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Assessment of iron and zinc intake and related biochemical parameters in an adult Mediterranean population from southern Spain: influence of lifestyle factors☆

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A hstract

This study assessed factors influencing iron and zinc intake and associated biochemical parameters in an adult population from southern Spain to identify patterns of intake and groups at risk for deficiency. A cross-sectional survey was done in Andalusia (southern Spain) to study nutrient intakes in a random sample of 3421 subjects (1747 men, 1674 women, age between 25 and 60 years). Blood samples were obtained for biochemical assays in a random subsample of 354 subjects (170 men, 184 women). Food consumption was assessed by 48-h recall. In blood samples, we measured red blood cells, haemoglobin (Hb), haematocrit, total iron binding capacity (TIBC) and plasma concentration of Fe and Zn. Information about educational level, smoking habit, alcohol consumption and physical exercise was collected with a structured questionnaire. Intakes were below two thirds of the recommended dietary allowances (RDA) in 22.45% of the sample for Fe and in 56.45% for Zn. Iron deficiency [two or more abnormal values for plasma Fe, TIBC, transferrin saturation and mean cell volume (MCV)] was found in 12.7% of the sample, and iron-deficient anaemia (low values for Hb, MCV, mean cell Hb and mean corpuscular Hb concentration) was found in 2.1%. In smokers, plasma levels of Fe were higher, and MCV was lower than in nonsmokers. Plasma Zn concentrations were below the reference value in 17.8% of the persons. Age and body mass index correlated inversely with plasma Zn $(P<.01)$. Gender, age, obesity, smoking, alcohol consumption and physical activity were associated with differences in nutrient intakes. Logistic regression analysis showed that female gender and older age were associated with the risk of low intakes of Fe and Zn. © 2009 Elsevier Inc. All rights reserved.

Keywords: Iron; Zinc; Adult; Status; Southern Spain

1. Introduction

Abundant information is available on dietary habits of the Spanish population and on the intakes of different foods and nutrients [\[1,2\].](#page-5-0) However, information on the influence of educational level, obesity, smoking habit, alcohol consumption and physical exercise on mineral status in the adult Spanish population is scarce, especially for Zn intake.

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The aims of this study were to evaluate the influence of demographic characteristics and lifestyle factors on nutritional status of Fe and Zn in the adult population in Andalusia (a western Mediterranean region in southern Spain), document the patterns of nutrient intake, identify groups at risk for dietary deficiency and suggest factors that may influence the status of these nutrients. It is hoped that this information will be useful in designing future health interventions aimed at modifying dietary habits.

2. Methods

2.1. Participants

The data reported here were obtained within the framework of a large-scale study in the region of Andalusia

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(southern Spain) [\[3](#page-5-0)–5]. A cross-sectional epidemiological survey was conducted with a representative random sample of adults between 25 and 60 years old living in the region of Andalusia, an 87597 -km² area with 2946228 inhabitants between the ages of 25 and 60 years at the time of the study [\[6\].](#page-5-0) Sampling was probabilistic and stratified and took place in several stages. The primary sampling unit was cities and towns (municipalities), the secondary unit was homes and the tertiary unit was individuals of either gender. The theoretical sample size was 3680 subjects for a sampling error of less than 5% and estimates at the 95% confidence level.

The actual sample consisted of 3421 individuals (1747 men, 1674 women), for a participation rate of 92.9% with valid observations. Participants were asked whether they had any acute or chronic illness and were included if they were (or appeared to be) in good health; pregnant and lactating women were excluded. The population of participants who consumed mineral or vitamin–mineral supplements (3.9%) was also excluded.

Blood samples were taken for biochemical analysis from a random subsample of 354 subjects (170 men, 184 women), who comprised approximately 10% of the sample.

The demographic characteristics of the sample; the sampling system; methods of food consumption assessment (48-h recall) [\[7\]](#page-5-0); blood sample collection for biochemical analysis and recording of level of education, smoking and drinking habits and physical exercise are described in detail elsewhere [3–[5\].](#page-5-0) The study protocol was approved by the Medical–Ethical Committee of the Health Council of the Andalusian Regional Government, and informed consent was obtained from each subject.

2.2. Analytical methods

Red blood cells (RBC) and haemoglobin (Hb) were determined with a Sysmex KX-21 automated haematology analyser (Sysmex, Kobe, Japan). Haematocrit (Hct) was determined by centrifugation at 12 000g for 5 min at 20°C. Total iron binding capacity (TIBC) was measured with commercial colorimetric kits from QCA (Amposta, Spain, ref. 991462). Seriscann Normal (ref. 994148) was used for quality control measures.

Mean cell volume (MCV), mean cell Hb (MCH) and mean corpuscular Hb concentration (MCHC) were calculated from the RBC, Hb and Hct.

The content of Fe and Zn in plasma was determined by atomic absorption spectroscopy (Perkin Elmer AAnalyst 300 spectrometer, Norwalk, CT, USA). Seronorm trace element assays (ref. 201405) (SERO, Billingstad, Norway) were used for Fe and Zn quality control measures. The value obtained for Fe was 1.23 mg/L (certified 95% CI, 1.19–1.35 mg/L), and that for Zn was 1.38 mg/L (certified 95% CI, 1.23–1.43 mg/L). For each element we used the mean of five separate determinations.

The percentage of transferrin saturation (TS) was calculated from the (plasma Fe/TIBC)×100. Reference values for all elements and biochemical analyses are shown in [Table 3](#page-2-0) [\[8\].](#page-5-0)

2.3. Statistical analysis

The crude experimental data were subjected to Student's t test for independent samples. Mineral intakes were adjusted for total energy using the energy-adjusted method [\[9\].](#page-5-0) Analysis of variance (ANOVA) $(2\times3$ -way) was used to test the differences in intake and analytical values between sexes and age groups and to look for interactions between age (as a three-level variable) and sex. Correlations between the variables were obtained with a partial correlation procedure after controlling by age and sex. Multiple logistic regression analysis was used to estimate the degree of association between intake or analytical values (dependent variable) and energy, sex, age, educational level, smoking, drinking and physical exercise. All analyses were done with version 12.0 of the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Differences were considered significant at the 5% probability level.

3. Results

Table 1 shows the contribution of the different food groups to Fe and Zn intake. Partial correlation coefficients after controlling by age and sex for the relationships between food and mineral intakes showed that the intakes of both elements correlated with intakes of most of the food groups considered here. The most notable correlations were those for grain products $(r=0.37$ for Fe and $r=0.41$ for Zn, $P<.001$ in both cases) and meat intake ($r=.25$ and $r=.31$ for Fe and Zn, respectively, $P<.001$ in both cases).

[Table 2](#page-2-0) shows mean intakes of energy, Fe and Zn and mean plasma levels of these minerals, together with their percentile distributions. Energy and mineral crude intakes were significantly higher in men than in women. However, when the intakes were adjusted for energy intake, the difference between sex was significant only for Zn. [Table 2](#page-2-0) also shows markers of Fe (plasma Fe, TIBC, TS, RBC, Hb,

 P indicates percentile.
 A ^a Mean values adjusted for energy intake.

^b Mean values were significantly different from those for the men ($P < 0.05$).

Hct, MCV, MCH and MCHC) and Zn status (plasma Zn) [\[10\]](#page-5-0) together with their percentile distributions. Mean biochemical values were within normal limits in both sexes.

Energy intake controlling by age and sex correlated with the intakes of Fe and Zn $(r=0.64$ and $r=0.56$, respectively, $P<0.01$ in both cases) and with RBC and Hb ($r= 0.12$ and $r= 0.14$, respectively, $P<05$ in both cases).

The occasional correlations between biochemical parameters and food intakes were weak. Iron intake correlated with RBC count $(r=0.13)$, Hb $(r=0.18)$ and Hct values $(r=0.12)$, with P<.05 in all cases.

Table 3 shows the percentages of our study population with intakes below two thirds of the recommended dietary allowance (RDA). The percentage of the population with low Zn intake was greater than the percentage with low Fe intake. Of note was the higher percentage of women than men at risk for deficient Fe intake. However, the percentages of men and women with plasma Fe concentrations below the reference value were higher than the percentages of both sexes with low plasma Zn concentration. As found for Fe intake, the proportion of participants with deficient plasma Fe levels was greater in women than in men.

Table 3 also shows the percentages of the population with laboratory results below the reference values. Based on the suggestion that the use of different markers simultaneously can provide a more accurate measure of Fe status [\[11\]](#page-5-0), we

Table 3

Percentage of the population with intakes below two thirds of the RDA [\[47\]](#page-6-0) and Zn, Fe and Hb concentrations, Hct, mean corpuscular volume, TIBC and percent TS below the reference values

	Total	Men	Women
Intake \leq 2/3 RDA			
\boldsymbol{n}	3421	1747	1674
Fe ^a	22.45	2.63	43.13
Zn^a	56.45	44.36	69.06

Biochemical parameters

% of the population with values below the reference values

^a The RDA for the Spanish population were used as reference values [\[47\].](#page-6-0) $\frac{b}{b}$ Reference values [8].

 \degree Defined as the existence of at least two abnormal values between four

markers of iron status (plasma Fe, TIBC, TS and MCV).
d Defined as below-normal values for Hb, MCV, MCH and MCHC.

Table 4

Effect of age, educational level, obesity, smoking, drinking and physical exercise on mean daily crude intakes and plasma concentrations of Fe and Zn

Mean values were significantly different from [†]25–39 years, ⁰obese, [‡]smokers, ^{||}drinkers and [¶]

^a One thousand eight hundred eighty persons with elementary school education only and 86 persons with no formal schooling.

b Two hundred twenty persons with elementary school education only and 10 persons with no formal schooling.

^c Obese: BMI >30 kg/m².

^d "Never" and "former" smokers.

^e Never drinks or drinks only on special occasions.

Sedentary: less than 1 h/week spent on leisure-time physical exercise.

considered individuals to be Fe-deficient if at least two of the following indicators were abnormal: plasma Fe, TIBC, TS and MCV. Individuals were considered to have iron-deficient anaemia if they had below-normal values for Hb, MCV, MCH and MCHC. As expected, the percentage of the irondeficient population was notably higher than the percentage population with iron-deficient anaemia. Moreover, the percentage of women with iron deficiency and iron-deficient anaemia was higher than the corresponding percentages in men.

When we compared mineral intakes across age groups (Table 4), we found that the oldest age group (50–60 years) had the lowest crude intake of Fe. However, mean plasma Fe concentration, Hb, MCV, MCH and percentage TS showed no significant changes with ageing.

Crude Zn intake decreased with age. In plasma, significant differences were found for Zn, with lowest values in the 50–60-year-old group (Table 4). Analysis of variance revealed a significant interaction only between age and sex for plasma Zn $(P<05)$. In addition, plasma Zn concentration correlated inversely with age $(r=-0.14, P<.01)$.

We also examined the possible effect of educational level, obesity, smoking habit, alcohol consumption and physical activity on the status of these minerals (Table 4). According to our results, Zn intake was lower in obese persons. Plasma Zn correlated inversely with body mass index (BMI) $(r=$ -0.13 , $P<01$). In smokers, drinkers and active people (those who devoted less than 1 h/week to physical exercise), higher intakes were found for Fe and Zn than in nonsmokers, nondrinkers and sedentary persons.

In contrast to the differences for intake when we compared participants for lifestyle variables, the results of the biochemical analyses showed the only significant differences to be higher plasma levels of Fe and lower MCV in nonsmokers than in smokers (Table 4).

Alcohol intake correlated with intakes of Fe $(r=0.29,$ P<.01) and Zn ($r = 0.20$, P<.01) and with Hb level ($r = 0.12$, $P<0.05$). The number of cigarettes smoked per day also correlated with Zn intake $(r=0.14, P<.05)$ and MCV $(r= 0.17, P< 01)$.

[Table 5](#page-4-0) shows the associations (odds ratio and 95% CIs) for Fe and Zn intake with energy intake, demographic and lifestyle factors. In addition to these results, we estimated the degree of association between biochemical parameters (dependent variables: plasma Fe, Hb, MCV, MCH, percentage TS and plasma Zn) and energy intake, demographic and lifestyle factors. The model adjusted for all variables failed to detect any significant associations.

4. Discussion

In southern Spain, as in other western countries, dietary Fe and Zn are provided mainly by grain products and meat Table 5

Odds ratios and 95% confidence intervals, adjusted for all variables in the table, for Fe and Zn intakes according energy intake, demographic and lifestyle factors

	Fe	Zn
Energy intake	OR (95% CI)	OR (95% CI)
$RDA < 100\%$	1.00	1.00
$RDA \ge 100\%$	$0.13(0.10-0.17)^*$	$0.09(0.07-0.12)$ *
Gender		
Male	1.00	1.00
Female	$37.21(29.23 - 47.38)^*$	$4.31 (3.35 - 5.52)^*$
Age (years)		
25-39	1.00	1.00
40-49	$1.25(0.98-1.60)$	1.72 $(1.29 - 2.31)^*$
50-60	$0.21 (0.16 - 0.28)^*$	$2.13(1.57-2.88)$ *
Obesity		
BMI \leq 0 kg/m ²	1.00	1.00
BMI \geq 30 kg/m ²	$0.73(0.57-0.95)$	$0.96(0.70-1.30)$
Educational level		
University	1.00	1.00
Secondary	$1.10(0.82 - 1.47)$	0.66 $(0.47-0.92)$ [*]
Primary/no schooling	$1.02(0.78-1.33)$	$0.75(0.55-1.02)$
Smoking habit		
Nonsmokers ^a	1.00	1.00
Smokers	$1.19(0.96 - 1.47)$	$0.89(0.71-1.12)$
Alcohol consumption		
Nondrinkers ^b	1.00	1.00
Drinkers	$0.75(0.61-0.91)^*$	$1.17(0.93-1.47)$
Physical exercise		
Active ^c	1.00	1.00
Sedentary	$1.18(0.95-1.46)$	$1.08(0.85-1.38)$

OR indicates odds ratios; CI, confidence intervals. All variables are adjusted for other variables in the table.
* $P<0.05$.

^a Nonsmokers and former smokers.
^b Never drinks or drinks only on special occasions.
^c Active: more than 1 h per week spent on leisure-time physical exercise.

[\(Table 1](#page-1-0)) [12–[16\].](#page-5-0) The correlations between energy intake and the intake of Fe [\[17\]](#page-5-0) and Zn support the notion that greater mineral intake was related with greater energy intake.

The intakes of Fe in southern Spain were similar to the mean values reported for the adult population of Spain overall [\[1\],](#page-5-0) Sweden [\[18\]](#page-6-0) and France [\[19\].](#page-6-0) However, Fe intakes in our study were slightly higher than those observed in adults in the Netherlands [\[18\],](#page-6-0) Belgium [\[20\]](#page-6-0), UK [\[14\]](#page-5-0) and Chile [\[16\]](#page-5-0) and slightly lower than in Denmark [\[18\]](#page-6-0) and the United States [\[21,22\]](#page-6-0). In women, Fe intake was lower than the values reported in Germany [\[18\]](#page-6-0), Russia [\[23\]](#page-6-0) and Ireland [\[12\]](#page-5-0) and was higher than in women from developing countries such as Nigeria [\[24\]](#page-6-0).

The intakes of Zn in southern Spain were similar to the mean values reported for the adult population of Spain overall [\[1\],](#page-5-0) Germany, Netherlands [\[18\]](#page-6-0), the United States [\[25\]](#page-6-0) and France [\[18,26\]](#page-6-0) but were slightly higher than those observed in adults in Sweden [\[18\]](#page-6-0), Ireland [\[12\]](#page-5-0), the United Kingdom [\[14\]](#page-5-0) and Chile [\[16\]](#page-5-0) and slightly lower than the intakes in Denmark [\[18\].](#page-6-0)

The higher energy-adjusted Zn intakes in men reflected their greater consumption of meat and grain products. Although the differences in crude Fe and Zn intakes between

men and women were significant, plasma concentrations were similar [\(Table 2](#page-2-0)).

The percentage of persons at risk for inadequate intake $(\leq 2/3$ or 66.6% RDA) of Fe in Andalusia (southern Spain) was lower than in Catalonia (northeastern Spain) [\[27\]](#page-6-0) and the Canary Islands [\[28\].](#page-6-0) A high percentage (39.3%) of the population in the United States was also found to be at risk for intakes lower than 70% of the RDA [\[25\].](#page-6-0)

The association between Fe intake and Hb in the adult population studied here has also been described by others in children aged 6–12 months [\[29\]](#page-6-0) and in a study with participants ranging in age from 6 months to 97 years [\[30\]](#page-6-0). The prevalence of anaemia (Hb \leq 12 mg/dl), as expected, was higher in women because of menstrual losses. The prevalence of anaemia among women in Andalusia is slightly lower than in women in Nepal [\[31\]](#page-6-0), Japan [\[32\]](#page-6-0) and Ireland, similar to the prevalence in the UK but higher than that in other European countries [\[33\].](#page-6-0) However, we note that participants in our study population ranged in age from 25 to 60 years, whereas the results reported by Hercberg et al. [\[33\]](#page-6-0) were obtained in menstruating women only. When we analysed the data only for women younger than 45 years, the prevalence of anaemia in our study population increased to 13.8%.

In the present study, the prevalence of iron-deficient anaemia ([Table 3](#page-2-0)) was similar to that reported by Deegan et al. [\[34\]](#page-6-0) in adolescents and lower than the prevalence found by Waldamann et al. [\[35\]](#page-6-0) in adult vegetarian women.

It is currently accepted that plasma Zn concentration is a valid indicator of whole-body Zn status in the absence of confounding factors, such as infection or stress [\[36\].](#page-6-0) Mean plasma Zn concentrations in the Andalusian population were similar to those reported in other regions of Spain [\[28,37\]](#page-6-0). However, the prevalence of low plasma Zn concentrations in southern Spain ([Table 3\)](#page-2-0) was lower than in Catalonia (northeastern Spain) [\[27\]](#page-6-0) and in Mexican women [\[38\]](#page-6-0) but much higher than the prevalence reported in the National Health and Nutrition Examination Survey II (NHANES II) study [\[39\]](#page-6-0) and in the Canary Islands [\[28\].](#page-6-0) In the latter study, the reference value (60 μ g/dl) was lower than the reference value we used (70 μg/dl), which may account for part of the considerable differences in prevalence between our study population and earlier studies.

The decrease in crude Zn intake with age [\(Table 3\)](#page-2-0) reflects mainly the decreased intake of meat in older age groups, as the intake of dairy products changes little with age. We found that in southern Spain, traditional dietary habits are stronger in older age groups, as has also been reported for a southern French population [\[40\].](#page-6-0) The decrease in Zn intake with age is reflected by lower plasma concentrations in older age groups [\[41,42\]](#page-6-0) and by the inverse correlation between plasma Zn concentration and age (see Results) [\[43\].](#page-6-0) The results of logistic regression analysis (Table 5) confirmed this finding.

In the present study, Fe status was not affected by obesity, but Zn status differed depending on BMI ([Table 4](#page-3-0)). The higher Zn intake in the nonobese population reflected the

greater consumption of grain and dairy products, which would account for the slightly higher mean values for plasma Zn concentration in this subgroup and for the inverse correlation between plasma Zn levels and BMI (see Results). This correlation was also described in pregnant women during the early weeks of gestation [\[44\]](#page-6-0) and in children aged 3–14 years [\[45\]](#page-6-0).

A possible explanation for the higher crude intakes of Fe and Zn in smokers [\(Table 4](#page-3-0)) is that energy intake was higher among smokers [9.90 (S.D., 3.79) MJ/day vs. 8.78 (S.D., 3.20) MJ/day; $P<.001$ for smokers and nonsmokers, respectively] because of their greater consumption of grain products and meat. The correlations between crude energy intake, total grain products and meat and crude intakes of each of the two elements (see Results) support this explanation. However, nonsmokers had higher mean plasma Fe concentrations than smokers, a finding that may be related with the higher vitamin C intake in nonsmokers [125.38 (S.D., 93.73) mg/day vs. 148.57 (S.D., 104.33) mg/ day; $P < 0.001$ for smokers and non-smokers, respectively].

The greater MCV in smokers [\(Table 4](#page-3-0)) was the result of the higher Hct [47.1 (S.D., 5.0) v. 45.4 (S.D., 4.3); $P<01$ in smokers and non-smokers, respectively]. Moreover, the number of cigarettes smoked per day correlated directly with MCV. These findings reflect the smoking-induced decrease pulmonary function.

As noted above with regard to the findings in the subgroup of persons who smoked, the greater crude intakes of the two elements in persons who drank [\(Table 4](#page-3-0)) reflected the greater energy intake in this population subgroup [drinkers 10.14 (S.D., 3.59) MJ/day v. non-drinkers 8.40 $(S.D., 3.18) MJ/day; P<0.01].$

The moderate mean intakes of alcohol in our population (one to two drinks per day) may help explain why no impairment was found in MCV among alcohol drinkers [3].

The greater energy intake in the active population (i.e., persons who spent more than 1 h per week on leisure-time physical activity) [active 9.76 (S.D., 3.35) MJ/day vs. sedentary 8.98 (S.D., 3.70) MJ/day; $P<.001$] accounted for the greater intakes of Fe and Zn in this subgroup. However, we should note that consumption of dairy products was also slightly higher in the active population [\[46,47\]](#page-6-0).

Although the interpretation of data from survey studies can be complex, our results provide an initial estimate of the nutritional status for Fe and Zn in the adult population of southern Spain. The percentages of persons found to be at risk for inadequate intake of Fe and Zn were worrisome [\(Table 3](#page-2-0)). Gender, age, obesity, smoking, alcohol consumption and physical activity were associated with differences in nutrient intakes. However, logistic regression analysis showed that the risk of low intake of Fe and Zn was associated only with energy intake, female gender and older age groups, and that the risks of Fe deficiency (in 12.7% of the population we studied), iron-deficient anaemia (2.1%) and hypozincemia (2.17%) were not associated with any of the other factors we investigated.

We therefore suggest that the intake of these minerals be increased in both genders and that recommendations to increase physical activity are in order. These measures should be aimed particularly at women of reproductive age, in whom menstrual blood loss increases the risk of iron deficiency and anaemia.

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