:20-27.
37. Collins LM, Dziak JJ, Li R. Design of experiments with multiple independent variables: a resource management perspective on complete and reduced factorial designs. *Psychol Methods* 2009;14:202-224.
38. Kim MH, Choi MK. Seven dietary minerals (Ca, P, Mg, Fe, Zn, Cu, and Mn) and their relationship with blood pressure and blood lipids in healthy adults with self-selected diet. *Biol Trace Elem Res* 2013;153:69-75.
39. Misiewicz A, Radwan K, Jeleń B, Karmoliński M. Level of total cholesterol and triglycerides in blood of workers involved in production of ferromanganese alloys. *Med Pr* 1993;44:371-377.
40. Longo M, Crosignani A, Podda M. Hyperlipidemia in chronic cholestatic liver disease. *Curr Treat Options Gastroenterol* 2001;4:111-114.
41. Ritzel U, Leonhardt U, Näther M, Schäfer G, Armstrong VW, Ramadori G. Simvastatin in primary biliary cirrhosis: effects on serum lipids and distinct disease markers. *J Hepatol* 2002;36:454-458.
42. Smith MR, Fernandes J, Go YM, Jones DP. Redox dynamics of manganese as a mitochondrial life-death switch. *Biochem Biophys Res Commun* 2017;482:388-98.
43. Lee B-K, Kim Y. Relationship between blood manganese and blood pressure in the Korean general population according to KNHANES 2008. *Environ Res* 2011;111:797-803.
44. Otsuka T, Takada H, Nishiyama Y, Kodani E, Saiki Y, Kato K, Kawada T. Dyslipidemia and the risk of developing hypertension in a working-age male population. *J Am Heart Assoc* 2016;5:e003053.
45. Prasad K, Dhar I, Caspar-Bell G. Role of advanced glycation end products and its receptors in the pathogenesis of cigarette smoke-induced cardiovascular disease. *Int J Angiol* 2015; 24:75-80.