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## Original

# Blood pressure of omnivorous and semi-vegetarian postmenopausal women and their relationship with dietary and hair concentrations of essential and toxic metals

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**Abstract**

***Objective:* This study aims to ascertain the relationships between mineral consumption, hair mineral content, and blood pressure.**

***Methods:* The study involved 26 postmenopausal women from enclosed religious communities, 14 were semi-vegetarians and 12 were omnivores. Mineral dietary assessment was performed using a 14-d precise weight method and Food tables. Hair mineral levels were measured by means Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES). Multivariable stepwise linear regression analyses were performed to find out the variables that affected most blood pressure.**

***Results:* In general terms, the omnivorous diet contained a significantly higher mineral content than the semi-vegetarian one. The mineral intake from both diets implied no health risk to the women studied, as their estimated daily intake (EDI) of toxic elements such as Cd and Pb was lower than their respective provisional tolerable weekly intake (PTWI) of these minerals. Hair of the semi-vegetarians contained higher amounts of Al ($p < 0.01$), Ba ($p < 0.01$), K ($p < 0.001$), Na ($p < 0.001$), Pb ($p < 0.001$) and Mn ($p < 0.01$) but lower levels of Ca ($p < 0.05$) and Zn ($p < 0.05$) than that of their omnivorous counterparts. The omnivores presented significantly higher systolic ($p < 0.01$) and diastolic ($p < 0.05$) pressures than the semi-vegetarians. Levels of hair Co ($R^2 = 0.328$; $p = 0.032$) and hair K ($R^2 = 0.409$; $p = 0.014$)) were explicative for systolic and diastolic blood pressure, respectively.**

***Conclusion:* Several dietary mineral and hair contents were higher in semi-vegetarian women suggesting that the hair is an important mineral excretion via contributing to maintain blood pressure at low levels.**

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Key words: *Minerals. Hair. Diet. Blood pressure. Postmenopausal.*

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**PRESIÓN ARTERIAL EN MUJERES OMNÍVORAS Y SEMIVEGETARIANAS POSTMENOPÁUSICAS Y SU RELACIÓN CON LOS METALES ESENCIALES Y TÓXICOS EN LA DIETA Y EN EL CABELLO**

**Resumen**

***Objetivo:* Se pretende establecer una relación entre consumo y niveles de minerales en cabello y tensión sanguínea en mujeres postmenopáusicas.**

***Métodos:* El estudio se ha realizado en 26 mujeres postmenopáusicas pertenecientes a dos comunidades religiosas de clausura, siendo 14 semivegetarianas y 12 omnívoras. La determinación de la ingesta de minerales se realizó mediante pesada precisa durante 14 días y las Tablas de Composición de Alimentos. Los niveles de minerales en cabello fueron determinados mediante Espectrometría de Masas con Fuente de Ionización de Plasma de Acoplamiento Inductivo (ICP-MS) y Espectrometría de Emisión Atómica con Fuente de Excitación de Plasma de Acoplamiento Inductivo (ICP-OES). Se realizó un análisis lineal múltiple por pasos para explicar los variables que más influían en la presión arterial.**

***Resultados:* En términos generales, la dieta omnívora posee un contenido en minerales significativamente superior a la semivegetariana. La ingesta mineral de ambas dietas no implica riesgo para la salud de las mujeres estudiadas ya que la ingesta diaria de elementos tóxicos como Cd y Pb, estimada (IDA) está por debajo de sus respectivas ingestas semanales tolerables provisionales (ISTP). En las semivegetarianas el cabello contienen cantidades mayores de Al ($p < 0,01$), Ba ($p < 0,01$), K ($p < 0,001$), Na ($p < 0,001$), Pb ($p < 0,001$) y Mn ($p < 0,01$) y niveles inferiores de Ca ($p < 0,05$) y Zn ($p < 0,05$) que las omnívoras. Éstas últimas, además presentan presiones arteriales superiores, tanto sistólica ($p < 0,01$) como diastólica ($p < 0,05$). Las concentraciones de Co ($R^2 = 0,328$; $p = 0,032$) y K ($R^2 = 0,409$; $p = 0,014$) en cabello fueron explicativas de los niveles de presión arterial sistólica y diastólica, respectivamente.**

***Conclusión:* Los resultados de varios minerales en la dieta y en el cabello de mujeres semivegetarianas sugieren que el pelo es una importante vía de excreción mineral, contribuyendo al mantenimiento de la presión sanguínea a niveles más adecuados.**

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Palabras clave: *Minerales. Cabello. Dieta. Presión sanguínea. Post-menopausia.*

## Introduction

Elderly persons often alter their nutritional habits, becoming vegetarians for religious, socio-cultural, economic and/or therapeutic motives.[1] However, following vegetarian diets it is not always easy to meet current recommendations for some nutrients, as minerals.[2] Vegetarian diets differ from one another according to the extent to which they may include animal products. In this regard, the vegan diet is the most restrictive, while the semi-vegetarian diet is the most permissive. Semi-vegetarians, also called partial vegetarians, or moderate vegetarians, consume certain foods of animal origin but usually exclude red meat from their diet.[3] Although vegetarian diets, associated with a low incidence of several chronic diseases, and are normally considered healthy, not all of them provide the same health benefits.[4]

The most common deficiencies documented in the elderly have been for zinc,[5] magnesium and calcium. Ca deficiency is mainly associated with bone resorption,[6] while magnesium deficiency increases muscle catabolism and cardiovascular risk.[7] Zn, Cu, Mg and Mn imbalances affect blood pressure values and are thus related to hypertension.[8] Other metals (e.g. As, Pb, Cd, and Hg) that have no known beneficial biological function may be harmful to health, and may even prove toxic at low doses following long-term exposure.

The mineral status of individuals has conventionally been determined by analysis of biological samples, most commonly blood. In recent years, however, the use of human scalp hair has become increasingly popular as a biomonitor of trace elements to determine nutritional status, as well as for diagnostic purposes.[9,10] The study of this metabolically inactive tissue permits an estimation of environmental exposure levels to minerals and investigation of the status and alterations of trace element concentrations in the body. Nonetheless, the limitations of hair mineral analysis, such as possible contamination by dust and/or sweat, and the effects of age, sex, and place of residence, must be considered together with its potential advantages.[11]

The aims of the present study were a) to assess the dietary mineral content of semi-vegetarian and omnivorous postmenopausal women; b) to monitor hair mineral content; and c) to study the possible relationships between blood pressure, dietary mineral content, and hair mineral concentrations.

## Material and methods

### *Study participants*

Volunteers had to fulfill the following eligibility criteria: a) age: women $\geq$ 45 years, b) postmenopausal, and c) BMI $\geq$ 18 kg/m$^2$. Taking into account the influence of degenerative diseases, sex, age, BMI, drugs, and smoking on blood pressure,[12] exclusion criteria included a) previous cardiovascular, metabolic, or systemic disease, b) treatment with any lipid-lowering, antihypertensive or anti-inflammatory drugs and/or hormone replacement therapy, and c) smoking habit.

Thirty volunteers were selected from among 40 nuns recruited in two enclosed convents from the same town in the centre of Spain and with a regular lifestyle and dietary habits. Two volunteers were excluded due to ongoing use of drug therapy. Three volunteers were 45 years old but were considered premenopausal. Five participants suffered from white coat hypertension. In addition, two volunteers were excluded for habitual use of hair cosmetics, and another two were excluded due to their very short scalp hair, which prevented hair sample collection. Thus, a total of 26 nuns (12 from an omnivorous enclosed community and 14 from another enclosed convent with semi-vegetarian food habits) were studied. Study protocols were approved by an Ethics Committee of the Universidad Complutense de Madrid, Spain, and research activities were performed in accordance with the principles laid down in the Helsinki Declaration.

### *Dietary assessment*

Food intake of each individual was estimated by the precise weighing method during a 14-day period.[13] Energy and nutrient intakes were calculated using food composition tables for raw weights of foodstuffs and compared with the Recommended Dietary Allowances for the Spanish population.[14] Daily intake of the toxic elements studied was calculated taking into account dietary composition and food consumption according to specialized literature.[15,16]

### *Anthropometric measures*

Trained personnel obtained body weight and height using standardized methodology. Body mass index (BMI) [(weight (kg)/height$^2$ (m$^2$)] was also calculated. Systolic and diastolic blood pressures were measured using a Hg sphygmomanometer, following WHO recommendations.[17]

### *Mineral concentrations in hair. Sample collection and analysis*

Scalp hair samples (1-3 cm) weighing approximately 1.0 g were taken from the occipital region, by cutting hair 2 cm from the hair root using stainless-steel scissors without vanadium and stored in plastic bags. Samples were washed to ensure accurate assessment of endogenous metal content. The washing procedure was carried out according to International Atomic Energy Agency (IAEA) recommendations.[18] Hair samples were first washed with ultrapure water, then washed

**Table I**
*Anthropometric characteristics and energy and macronutrients intakes in omnivore and semi-vegetarian postmenopausal women*

| | *Omnivores (n = 12)* | | *Semivegetarians (n = 14)* | | *Significance* |
|---|---|---|---|---|---|
| | *Mean ± SD* | *Median (P25-P75)* | *Mean ± SD* | *Median (P25-P75)* | *p-value* |
| Age (years) | 71.4 ± 6.4 | 71.5 (67.3-77.8) | 65.78 ± 11.07 | 65.5 (55.8-75.0) | ns |
| Weight (kg) | 59.3 ± 7.7 | 57.2 (55.0-67.4) | 54.31 ± 9.30 | 54.6 (46.1-61.7) | ns |
| Height (cm) | 153.0 ± 7.0 | 154 (149-156) | 153.0 ± 7.0 | 153 (148-157) | ns |
| BMI (kg/m$^2$) | 25.3 ± 2.7 | 25.5 (24.0-26.4) | 23.2 ± 3.4 | 22.6 (20.1-25.2) | ns |
| Systolic blood pressure (mmHg) | 145.8 ± 20.7 | 145 (130-168) | 126.4 ± 16.1 | 120 (118-140) | ** |
| Diastolic blood pressure (mmHg) | 80.8 ± 7.9 | 80 (72-90) | 67.1 ± 8.7 | 60 (60-70) | * |

Values are mean ± SD and Median (Percentile 25-Percentile 75) of indicated number of volunteers. ns: non-significant; **p < 0.01.

three times with acetone, and finally washed once again with ultrapure water. The samples were then oven-dried at 100 ºC.

A 250 ± 0.1 mg portion of each sample was weighed and introduced into a high-pressure, enclosed, Teflon decomposition vessel. Five millilitres of a 2.5:0.25 $HNO_3$ and $H_2O_2$ (v/v) mixture were carefully added to each sample and the vessels were gently shaken, sealed and digested in a microwave oven at 330 W for 10 min.

Samples were wet ashed according to the method of González-Muñoz et al.[10] Multi-element analysis of Al, Ba, Cd, Co, Cr, Mn, Mo, Ni, Pb, Sb, Se, Sr, and V was performed with Inductively Coupled Plasma Mass Spectrometry (ICP-MS), 810 Bruker Corporation (Billerica, MA, USA). Other elements were analyzed with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES), Perkin Elmer Model Optima 3300 DV, (Palo Alto, CA, USA) using the multi-element method described by Gonzalez-Muñoz et al.[10] Elements with the highest isotopic abundance, free from isobaric and polyatomic interference, were selected for ICP-MS spectrometry. The validation process of the methods based on ICP-OES and ICP-MS techniques was performed according to Eurachem guidelines,[19] with regard to accuracy, precision, sensitivity, and linearity using the experimental setting that provided the optimal conditions. The reagents and methods employed have been described in greater detail by González-Muñoz et al.[10]

*Statistical analysis*

This study was designed to have a power of 80% to detect a 25% relative difference between mineral hair concentrations considering a pooled SD of 25% for most minerals using the Mann-Whitney U test for group comparison [PASS 2008 program, NCSS, (Kaysville, Utah, USA)]. To perform main factor analysis, hair mineral contents, when necessary, were natural logarithmic transformed. For systolic and diastolic blood pressure variables, a stepwise multiple regression procedure was used to identify variables that explained the systolic and diastolic blood pressure variability of the postmenopausal women studied. Explicative variables considered for systolic or diastolic blood pressures were a) and the consumption of cereals, dairy products, eggs, oils, vegetables, pulses, fruits, meat and derivates, fish and derivates, and sugar; b) the intakes of energy, carbohydrates, protein, fat, cholesterol, fibre, and minerals and the energy contribution of carbohydrates, protein, fat, SFA, MUFA and PUFA omega-6 and omega-3; c) the mineral content in hair. The SPSS statistical package (version 15.0, Chicago, IL., USA) was used to analyze the data.

## Results

*Anthropometrical characteristics*

Table I summarizes the anthropometrical characteristics and systolic and diastolic blood pressure values of the population studied. No significant differences in anthropometrical parameters were detected between the two groups of women. However, the semi-vegetarians, of whom 25% were overweight, tended to have lower BMI values than their omnivorous counterparts, of whom 50% were overweight (data not shown). Significant differences between the omnivorous and semi-vegetarian women were observed with regard to blood pressure. The omnivores had significantly higher ($p < 0.01$) systolic and ($p < 0.05$) diastolic pressures than the semi-vegetarians.

*Dietary intakes*

Energy intakes in both communities were not significantly different; However, differed in their protein ($p < 0.01$), total PUFA ($p < 0.01$), PUFA omega-6, and PUFA omega-3 energy contributions (all $p < 0.01$) (table II).

Intake of components of several food groups varied significantly between the two diets (table III). The

**Table II**

*Intake by food group in omnivore and semi-vegetarian postmenopausal women*

| | *Omnivores (n = 12)* | | *Semivegetarians (n = 14)* | | *Significance* |
|---|---|---|---|---|---|
| | *Mean ± SD* | *Median (P25-P75)* | *Mean ± SD* | *Median (P25-P75)* | *p-value* |
| Energy (kJ/d) | 7,632 ± 1,377 | 7,671 (6,543-8,524) | 7,335 ± 774 | 7,343 (6,778-7,653) | ns |
| Carbohydrates (g/d) | 218 ± 54.0 | 210 (189-250) | 197 ± 28.9 | 203 (177-212) | ns |
| Protein (g/d) | 69.7 ± 13.4 | 67.4 (61.2-77.8) | 52.0 ± 6.4 | 52.5 (47.9-54.3) | ** |
| Fat (g/d) | 86.8 ± 15.7 | 88.7 (78.4-98.5) | 90.1 ± 8.2 | 88.7 (83.8-94.0) | ns |
| Carbohydrates (%En) | 43.6 ± 8.9 | 43.8 (42.0-47.3) | 42.0 ± 3.0 | 43.0 (42.0-45.0) | ns |
| Protein (% En) | 14.8 ± 3.6 | 14.4 (13.0-16.5) | 11.7 ± 1.4 | 11.4 (11.0-12.0) | ** |
| Fat (%En) | 41.6 ± 8.4 | 43.3 (38.5-46.8) | 46.4 ± 3.0 | 46.7 (45.5-48.3) | ns |
| SFA (%En) | 11.4 ± 2.2 | 11.7 (10.0-13.0) | 11.9 ± 1.7 | 12.0 (11.0-13.0) | ns |
| MUFA (%En) | 21.3 ± 4.6 | 21.2 (19.0-23.3) | 20.6 ± 1.2 | 20.0 (19.8-21.3) | ns |
| PUFA (%En) | 6.4 ± 2.3 | 6.5 (4.9-7.3) | 10.7 ± 0.7 | 10.5 (10.4-11.0) | ** |
| PUFA omega-6 (%En) | 5.8 ± 2.3 | 5.2 (4.0-7.3) | 9.8 ± 0.67 | 9.8 (9.5-9.9) | ** |
| PUFA omega-3 (%En) | 0.40 ± 0.33 | 0.40 (0.30-0.60) | 0.65 ± 0.18 | 0.68 (0.56-0.73) | ** |
| Omega-6/omega-3 | 14.9 ± 2.8 | 15.0 (12.8-16.0) | 15.1 ± 1.9 | 15.0 (14.0-17.0) | ns |
| Fibre (g/d) | 19.8 ± 2.1 | 19.9 (19.0-22.0) | 21.5 ± 2.1 | 20.5 (19.0-22.5) | ns |
| Cholesterol g/d | 0.48 ± 0.08 | 0.50 (0.46-0.50) | 0.40 ± 0.03 | 0.40 (0.39-0.40) | ns |

Values are mean ± SD and median (Percentile 25-Percentile 75) of indicated number of volunteers. ns, non-significant; ***(p < 0.001); **(p < 0.01); * (p < 0.05)

**Table III**

*Intake (g) by food group in omnivore and semi-vegetarian postmenopausal women*

| | *Omnivores (n = 12)* | | *Semivegetarians (n = 14)* | | *Significance* |
|---|---|---|---|---|---|
| | *Mean ± SD* | *Median (P25-P75)* | *Mean ± SD* | *Median (P25-P75)* | *p-value* |
| Cereals | 159 ± 20.7 | 158 (171-141) | 133 ± 22.1 | 137 (120-144) | *** |
| Dairy products | 450 ± 52.3 | 470 (435-480) | 505 ± 86.5 | 495 (480-559) | ** |
| Eggs | 73.1 ± 5.6 | 74.5 (69.3-78.0) | 64.3 ± 9.9 | 66.0 (62.5-68.5) | * |
| Oils | 49.4 ± 9.0 | 47.5 (43.3-56.3) | 59.1 ± 5.7 | 60.0 (58.0-63.5) | *** |
| Vegetables | 247 ± 40.5 | 238 (228-257) | 370 ± 56.0 | 374 (335-403) | *** |
| Pulses | 19.0 ± 2.7 | 19.5 (17.5-20.3) | 14.7 ± 2.0 | 15.0 (13.5-15.8) | *** |
| Fruit | 260 ± 23.9 | 258 (250-274) | 275 ± 26.3 | 271 (258-287) | ns |
| Meat and derivates | 49.7 ± 11.3 | 49.95 (44.5-52.8) | 0.0 ± 0.0 | 0.0 (0.0-0.0) | *** |
| Fish and derivates | 53.8 ± 6.3 | 53.0 (51.0-54.3) | 56.5 ± 9.7 | 55.3 (53.6-62.4) | * |
| Sugar | 39.5 ± 6.9 | 39.5 (36.1-44.3) | 19.0 ± 1.7 | 18.6 (18.1-19.8) | *** |

Values are mean ± SD and median (Percentile 25-Percentile 75) of indicated number of volunteers. ns, non-significant; ***(p < 0.001); **(p < 0.01); * (p < 0.05).

omnivorous women consumed more cereals ($p < 0.001$), dairy products ($p < 0.01$), eggs ($p < 0.05$), meat ($p < 0.001$), pulses ($p < 0.001$), and sugar ($p < 0.001$), but less fish ($p < 0.05$) and fewer oils ($p < 0.001$) and vegetables ($p < 0.001$) than the semi-vegetarians.

The daily dietary intake of minerals by the postmenopausal women is presented in table IV along with recommended dietary allowances (RDA) and provisional tolerable weekly intake (PTWI) values. Except for I, and K all mineral intakes differ between groups. The omnivores ingested more Ca ($p < 0.05$), Cd ($p < 0.01$), Cr ($p < 0.001$), Fe ($p < 0.001$), Mg ($p < 0.001$), Na ($p < 0.001$), Ni ($p < 0.001$), and Zn ($P < 0.01$) and less Mn ($p < 0.001$) and Pb ($p < 0.01$) than the semi-vegetarian women.

### *Hair mineral levels*

The omnivores had higher concentrations of Cd ($p < 0.01$), Co ($p < 0.01$), and Zn ($p < 0.05$), but lower levels of Al ($p < 0.01$), Ba ($p < 0.05$), K ($p < 0.001$), Mn ($p < 0.01$), Na ($p < 0.001$), and Pb ($p < 0.01$) than the semi-vegetarians (table V).

**Table IV**
*Dietary intake of metals and metalloid of omnivore and semivegatarian postmenopausal women*

| | *Omnivores (n = 12)* | | *Semivegetarians (n = 14)* | | *Significance* | | |
|---|---|---|---|---|---|---|---|
| | *Mean ± SD* | *Median (P25-P75)* | *Mean ± SD* | *Median (P25-P75)* | *p-value* | *RDA[14+]* | *PTWI (µg/kg/bw/week)* |
| Ca (mg/d) | 893 ± 104 | 860 (843-899) | 786 ± 133 | 820 (753-858) | * | 800 | nd |
| Cd (µg/d) | 15.0 ± 1.6 | 15.0 (11.9-16.5) | 11.0 ± 1.7 | 11.0 (10.1-11.9) | ** | | 7 |
| Cr (µg/d) | 103 ± 18.6 | 98.9 (88.5-110.3) | 74.1 ± 10.4 | 74.4 (68.1-79.4) | *** | | 1,050 |
| Fe (mg/d) | 10.4 ± 1.7 | 10.5 (10.0-11.2) | 8.7 ± 1.1 | 9.0 (8.0-9.0) | *** | 10 | 5,600 |
| I (µg/d) | 466 ± 116 | 490 (451-490) | 502 ± 96.2 | 490 (466-516) | ns | 110 | nd |
| K(mg/d) | 2,806 ± 241 | 2,900 (2,600-2,900) | 2,811 ± 284 | 2,900 (2,600-2,900) | ns | 3,500 | nd |
| Mg (mg/d) | 243 ± 59.1 | 253 (218-275) | 198 ± 27.9 | 180 (170-195) | *** | 350 | nd |
| Mn (µg/d) | 1,857 ± 201 | 1,866 (1,782-1,982) | 2,204 ± 311 | 2,218 (2,012-2,377) | *** | | 1,100 |
| Na (mg/d) | 2,866 ± 724 | 2,900 (2,475-3,225) | 2,279 ± 232 | 2,300 (2,200-2,400) | *** | 2,40[0++] | nd |
| Ni (µg/d) | 307 ± 74.1 | 301 (285-314) | 211 ± 28.2 | 213 (194-226) | *** | | 35 |
| Pb (ng/d) | 2.46 ± 0.15 | 2.48 (2.39-2.54) | 2.80 ± 0.40 | 2.70 (2.60-3.00) | ** | | 0.025 |
| Zn (mg/d) | 8.37 ± 1.66 | 8.50 (7.75-9.00) | 6.75 ± 1.02 | 7.05 (6.20-7.50) | ** | 15 | 4,900 |

*RDA: Spanish recommended dietary allowance. PTWI: Provisional Tolerable Weekly Iintake. Values are mean ± SD and Median (Percentile 25-Percentile 75) of indicated number of volunteers. nd: no determined; ns, non-significant; ***(p < 0.001); **(p < 0.01); *(p < 0.05).

**Table V**
*Metals and metalloids hair levels (µg/mg) of omnivore and semivegetarian post-menopausal women*

| | *Omnivores (n = 12)* | | *Semivegetarians (n = 14)* | | *Significance* |
|---|---|---|---|---|---|
| | *Mean ± SD* | *Median (P25-P75)* | *Mean ± SD* | *Median (P25-P75)* | *p-value* |
| Al | 5.7 ± 4.3 | 4.5 (1.8-9.1) | 11.1± 5.1 | 12.4 (5.5-14.6) | ** |
| Ba | 0.23 ± 0.09 | 0.23 (0.14-0.32) | 0.39 ± 0.19 | 0.37 (0.21-0.49) | * |
| Ca | 565 ± 355 | 413 (294-966) | 357 ± 119 | 373 (270-460) | ns |
| Cd | 0.033 ± 0.010 | 0.033 (0.022-0.034) | 0.019 ± 0.010 | 0.019 (0.010-0.021) | ** |
| Co | 0.30 ± 0.54 | 0.039 (0.026-0.345) | 0.02 ± 0.01 | 0.017 (0.013-0.025) | ** |
| Cr | 0.35 ± 0.19 | 0.27 (0.25-0.54) | 0.39 ± 0.14 | 0.36 (0.29-0.54) | ns |
| Cu | 12.2 ± 3.0 | 12.4 (9.8-14-9) | 11.2 ± 1.2 | 10.9 (10.3-12.1) | ns |
| Fe | 10.2 ± 6.0 | 9.8 (6.0-11.4) | 13.1 ± 6.3 | 11.0 (9.6-16.7) | ns |
| K | 7.90 ± 3.9 | 6.10 (4.7-13.0) | 26.5 ± 16.6 | 22.8 (17.0-32.2) | *** |
| Mg | 30.2 ± 11.4 | 27.0 (24.5-40.9) | 34.7 ± 17.3 | 33.3 (20.4-43.0) | ns |
| Mn | 0.13 ± 0.15 | 0.076 (0.061-0.104) | 0.28 ± 0.25 | 0.191 (0.119-0.356) | ** |
| Mo | 0.50 ± 0.53 | 0.230 (0.153-0.723) | 0.63 ± 0.65 | 0.28 (0.18-1.25) | ns |
| Na | 28.7± 18.9 | 24.0 (12.2-44.7) | 77.2 ± 36.4 | 87.1 (51.4-97.5) | *** |
| Ni | 0.94 ± 0.33 | 0.89 (0.71-0.99) | 0.83 ± 0.28 | 0.76 (0.59-1.07) | ns |
| Pb | 0.81 ± 0.44 | 0.95 (0.36-1.15) | 2.01 ± 1.17 | 1.87 (0.97-2.81) | ** |
| Se | 0.52 ± 0.23 | 0.55 (0.35-0.68) | 0.61 ± 0.12 | 0.61 (0.53-0.70) | ns |
| Sb | 0.041 ± 0.03 | 0.041 (0.017-0.055) | 0.062 ± 0.08 | 0.036 (0.019-0.048) | ns |
| Sr | 1.4 ± 1.2 | 0.80 (0.57-2.78) | 1.0 ± 0.81 | 0.77 (0.39-1.53) | ns |
| V | 0.041 ± 0.022 | 0.032 (0.022-0.063) | 0.059 ± 0.027 | 0.052 (0.037-0.081) | ns |
| Zn | 222 ± 47.1 | 229 (214-257) | 187 ± 43.2 | 189 (147-225) | * |

Values are mean ± SD and Median (Percentile 25-Percentile 75) of indicated number of volunteers. ns, non-significant; ***(p < 0.001); **(p < 0.01); *(p < 0.05).

*Multivariant studies*

Table VI shows the linear stepwise regression models for systolic and diastolic blood pressures. Three models were assessed: a) food group consumption; b) energy, micronutrients, fibre, alcohol consumption and c) hair mineral concentrations were considered. The food group consumption models explained the 24.5% (p = 0.010) and 28.3% (p = 0.005) of systolic and diastolic blood pressures data variance, respectively. Meat and fat con-

**Tabla VI**
*Linear stepwise regression models for systolic and diastolic blood pressures of postmenopausal women*

| *Models* | *Independent variable* | *Coefficients* | *Significance* | *β-coefficient* |
|---|---|---|---|---|
| | *Considering food groups consumption* | | | |
| Systolic blood pressure | $R^2 = 0.245$; F: 10.35; p = 0.010 | | | |
| | Intercept | 49.4 ± 18.7 | 0.046 | |
| | Meat consumption (g) | 0.68 ± 1.64 | < 0.001 | 0.872 |
| | Oil and fat consumption (g) | 1.30 ± 0.47 | 0.012 | 0.570 |
| Diastolic blood pressure | $R^2 = 0.283$; F: 9.46; p = 0.005 | | | |
| | Intercept | 67.4 ± 2.9 | < 0.001 | |
| | Meat consumption (g) | 0.26 ± 0.09 | 0.005 | 0.532 |
| | *Considering energy, nutrients, mineral, fibre, and cholesterol consumptions* | | | |
| Systolic blood pressure | $R^2 = 0.982$; F: 67.05; p < 0.0001 | | | |
| | Intercept | 83.5 ± 13.2 | 0.011 | |
| | SFA consumption (g/d) | 3.80 ± 0.53 | < 0.001 | 1.029 |
| | Cr consumption (μg/d) | 0.89 ± 0.10 | < 0.001 | 0.844 |
| | PUFA contribution (%en) | -4.50 ± 0.63 | < 0.001 | -0.450 |
| | Cholesterol consumption (mg/d) | -0.180 ± 0.047 | 0.009 | -0.595 |
| | I consumption (μg/d) | 0.054 ± 0.019 | 0.026 | 0.307 |
| Diastolic blood pressure | $R^2$=0.881; F: 19.74; p<0.0001 | | | |
| | Intercept | 58.8 ± 11.3 | 0.001 | |
| | Cr consumption (μg/d) | 0.30 ± 0.05 | < 0.001 | 0.746 |
| | Na consumption (mg/d) | 9.6 ± 1.9 | 0.004 | 0.651 |
| | Protein contribution (%en) | -2.2 ± 0.6 | 0.008 | -0.458 |
| | *Considering hair minerals* | | | |
| Systolic blood pressure | $R^2 = 0.328$; F: 5.87; p = 0.032 | | | |
| | Intercept | 171.0 ± 13.3 | < 0.001 | |
| | Hair LnCo (μg/mg) | 9.1 ± 3.7 | 0.032 | 0.573 |
| Diastolic blood pressure | $R^2$=0.409; F: 8.291; p = 0.014 | | | |
| | Intercept | 81.9 ± 3.3 | < 0.001 | |
| | Hair K (μg/mg) | -0.37 ± 0.13 | 0.014 | -0.639 |

Values are the coefficient ± standard errors; P: P values for $R^2$ significance, Ln: Natural logarithmic.

sumption by one hand and meat by the other were positively associated with the systolic and diastolic blood pressures, respectively. The second models explained the 98.2% (p < 0.001) and 88.1% (p < 0.001) of systolic and diastolic blood pressure data variance, respectively. SFA, Cr and I consumptions were positively associated while the energy contribution of PUFA and the cholesterol consumption negatively with systolic blood pressure. Cr and Na consumptions were positively associated but the energy contribution of protein negatively with diastolic blood pressure. The hair mineral content models explained the 32.8% (p = 0.032) and 40.9% (p = 0.014) of systolic and diastolic blood pressure variance data, respectively. Co (as Ln values) in hair was positively associated with systolic blood pressure while K hair negatively with systolic blood pressure.

## Discussion

Blood pressure levels of the semi-vegetarians coincide with those of vegetarians studied by Myers and Champagne.[20] Vegetarians have been reported to display lower BMI values and blood pressure levels, and lower incidence rates of type 2 diabetes, colon cancer, and lower energy and macronutrient intakes than non-vegetarians.[2]

The two diets, rich in fat and relatively poor in carbohydrates, reflected present Spanish eating habits.[21] Although their fatty acid contributions and profiles differed, the omega-6/omega-3 ratios of the two diets did not vary significantly. This omega-6/omega-3 fatty acids ratio is known to affect blood pressure, while other fatty acids and sources of dietary energy and minerals have little or no influence over blood pressure values[22]. Vegetarians diets offer a number of nutritional benefits including lower levels of saturated fat, cholesterol, and animal protein as well as higher levels of carbohydrates, fibre, magnesium, potassium, folate, antioxidants such vitamins C and E, and phytochemicals.[2] Omnivorous diets tend to have more cholesterol and less fibre than semi-vegetarian ones. The consumption of meat and meat derivates and oil by one hand and that of SFA, Cr, I, and PUFA (%En) and cholesterol by other hand were associated with systolic blood pressure levels. Diastolic pressure appears associated by one hand with meat consumption and by other hand with Cr, Na consumption and energy protein contribution.

These facts clearly explain the differences between the food groups intakes observed in both studied groups. Although both diets were mixed diets rich in fruits, vegetables, eggs, milk and fish but the semi-vegetarians' one was absent of meat-group items.

In some cases, intake of the essential elements was below the levels recommended for adults in Spain.[14] Data from a number of studies performed in Western countries indicate that mean intake of Mg and Zn by the elderly is below recommended levels.[7,23] Results of the present study show that the omnivores and semi-vegetarians consumed only 69% and 57% of the RDA for Mg, respectively, and 56% and 55% of the RDA for Zn, respectively. The semi-vegetarians consumed low but adequate levels of Na, while the omnivores consumed somewhat higher than recommended levels of this mineral.[17] Dietary intake of iron by semi-vegetarian women was lower than RDI[14] and 13% of these individuals did not reach appropriate Fe intakes. Moreover, given the characteristics of their diet, the semi-vegetarian women would necessarily have less bioavailable dietary Fe than their omnivorous counterparts.[24] The omnivores consumed more Ca than the semi-vegetarians, and only 26% had an intake below the RDI, as compared with 33% of the semi-vegetarians. The main sources for Ca in the standards Western diet are milk and other dairy products.[25] The fact that the omnivorous women consumed a significantly greater amount of dairy products than the semi-vegetarians would explain their higher Ca levels.

The omnivores in the present study consumed significantly more cereals than the semi-vegetarians, and grains contributed more Cd,[16] Mg[25] and Cr[26] to the diet than any other food group. According to Anderson and Kozlovsky,[27] intake of Cr is highly correlated with intake of K, total fat, saturated fat and Na. The semi-vegetarians may have consumed less Fe, Na, and Zn than the omnivores as a result of their meatless diet.

In order to assess the health risk associated with the estimated intake levels mentioned above, these consumptions were compared with the current provisional tolerable weekly intake (PTWI) values for these elements.[28] The estimated intake of the toxic elements Cd and Pb in both groups was lower than the PTWI values for these minerals and was similar to that reported in other countries. For this reason, these intake levels do not represent a health concern for the women of the present study. Nevertheless, although intake of Ni was 18% below PTWI levels in the semi-vegetarians, it reached PTWI levels in the omnivores. Excess Ni decreases tissue levels of Mg, Mn, and Zn.[29] Nonetheless, there is little information available regarding either chronic or acute effects of excess dietary intake of Ni.[29]

Cd intake is positively correlated with several chronic diseases, particularly hypertension. As previously mentioned, the omnivorous women consumed significantly more of this metal than their semi-vegetarian counterparts.[30] The omnivores consumed more Na and less K than the semi-vegetarian women. Several epidemiological studies[31] have reported the beneficial impact of reducing salt intake on hypertension. A low intake of K has been related to hypertension and cardiovascular diseases.[32] The semi-vegetarians had lower blood pressure levels than the omnivores. In this regard, our findings concur with those of Cianciaruso.[33] Elliot et al.[34] found that dietary Ca and Mg values are inversely correlated with blood pressure. The omnivorous women although consumed more Mg and Ca than the semi-vegetarians, displayed higher blood pressure levels. Not clear explanation can be drawn but Ca may mitigate some of the toxicity ascribed to Cd[35] in omnivore women.

Bibliographic data regarding the ranges of toxic and essential metal concentrations are influenced by numerous parameters, including gender, age, income, dietary habits, and environmental status (food, air, water, soil), which are not always taken into account by investigator.[36]

The values recorded in the hair samples from the groups studied were within the normal Spanish range. This is an important finding, since according to Durnicz-Sokolowska et al.,[9] hair concentrations of bioelements that are outside the reference range may be indicative of various pathological conditions. Deficiencies in essential trace elements and/or high levels of toxic metals may, thus, be involved in the development of heart disease. In addition, toxic metals may also reduce absorption of essential elements.[37]

Hair concentrations of Ca, Fe, Mg, Mn, and Na were lower in the women of the present study than in another group of 60 non-smoking Spanish women aged 52-78 who consumed a Mediterranean diet.[38] Touyz and Schiffrin[39] found that Mg concentrations in erythrocytes and hair decrease with age. Arnaud et al.[40] concluded that that Se concentrations decrease in the elderly. The present data regarding Se values in hair coincide with the range previously cited (0.002-6.6 mg/g. Se levels in hair decrease with age as is the case in nails.[41] Hair Cd levels in the women of the present study were lower than those reported for developing countries, but Pb levels were similar to those of individuals in developed countries.[42]

The fact that the omnivores in the present study had significantly higher blood pressure levels than the semi-vegetarians may be related to their hair levels of certain minerals.

Al is naturally present in many foods. A certain amount of Al in many plant foods due to inappropriate harvesting techniques or soil contamination. In general, vegetables are better sources of Al than animal foods.[23] The high Al values in the hair of the semi-vegetarians may be due to the fact that these women consumed more of most vegetables than their omnivorous counterparts. The amount of Al in tap water, which varies between municipalities depending on the quantity of aluminium salt used by the local water-purification treatment plant, did not affect our results, as study participants lived in the same town and drank the same water.[43]

Hair concentrations of Ba, an element mainly present in plant foods, were 70% higher in the hair of the semi-vegetarians than in that of the omnivores, although the amount consumed by the former women did not appear to be harmful. In cases of intoxication, Ba ions competitively block the passive efflux of K ions and cause the Na-K ion pump to act continuously, producing an intracellular accumulation of K and extracellular hypokalaemia.[44] As discussed earlier, present data suggest that hair works as a secretory system, helping the body to eliminate both Ba and K.

The higher hair levels of Ca and Na in the omnivores may indicate that the diet of these women contained a greater quantity of these elements than that of the semi-vegetarians.[45] K intake and hair levels in the semi-vegetarians were both significantly higher than the corresponding values for the omnivores. These results are complex and somewhere paradoxical. As previously noted, elimination of K via the hair in the semi-vegetarians was probably caused by excretion of Ba. In a previous paper[10] we found that hair concentrations of Na were significantly higher in hypertensive individuals than in normotensive subjects. Results of the present study contrast with those expected, as aldosterone is known to promote retention of K, lowering levels of that element in hair. Hair follicle cells express aldosterone and Na excretion is aldosterone-dependent,[46] which contributes to the excretion of Na and the accumulation of K in the hair. These facts help to explain why the semi-vegetarians of the present study displayed lower blood pressure levels than the omnivores. Complete vegetarians present lower blood pressure levels than omnivores, possibly because K-rich vegetarian diets contain low levels of Na.[33]

Cd is harmful to human health, and previous publications have reported that high levels of Cd cause hypertension.[47] Cd increases blood pressure by raising plasma renin activity and modifying catecholamine metabolism or by inducing sodium retention by directly influencing the proximal renal tubules.[46] Hair Cd values were lower ($p < 0.01$) in the semi-vegetarians, at least partially explaining, the lower blood pressure levels of this group. The semi-vegetarians presented higher hair Pb levels ($p < 0.01$) and had a higher estimated intake of this metal than the omnivores. It may be relevant that the semi-vegetarian women lived in the countryside, near a main road. Tetraethyl lead was once routinely added to gasoline as an antiknock agent and certain vehicles may still use leaded gasoline (e.g. tractors). As Pb may share and compete for some of the same cellular transport and absorption receptors as Fe, diets with reduced Fe bioavalilability could potentially enhance retention of this element,[49] at least partially explaining the similar hair levels of Fe in both groups of women.

As the omnivores presented higher hair levels of Co than their semi-vegetarian counterparts, hair may represent a route of Co excretion, effectively reducing the bioavailability of this mineral. Co enhances the activity of hypoxia-inducible factor (HIF),[50] and histological examination reveals that Co reduces proteinuria as well as kidney damage. Co increases the expression of HIF-regulated genes such as erythropoietin, vascular endothelial growth factor and heme oxygenase-1.

Vegetables may be rich in Mo if they are grown in neutral or alkaline soils high in available Mo. The semi-vegetarians tended to present higher levels of Mo in hair than the omnivores, probably as a result of their high vegetable intake.[51]

Almost certainly as a result of their higher Mn intake, the semi-vegetarians displayed, through their elevated hair manganese levels, a higher Mn status than the omnivores.[52] On the other hand, iron deficiency may increase Mn absorption and further increase the body-burden of Mn, especially in vegetarians.[53] Mn activates nitric oxide synthase (NOS I), which converts L-arginine into L-citrulline and $NO^{\bullet}$, and produces $O_2^{\bullet-}$ in the absence of L arginine. Nitric oxide has been implicated in many physiopathological conditions, including hypertension.[54]

Vegetarian diets usually provide a lower amount of bioavailable Zn than omnivorous diets. Plant foods rich in Zn, such as legumes, whole grains, nuts, and seeds are also high in phytic acid, an inhibitor of Zn bioavailability.[49] Low dietary intake of Zn by vegans was attributed to heavy reliance on fruits and vegetables that are poor sources of Zn.[3] Zn intake is especially correlated with that of protein and largely depends on the protein source. As a consequence of their diet, the semi-vegetarians had lower hair Zn concentrations than the omnivores. Zn levels in the hair of vegetarians are low, compared with those of omnivores.[49] However, Ball and Ackland[55] conclude than ovolactovegetarians did not have a significantly greater risk of low Zn status than omnivores. Excessive Zn intake may contribute to elevating systemic BP levels in normotensive individuals, presumably as a result of the oxidative stress produced by superoxide substrates.[56] The mechanism involved is due likely to a decrease in the action of the vasodilator NO through the formation of peroxynitrite.

Among mineral in hair, Co for systolic blood pressure and K for diastolic blood pressure appear as good explicative models.

## Conclusions

Although limitations of hair mineral analysis could be influenced by dust and/or sweat, age, sex, and place of residence,[11] the strict population selection, the hair sampling and the methodological criteria followed in the present paper permit us to conclude that semi-vegetarian women presented higher Al, Ba, K, Na, Pb and Mn but lower levels of Ca and Zn hair levels than their omnivorous counterparts suggesting the influence of their diet. Differences in hair mineral values between the semi-vegetarians and omnivores of the present study explain, at least partially, the higher systolic and diastolic pressures found in the semi-vegetarians. Meat, SFA, Cr and I consumptions and Co in hair were positively associated, while K in hair negatively with the systolic and diastolic blood pressures. Further studies are needed to better comprehend the relationship between hair mineral content and blood pressure and the mechanisms involved in their regulation.

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