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Original article

Serum metal levels in a population of Spanish pregnant women

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A B S T R A C T

Objective: To describe serum levels of calcium, copper, selenium, magnesium, iron and zinc and evaluate their relationship with maternal socio-demographic characteristics and dietary variables in women in the first trimester of pregnancy.
Method: Cross-sectional study with 1279 participants from the INMA cohorts.
Results: The concentrations of the elements analyzed were within the normal range. Associations with higher levels of these metals were found for calcium with white meat intake (p = 0.026), for copper with excess body weight (p < 0.01), low social class (p = 0.03) and being multipara (p < 0.01), for magnesium with being over 35 years old (p = 0.001), high social class (p = 0.044), primiparous status (p = 0.002) and low daily intake of bread (p = 0.009) and legumes (p = 0.020); for zinc with university education (p = 0.039) and residence in Gipuzkoa (p < 0.01), and for selenium with residence in Valencia (p < 0.01), university education (p = 0.001), vitamin B_6 supplementation (p = 0.006), fish intake (> 71 g/day) (p = 0.014) and having been born in Spain (p = 0.001). Further, lower iron levels were associated with being overweight (p = 0.021) or obese (p < 0.001) and vitamin B_{12} supplementation (p = 0.006).
Conclusions: Our results suggest that trace elements in the analyzed cohorts are adequate for this stage of pregnancy. The variability in these elements is mainly linked to socio-demographic and anthropometric variables.

Niveles de metales en suero en una población de mujeres embarazadas españolas

Palabras clave:
Suero
Oligoelementos
Embarazo

R E S U M E N

Objetivo: Describir las concentraciones de calcio, cobre, selenio, magnesio, hierro y zinc en muestras de suero de gestantes en el primer trimestre y evaluar la relación con las características sociodemográficas maternas y las variables de dieta.
Método: Estudio transversal con 1279 participantes de las cohortes INMA.
Resultados: Las concentraciones de los elementos analizados estuvieron dentro de los límites de referencia. El calcio se asoció con el consumo de carne blanca (p = 0,026). Los valores elevados de cobre se asociaron con tener exceso de peso (p < 0,01), clase social baja (p = 0.03) y ser multípara (p < 0,01). Los valores más elevados de magnesio se asociaron con tener más de 35 años (p = 0,001), clase social alta (p = 0,044), ser primípara (p = 0,002) y bajo consumo diario de pan (p = 0,009) y legumbres (p = 0,020). El zinc se asoció con tener estudios universitarios (p = 0,039) y con la cohorte de Gipuzkoa (p < 0,01). Los valores más altos de selenio se asociaron con la cohorte de Valencia (p < 0,01), tener estudios universitarios (p = 0,001), tomar suplementos de vitamina B_6 (p = 0,006), consumo de pescado >71 g/día (p = 0,014) y ser española (p = 0,001). Los valores más bajos de hierro se asociaron con tener exceso de peso (p = 0,021) u obesidad (p < 0,001) y con tomar suplementos de vitamina B_{12} (p = 0,006).
Conclusiones: Nuestros resultados sugieren que los oligoelementos en las cohortes analizadas son adecuados para esta etapa del embarazo. La variabilidad de estos elementos está asociada principalmente a las variables sociodemográficas y antropométricas.

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Introduction

Trace elements are bioelements that play a significant role in keeping the body healthy.[1] The human body cannot produce them, and therefore, we need to obtain them at appropriate doses from our diet. Both deficiency and excess may have a negative impact on body functions and the body's needs vary depending on the stage of the growth. Nutrition is vital during pregnancy for both the expectant mother and the developing fetus. Notably, the nutritional requirements of pregnant women differ from those of non-pregnant women of the same age. They also vary according to the week of pregnancy, with few differences compared to non-pregnant women during the first trimester; demands increasing during the second trimester; and a sharp rise during the third trimester, due to the rapid development of the fetus. Daily dietary reference intakes (DRIs) for pregnant women in Spain, published by the Spanish Federation of Nutrition, Food and Dietetics Societies[2] in 2010 were: 1000-1300 mg/d for calcium (Ca), 350-400 mg/d for magnesium (Mg), 1000 μg/d for copper (Cu), 27 mg/d for iron (Fe), 60 μg/d for selenium (Se) and 11-13 mg/d for zinc (Zn). Serum levels in the following ranges are considered normal: Ca 8.5-10.2 mg/dL, Fe 60-180 μg/dL, Mg 0.75-1.25 mmol/L, Cu 70-140 μg/dL, Se 70-150 ng/mL and Zn 0.66-1.10 μg/mL.[3]

Fe is necessary for proper development of the placenta, as well as bone and organ formation in the fetus. Iron-deficiency anemia is relatively common among pregnant women and may increase the risk of some complications, including preterm delivery and low infant birth weight. To prevent maternal anemia, it is recommended that pregnant women take a daily oral supplement of 30 to 60 mg of elemental Fe.[4]

Zn is important for protein synthesis, cell division, and nucleic acid metabolism. Deficiency in this element is manifested in different ways, depending on its severity, but in particular, may have a negative impact on the nervous and reproductive systems. Cu is essential for oxidative metabolism, cell growth, development of connective tissue and hemoglobin synthesis.[5] Deficiency in this element can cause bone deformations, as well as cardiovascular problems. Se is part of the key enzyme glutathione peroxidase, seems to be involved in platelet function and helps to neutralize heavy metals such as mercury, lead, arsenic and cadmium. Further, deficiency in this element affects all components of the immune system. During pregnancy, there is active transport to the fetus through the placenta and Se deficiency is reported to be associated with a variety of adverse outcomes including miscarriage, preterm delivery, gestational diabetes and preeclampsia.[6]

Mg is a key element for fetal growth and is one of the most abundant nutrients in the human body. It is involved in cellular respiration, protein synthesis, maintenance of cardiovascular health, regulation of cellular function and the action of collagen, among many other functions. Deficiency in Mg during pregnancy has been associated with preeclampsia and preterm delivery, as well as low infant birth weight.[7]

Lastly, Ca is another element necessary for normal fetal growth.[8] Among its functions, in addition to providing structure and rigidity to the bones, it plays a key role in muscle contractility, the transmission of signals from the brain nerves to the rest of the body, the circulation of the blood and the production of hormones and enzymes for various body functions. Lower maternal serum Ca levels have been associated with pregnancy-induced hypertension and preeclampsia.

The objective of this work was to describe serum levels of Ca, Fe, Mg, Se, Cu and Zn, and evaluate their relationship with maternal socio-demographic, anthropometric and dietary variables in women from cohorts from Valencia and Gipuzkoa (Spain) in their first trimester of pregnancy.

Method

Study population

Study participants were enrolled in the INMA Project (from the Spanish: Infancia y Medio Ambiente, meaning childhood and the environment: www.proyectoinma.org). A cross-sectional study was designed. We gathered information from women in the INMA Valencia and Gipuzkoa cohorts during their routine check-up in the first trimester of pregnancy (Valencia n = 855; Gipuzkoa n = 638). The final study population was composed of 1279 women (Valencia n = 656, Gipuzkoa n = 623). Samples were collected between February 2004 and June 2005 in Valencia and between May 2006 and February 2008 in Gipuzkoa, and Mg was only analyzed in the Valencia cohort (n = 656). Informed consent was obtained from all the participants before inclusion. The hospital ethics committees of each area approved the research protocols.

Socio-demographic and dietary characteristics

Data on socio-demographic characteristics were collected through questionnaires. Occupational status data were classified into two categories according to whether the women were in work. Social class was based on occupation and five categories were considered in accordance with the Spanish adaptation of the British Registrar General's Social class classification (I being the highest socioeconomic position and V the lowest). In order to increase the statistical power for the analysis, social class was further grouped into two categories: manual worker or lower class (IV and V) and non-manual worker or higher class (I-III). Educational level was classified according to the highest level attained into three categories: up to primary school, secondary school and university. Body mass index (BMI) before pregnancy was classified as underweight: < 18.5 km/m^2; normal weight: 18.5-24.9 kg/m^2; overweight: 25-29.9 kg/m^2; or obese: $\geq$ 30 kg/m^2. Smoking status information was classified as: smoker during pregnancy, quit at the beginning of pregnancy, ex-smoker (quit earlier than at the beginning of pregnancy) or never-smoker. The daily consumption of alcohol was recorded as grams of alcohol consumed daily and was subsequently categorized into two categories: $\leq$ 5 g/day and > 5 g/day. Food and mineral or vitamin supplement intake was also evaluated using a food-frequency questionnaire (FFQ) during pregnancy. The FFQ used was a version of Willett's questionnaire adapted for use in a Spanish population[9]. Regarding vitamin and mineral supplementation, information was collected on the intake of vitamin B6,vitamin B12, vitamin D,Ca,Fe,Mg and Zn supplements. The food frequency variables were categorized at the median for the analysis.

Chemical analysis

1) Sample preparation

Serum samples were prepared for ICP-MS analysis by a direct alkali dilution method. Briefly, samples were diluted 1:15-50 with an alkali solution consisting of 2% butanol (Honeywell Research Chemicals, Seelze, Germany), 0.05% EDTA (Sigma-Aldrich, St. Louis MO, USA), 0.05% Triton X-100 (Sigma- Aldrich),1% NH4OH (Romil,Cambridge,UK) and 20 μg/g of the internal standards Sc,Ge and Rh. Finally, the samples were vortexed and analyzed.

2) Instrumentation and analysis

An inductively coupled plasma mass spectrometry system (Agilent 7700x ICP-MS, Agilent Technologies, Tokyo, Japan) equipped with an octopole reaction system, employing a collision/reaction cell, was used for measuring concentrations of six elements in human serum: Ca, Mg, Fe, Cu, Zn, and Se. Two modes were used for the analysis of Se: collision mode using helium and reaction mode using hydrogen gas. The hydrogen mode has certain benefits over using helium for Se analysis. Hydrogen removes doubly charged species, and due to its smaller size, it can increase the sensitivity of Se measurements.

3) Quality control

The level of detection for each element was estimated as three times the standard deviation (SD) of blanks (alkali solution) and a signal-to-noise ratio of 3. The accuracy and precision of each measurement was verified by analyzing commercially available reference materials: Seronorm human serum lot MI0181 (SERO, Billingstad, Norway) and NIST animal serum SRM 1598a (National Institute of Standards and Technology, Gaithersburg MD, USA).

Blanks and reference materials were treated along with the serum samples collected and analyzed at the beginning, in the middle and at the end of each run. Metal concentrations were corrected according to the variations in the three daily measurements of the Seronorm reference material. The correction was performed by adding to each measurement the difference between the daily mean of the reference measures and the overall mean of the reference measures. These measurements were performed at the Institute of Environmental Medicine at the Karolinska Institutet (Sweden).

Statistical analysis

Descriptive statistics were calculated for each metal. A bivariate analysis was carried out between each metal and the defined variables overall and by cohort. Student's t-test or analysis of variance was performed depending on the number of categories of the independent variable (see Appendix online). Multivariable linear regression models were built to estimate the association between serum levels of metals and the independent variables. The variables with a $p \leq 0.20$ in the bivariate analysis were initially included in the models and those that were statistically significant ($p < 0.05$) were kept in the model, following a backward selection procedure. Statistical analyses were carried out using IBM SPSS for Windows, version 24.

We also performed permutational multivariate analysis of variance to test the null hypotheses of no association between multivariate variance in serum levels of Ca, Cu, Fe, Se and Zn, and socio-demographic and dietary variables. To this end, two multivariate linear models were built: the first only considered dietary explanatory variables; and the second, only socio-demographic explanatory variables. The method for variable selection that we applied was stepwise backward regression. Finally, we also built a joint (socio-demographic-dietary) model with the variables selected in the former step and then applied the method of Borcard et al.[10] to partition multivariate variance in serum levels of the metals into components explained by socio-demographic factors, by dietary factors, and by a shared fraction.

Results

We analyzed data from serum samples of 1279 women (51.3% from the Valencia cohort). Sample size, percentages and missing values of all the variables are shown in Table 1. Overall, 92% had been born in Spain, 61.3% were 30 years old or older, with a mean age of 30.7 years (31.3 and 30.1 years for the Gipuzkoa and Valencia cohorts respectively), 50.1% lived in urban areas, 77.2% had secondary or university studies, 78.5% were active workers, 51% belonged to the lowest social class and 55% were primipara. In relation to other variables, 24.7% had excess body weight, 17.4% smoked during pregnancy, 14.9% had smoked but quit before pregnancy and 1.1% consumed more than 5 g of alcohol per day.

Sample size, missing data, mean, SD, minimum, maximum and percentiles (25th, 50th and 75th) for each metal are presented in Table 2 by cohort. Mean and standard deviation of the levels were 94030.7 ± 4278.0 μg/L for Ca, 1614.8 ± 279.5 μg/L for Cu, 1113.9 ± 325.7 μg/L for Fe, 17032.5 ± 1006.4 μg/L for Mg, 79.56 ± 9.64 μg/L for Se and 642.1 ± 110.8 μg/L for Zn.

Table 3 summarizes the results of multiple linear regression models for each metal. Ca levels were related to daily white meat consumption ($p = 0.026$). Higher levels of Cu were related to excess body weight ($p < 0.001$), low social class ($p = 0.003$) and being multipara ($p < 0.001$). On the other hand,lower levels of Cu were related to bread intake of less than 42 g /day ($p = 0.013$) and residence in Gipuzkoa, after adjustment for the other variables. With reference to Se, higher levels were related to residence in Valencia ($p < 0.001$), having a university degree ($p = 0.001$),taking B6 vitamin supplements ($p = 0.006$) and a fish intake of more than 71 g/day ($p = 0.008$), after adjustment for the other variables. Women born outside Spain showed lower Se levels ($p < 0.001$). In regard to Fe, lower levels were related to having excess body weight ($p = 0.021$) or obesity ($p < 0.01$) and taking vitamin B12 supplements ($p = 0.006$). Higher levels of Mg were associated with being over 35 years old ($p = 0.001$), high social class ($p = 0.044$), primiparous status ($p = 0.002$) and low daily bread ($p = 0.009$) and legume ($p = 0.020$) intake, after adjustment for the other variables.

Finally, higher levels of Zn were related to having a university degree ($p = 0.039$) and being from the Gipuzkoa cohort ($p < 0.001$), and lower levels of Zn were related to being over 35 years old ($p = 0.027$), after adjustment for the other variables.

Table 4 shows that the multivariate variance in serum levels of Ca, Cu, Fe, Se and Zn was associated with body mass, parity, educational level and country of origin (socio-demographic model) and also with white bread and vitamin B6 intake (dietary model). BMI and country of origin emerge as the most important among the explanatory variables considered. Although the influence of both socio-demographic conditions and diet on serum levels appears to be rather modest, the partitioning of variance in the joint model (Fig. 1) suggests that, overall, socio-demographic conditions are more influential than diet itself. Table 5 summarizes the results of previous studies.

Discussion

We analyzed trace element levels in pregnant women in their first trimester of pregnancy. Our results reveal that the main variables related to these levels are BMI, social class and cohort. According to Abbassi-Ghanavati et al.[11], serum Ca between 88,000 μg/L and 106,000 μg/L can be considered normal and our results fall within this range (94,030.7 μg/L). Further, the Ca levels in our sample of pregnant women are in line with those observed in several previous studies[7,12,13] (with values of 95,900 μg/L, 97,000 μg/L and 92,000 μg/L respectively). On the other hand, they are a little higher than those obtained by Kanagal et al.[14] (89,700 μg/L), and Punthumapol and Kittichotpanich[15] (89,900 μg/L), and much higher than those found in a study carried out in China by Tong[16] (21,600 μg/L). Some studies have shown Ca levels to be related to body weight, levels being significantly lower in those with excess body weight,[17,18] but we did not find any such relationship in our study. Regarding age, unlike other authors,[18] we found an association close to statistical significance, levels decreasing with increasing age ($p = 0.077$).

Table 1
Maternal anthropometric, socio-demographic, and dietary characteristics.

Variable	Categories Mean[a]	N	% SD[a]	Missing data
		1279	100	
Socio-demographic variables				
Cohort	Gipuzkoa	623	48.7	0
	Valencia	656	51.3	
Season	Spring	332	26.0	0
	Summer	350	27.4	
	Autumn	303	23.7	
	Winter	294	23.0	
Country of birth	Spain	1175	91.9	0
	Other	104	8.1	
Age (years)		30,7[a]	4.1[a]	
Age-cat (years)	<25	84	6.6	0
	25-29	412	32.2	
	30-34	570	44.6	
	≥35	213	16.7	
Residence	Urban	636	50.1	10
	No urban	633	49.9	
Physical activity before pregnancy	<1 hour/week	768	60.0	16
	1-3 hours/week	316	24.7	
	>4 hours/week	179	14.0	
Social class	No manual	629	49.2	0
	Manual	650	50.8	
Educational level	Up to Primary	291	22.8	2
	Secondary	511	40.0	
	University	475	37.2	
Mother's smoking habit	Never-smoker	526	42.3	37
	Ex-smoker	315	25.4	
	Quit at the beginning of pregnancy	185	14.9	
	Smoker in pregnancy	216	17.4	
Mother alcohol consumption	≤5 g/day	1249	97.7	
	>5 g/day	14	1.1	
Mother alcohol consumption (g/day)		0.28[a]	1.1[a]	
Parity	Primipara	699	54.7	0
	Multipara	580	45.3	
Gestation week		13.10*	1.27*	
BMI (kg/m[2])	Underweight	50	3.9	1
	Normal weight	912	71.4	
	Overweight	225	17.6	
	Obese	91	7.12	
Marital situation	Live with father	1263	98.7	0
	No live with father	16	1.3	
Occupational status	Employed	1002	78.5	2
	Unemployed	275	21.5	
Diet variables				
Calories (KJ/day)	≤2000	516	40.3	0
	> 2000	747	58.4	
Proteins (g/day)	≤99.50	461	36.5	16
	>99.50	802	63.5	
Fat (g/day)	≤89.5	726	57.5	16
	>89.5	537	42.5	
Carbohydrates (g/day)	≤250.50	687	54.4	16
	>250.50	576	45.6	
Dairy products (g/day)	≤455	676	53.5	16
	>455	587	46.5	
Red meat (g/day)	≤53.50	399	31.6	16
	>53.50	864	68.4	
White meat (g/day)	≤30.45	551	43.6	16
	>30.45	712	56.4	
Fish (g/day)	≤71	698	55.3	16
	>71	565	44.7	
Cereals and pasta (g/day)	≤90.5	723	57.2	16
	>90.5	540	42.7	
Legumes (g/day)	≤50	561	44.4	16
	>50	702	55.6	
Potatoes (g/day)	≤61.5	288	47.0	667
	>61.5	324	52.9	
Bread (g/day)	≤42	571	45.2	16
	>42	692	54.8	
Supplement variables				
Supplement Vit B_{12}	No	354	27.7	0
	Yes	925	72.3	

Table 1 (*Continued*)

Variable	Categories Mean[a]	N	% SD[a]	Missing data
Supplement Vit B_6	No	888	69.4	0
	Yes	391	30.6	
Supplement Vit D	No	962	75.2	0
	Yes	317	24.8	
Supplement Ca	No	915	71.5	0
	Yes	354	28.5	
Supplement Fe	No	836	65.4	0
	Yes	442	34.6	
Supplement Mg	No	903	70.6	0
	Yes	376	29.4	
Supplement Zn	No	903	70.6	0
	Yes	376	29.4	

BMI: Body mass index; SD: standard deviation.
[a] Mean and standard deviation.

Table 2
Concentrations of Ca, Cu, Fe, Mg, Se and Zn (μg/L) in serum samples during the first trimester of pregnancy by cohort and the whole sample. t-test independent samples analysis.

	Cohort	N	Missing data	Mean	SD	Min	P25	P50	P75	Max	p
Ca (μg/L)	Gipuzkoa	620	3	93748.46	5176.31	77547.12	90629.34	93249.86	96037.01	129211.7	
	Valencia	656	0	94297.40	3186.93	86610.39	92255.24	94092.87	96138.34	113686.1	
	Total	1276	3	94030.67	4278.00	77547.12	91648.91	93668.1	96103.3	129211.7	0.022[a]
Cu (μg/L)	Gipuzkoa	622	1	1620.35	291.36	520.29	1438.82	1604.02	1791.99	3004.92	
	Valencia	656	0	1609.62	267.84	642.42	1438.86	1612.59	1783.43	2563.56	
	Total	1278	1	1614.84	279.48	520.29	1438.74	1608.887	1787.08	3004.92	0.493
Fe (μg/L)	Gipuzkoa	622	1	1113.00	319.78	160.64	889.54	1099.11	1289.85	2369.89	
	Valencia	654	2	1114.88	331.40	234.16	880.62	1101.78	1325.09	2514.47	
	Total	1276	3	1113.96	325.66	160.64	888.30	1099.46	1304.38	2514.47	0.918
Mg (μg/L)	Valencia	656	0	17032.46	1006.40	13665.48	16363.53	17064.49	17700.25	20297.71	-
Se (μg/L)	Gipuzkoa	623	0	77.07	10.62	37.99	70.17	76.01	82.64	145.34	
	Valencia	656	0	81.93	7.92	48.45	76.35	81.27	86.40	115.12	
	Total	1279	0	79.56	9.64	37.99	73.61	79.05	85.15	145.34	< 0.001[a]
Zn (μg/L)	Gipuzkoa	561	59	673.79	102.80	307.75	601.87	666.28	730.34	1098.17	
	Valencia	629	27	613.84	110.17	349.81	544.63	600.48	667.37	1390.13	
	Total	1190	86	642.10	110.83	307.75	569.20	633.45	705.78	1390.13	< 0.001[a]

P: percentile; SD: standard deviation.
[a] Significant p-values.

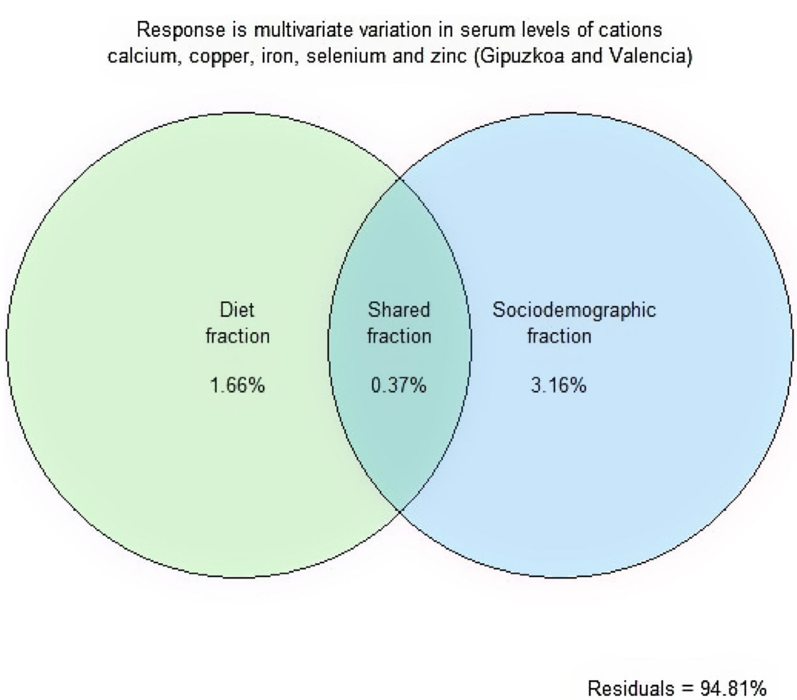

Figure 1. Venn diagram showing how multivariate variation in serum levels of Ca, Cu, Fe, Se and Zn was partitioned among a diet component and a socio-demographic component. Numbers are R^2 values (%). The diet component includes white bread intake and vitamin B_6 intake. The socio-demographic component includes body mass index, parity, education level and country of origin.

Table 3
Linear regression model for association between levels of Ca, Cu, Se, Mg, Fe, Zn and sociodemographic and dietary variables.

Dependent variable	Independent variables	β (95%CI)	p
Ca	Cohort (Gipuzkoa vs. Valencia)	216.22 (−24.02; 456.61)	0.078
	White meat consumption (≤30.45 g/day vs. >30.45 g/day)	549.44 (65.40; 1033.48)	0.026
Cu	Cohort (Gipuzkoa vs. Valencia)	− 29.96 (−49.36; −10.59	0.002
	BMI (kg/m^2)		
	18.5-25	-	
	< 18.5	−69.98 (−143.18; 11.21)	0.094
	25< 30	102.62 (63.26; 141.99)	< 0.001
	≥35	170.40 (111.71; 229.08)	< 0.001
	Social class (no manual vs. manual)	45.32 (15.14; 75.50)	0.003
	Parity (primipara vs. multipara)	92.31 (62.43; 122.18)	< 0.001
	Bread consumption (≤42 g/day vs. >42 g/day)	−49.35 (−88.19; −10.52)	0.013
Se	Cohort (Gipuzkoa vs. Valencia)	4.69 (3.40; 5.98)	< 0.001
	Country of birth (Spain vs. Other)	−3.27 (−5.17; −1.40)	0.001
	Educational level		
	Primary	-	
	Secondary	0.99 (−0.36; 2.34)	0.149
	University	2.29 (0.89; 3.69)	0.001
	Fish consumption (≤71 g/day vs. >71 g/day)	1.29 (0.26; 2.33)	0.014
	B_6 vitamin supplementation (no vs. yes)	1.88 (0.54; 3.22)	0.006
Fe	Cohort (Gipuzkoa vs. Valencia)	31.96 (−7.08; 71.00)	0.109
	BMI (kg/m^2)		
	18.5-25	-	
	< 18.5	56.37 (−36.17; 148.92)	0.232
	25< 30	−55.48 (−102.72; −8.24)	0.021
	≥35	−143.82 (−213.61; −73.93)	< 0.001
	B_{12} vitamin supplementation (no vs. yes)	−60.93 (−104.61; −17.24)	0.006
Mg	Age (years)		
	25-29	-	
	< 25	47.95 (−320.39; 224.50)	0.730
	30-34	19.35 (−164.78; 203.07)	0.836
	>35	423.22 (167.52; 678.93)	0.001
	Social class (no manual vs. manual)	−163.73 (−323.15; −4.31)	0.044
	Parity (primipara vs. multipara)	−256.18 (−421.60; −90.76)	0.002
	Bread consumption (≤42 g/day vs. >42 g/day)	−241.70 (−423.59; −59.82)	0.009
	Legumes consumption (≤50 g/day vs. >50 g/day)	−182.36 (−335.97; −28.76)	0.020
Zn	Cohort (Gipuzkoa vs. Valencia)	−28.21 (−34.60; −21.82)	< 0.001
	Age (years)		
	25-29	-	
	< 25	−4.89 (−31.07; 21.28)	0.714
	30-34	−1.46 (−15.59; 12.66)	0.839
	>35	−20.57 (−38.80; −2.34)	0.027
	Educational level		
	Primary	-	
	Secondary	4.35 (−11.72; 20.41)	0.596
	University	17.85 (0.89; 34.80)	0.039

BMI: body mass index; 95%CI: 95% confidence interval.

Table 4
PERMANOVA. The response or outcome, which is constituted by multivariate variation in serum levels of Ca, Cu, Fe, Se and Zn (for both cohorts, Gipuzkoa and Valencia), is explained in terms of either a purely diet model or in terms of a purely socio-demographic model. Adjusted sums of squares and permutation-based *p*-values are reported.

Variable	Df	SS	R^2	F	p
Diet model					
White bread intake	1	40.3	0.0075	8.20	0.001
Vitamin B6 intake	1	33.6	0.0063	6.84	0.001
Residual	1069	5246.0	0.9796	-	-
Total	1071	5355.0	1.0000	-	-
Socio-demographic model					
Body mass index	1	61.1	0.0114	12.57	0.001
Parity	1	36.2	0.0068	7.45	0.001
Education level	2	28.4	0.0053	2.93	0.003
Country of origin	3	55.5	0.0104	3.81	0.001
Residual	1064	5166.1	0.9647	-	-
Total	1071	5355.0	1.0000	-	-

Regarding Mg, our results (17,000 μg/L) are within the normal range for Mg of between 16,000 μg/L and 22,000 μg/L[11]. The levels we observed were similar to those reported by Kanagal et al.[14] (15,700 μg/L) and slightly lower than those observed by Punthumapol and Kittichotpanich[15] (20,400 μg/L), Arun et al.[7] (20,300 μg/L) and Sukonpan and Phupong[12] (20,700 μg/L). In contrast, a study carried out in China by Li[19], showed levels of serum Mg well below those obtained in this study (9800 μg/L).

The mean Cu level in our study population (1615 μg/L) was within the normal range of between 1120 μg/L and 1990 μg/L[11]. Similar levels have been observed in other studies[20–22] (1614 μg/L, and 1650 μg/L and 1750 μg/L, respectively). On the other hand,

Table 5
Trace elements concentrations in serum samples from women in different studies.

	Author	Year	Country	Sample size	Mean (SD)
Ca (μg/L)	Sukonpan[12]	2005	Thailand	40	97000 (7000)
	Ainy[13]	2006	Iran	48	92000 (6000)
	Punthumapol[15]	2008	Thailand	36	89900 (3100)
	Tong[16]	2010	China	90	21600
	Kanagal[14]	2014	India	60	89700 (6900)
	Arun[7]	2017	Nepal	35	95900 (6200)
Mg (μg/L)	Sukonpan[12]	2005	Thailand	40	20700
	Li[19]	2010	China	100	9800
	Kanagal[14]	2014	India	60	15700 (7200)
	Arun[7]	2017	Nepal	35	20300 (1600)
	Punthumapol[15]	2008	Thailand	36	20400 (1900)
Cu (μg/L)	Awadallah[21]	2004	Jordan	52	1750 (420)
	Zhang[23]	2013	China	2380	1026
	Jariwala[20]	2014	India	42	1614 (295)
	Choi[22]	2016	Korea	245	1650
	Polanska[24]	2017	Poland	539	1980 (570)
Zn (μg/L)	Awadallah[21]	2004	Jordan	52	770 (160)
	Izquierdo[26]	2007	Spain	159	654 (12.9)
	Zhang[23]	2013	China	2380	920
	Jariwala[20]	2014	India	42	514 (149)
	Shen[28]	2015	China	1447	900
	Choi[22]	2016	Korea	245	570
	Khoushabi[27]	2016	Iran	60	749
	Polanska[24]	2017	Poland	539	910 (270)
	Liu[34]	2017	China	1400	740
Fe (μg/L)	Awadallah[21]	2004	Jordan	52	690 (260)
	Zhang[23]	2013	China	2380	900
	Jariwala[20]	2014	India	42	1132 (519)
	Shen[28]	2015	China	1447	800
	Khoushabi[27]	2016	Iran	60	744
	Liu[34]	2017	China	1400	1315
Se (μg/L)	Kantola[31]	2004	Finland	216	81 (27)
	Izquierdo[26]	2007	Spain	159	99.59 (21.7)
	Ejezie[32]	2012	Nigeria	120	112.3
	Jariwala[20]	2014	India	42	70 (15)
	Choi[22]	2016	Korea	245	94
	Liu[34]	2017	China	1400	77.6

SD: standard deviation.

Cu levels considerably different from ours have been observed in China[23] and in Poland[24] (1026 μg/L and 1980 μg/L, respectively). Wilson et al.[25] reported significantly higher levels in obese women than underweight, normal weight or overweight women, as in our study. We found a trend related to BMI (with the highest levels in obese women), while others have observed an opposite trend.[17]

Again, in the case of Zn, the levels we observed (642 μg/L) lie within the normal range of between 570 μg/L and 880 μg/L.[11] These results were in agreement with those found in previous studies in Spain[26] and Iran[27] (654 μg/L and 749 μg/L, respectively). Studies carried out in China[23,28] and Poland[24] showed higher levels (920 μg/L, 900 μg/L and 910 μg/L, respectively), while studies carried out in Korea[22] and India[20] showed lower levels (570 μg/L and 514 μg/L respectively). We found an association between Zn and educational level, which could be an indicator of social class. Nevertheless, other authors did not find this pattern[29].

As for Se, our results were consistent with studies carried out in Spain[30] and Finland[31] with reported values of 81 μg/L, while a study from India[20] found lower values of 70 μg/L. On the other hand, higher values were found in Korea,[22] Spain[26] and Africa[32] (94 μg/L, 99.59 μg/L and 112.3 μg/L, respectively). In our study, Se levels appear to be related to fish consumption, which is consistent with other research findings.[33]

Fe levels between 720 μg/L and 1430 μg/L can be considered normal,[11] and our results are in that range (1114 μg/L). Similar results have been reported in China[23] and India[20] (900 μg/L and 1132 μg/L respectively), while some studies have shown slightly lower values[21,27,28] (690 μg/L, 800 μg/L and 744 μg/L respectively), and another study in China,[34] found values slightly higher than ours (1500 μg/L). We observed that being overweight or obese was associated with significantly lower serum Fe levels, a pattern that has been observed previously[15,25].

Social inequalities in dietary habits have been widely described and indicate that the lowest social classes tend to have less balanced and less healthy diets. Pregnant women with lower social status have fewer healthy habits, including a less healthy diet, more harmful behaviors, and poorer monitoring during pregnancy. Indeed, the role of education as a social determinant of diet has been confirmed by several authors as the most robust independent predictor of healthy dietary habits.[35]

The strengths of the study are the sample size (n = 1279) and the fact that we gathered data on and adjusted models for a large number of factors in the first trimester, especially socio-demographic characteristics. Regarding its limitations, the descriptive and cross-sectional character of the design means that we cannot ascertain causality in the associations observed, measurements were taken at only one point during pregnancy, and the spectroscopy measurements of the metals were element-specific not species-specific. Another limitation is that the information regarding some of the behavioral variables (smoking and alcohol consumption) was self-reported, an approach which may underestimate the prevalence of socially disapproved behaviors.

Conclusions

Our results indicate that the Ca, Cu, Se, Fe, Mg and Zn levels in both cohorts were adequate and within the ranges considered normal for pregnant women. More than half of the sample did not take supplements, except in the case of vitamin B_{12} (72%). Among the variables considered, those with the best explanatory value of the levels of these trace elements were anthropometric and socio-demographic variables and within these, BMI, social class, educational level and cohort. This study will serve as a basis for future research, in particular, to identify trends in these elements through pregnancy and their possible relationship with the later development of the child.

What is known about the topic?

Poor nutritional status is known to carry a higher risk of disease. This risk is greater during pregnancy since not only maternal health is at stake, but also the proper development of the fetus. Therefore the establishment and knowledge of reference values is of vital importance.

What does this study add to the literature?

This study provides relevant data on serum metal levels in pregnant women and their relationship with socio-demographic and anthropometric characteristics, supplementation during pregnancy and diet. The follow-up of clinical variables during pregnancy and the knowledge of socio-demographic conditions, supplementation, vitamins, minerals and diet of pregnant women are vital to ensure adequate care during pregnancy. This article will serve as a basis for future work assessing the evolution of these trace elements throughout pregnancy in order to improve perinatal outcomes and subsequent child development.

What are the implications of the results?

It is necessary to implement measures to intervene in healthy lifestyles (healthy diet, not smoking, avoiding a sedentary lifestyle, etc.) as well as care and attention during pregnancy. A balanced diet must be sufficient to cover the needs in a way that it contains all nutrients (proteins, carbohydrates, fats, vitamins and minerals) in adequate amounts. The requirements of minerals (Ca, Cu, Fe, Mg, Zn, Se, etc.) and vitamin (B12) are adequately covered by the consumption of raw fruit and vegetables, whole grains, raw olive oil and dairy products. On the other hand, it may be advisable to pay special attention to pregnant women with excess weight in relation to Fe control, as well as vitamin B12 supplementation.

Fish consumption, avoiding the most mercury-contaminated species (swordfish, shark, bluefin tuna), has a beneficial effect on health due to its high content in proteins of great nutritional value, omega-3 fatty acids, vitamin D and antioxidant substances (including Se). Considering that the variability of all these elements is mainly associated with socio-demographic and anthropometric variables, it is necessary to promote healthy habits and lifestyles, as well as self-care during pregnancy.

Editor in charge

Juan Alguacil.

Transparency declaration

The corresponding author on behalf of the other authors guarantee the accuracy, transparency and honesty of the data and information contained in the study, that no relevant information has been omitted and that all discrepancies between authors have been adequately resolved and described.

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Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication that could have influenced its outcome.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gaceta.2021.07.006.

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