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Daily dietary intake of chromium in southern Spain measured with duplicate diet sampling

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We measured daily dietary Cr intake in southern Spain by sampling duplicate diets for seven consecutive days in different population groups. Cr was determined by electrothermal atomization–atomic absorption spectrometry. The samples were mineralized in a digestion block with HNO_3, $HClO_4$ and V_2O_5. A total of 161 duplicate diets from twenty-three subjects were analysed, and mean levels of Cr intake ranged from 9·39 to 205·16 μg/d. Mean Cr intake (100 μg/d) was similar to levels found for most other countries, and was within the range recommended by the National Research Council for a safe and adequate daily intake (50–200 μg/d). Chromium intake correlated significantly with energy, protein and carbohydrate intake, and with the daily intake of Zn, Fe, Mg, K, Na, Ca and nicotinic acid in the diets analysed.

Dietary chromium: Duplicate diet: Nutrient intake: Correlation

Cr, an essential element in human nutrition, is involved in carbohydrate and lipid metabolism (Prasad, 1985). The most frequent manifestation of chromium deficiency is altered glucose tolerance (Mertz, 1995). This nutrient has also been associated with diabetes and cardiovascular disease. Some authors have reported beneficial effects of dietary supplementation with chromium, particularly in groups in which deficiencies are frequent (Van Cauwenbergh *et al.* 1996). The National Research Council (1989) considered a varied, balanced diet the best way to guarantee adequate Cr intake; the recommended dietary intake for adults was set at 50–200 μg/d. However, at least one study noted the lack of reliable methods to evaluate chromium status in living organisms, and have called for further research in dietary Cr levels (Wood *et al.* 1995).

Estimating the dietary intake of Cr (or any other trace element) is made difficult by the various sources of error and the lack of advice as to how to avoid them (Pennington *et al.* 1986; Southgate *et al.* 1989; Nielsen, 1994). Among the methods proposed thus far, chemical analysis of duplicate diets provides the most precise and reliable data (Anderson & Kozlovsky, 1985; Cameron & Van Staveren, 1988; Nielsen 1994; Van Cauwenbergh *et al.* 1996; Biego *et al.* 1998, 1999).

In this study we evaluated the dietary intake of Cr in southern Spain by chemically analysing duplicate diets consumed by members of different population groups. To date, no data on micronutrient intake have been published for this population, and it has thus not been possible to determine whether dietary supplies can be considered adequate in this population on the basis of National Research Council recommendations (1989). In addition we looked for possible correlations between Cr intake and the intake of energy and macronutrients.

Materials and methods

Sampling strategies

To determine the dietary intake of trace elements, study designs that include collection and preparation of foods ready for consumption (by cooking) are believed to produce the most realistic and reliable results (Anderson *et al.* 1993; Robberecht *et al.* 1994; Van Cauwenbergh *et al.* 1996). Therefore, duplicate meals, drinking water, beverages and between-meal snacks were collected over 24 h periods in different places in southern Spain:

- Acuartelamiento de Cervantes, Grupo de Operaciones Especiales Santa Fe II (Granada). The dining hall of this military base serves meals to 115–120 persons.
- Colegio Mayor Jesús-María (Granada), a university residence providing full board to 190–200 students.
- Families consisting of two to ten members residing in the city of Granada.

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Table 1. Instrument parameters for Cr determination in diets by electrothermal atomization atomic absorption spectrometry

Wavelength (nm)	357·9
Slit width (nm)	0·7
Atomization system	Stabilized temperature platform furnace
Ashing temperature (°C)	1650
Atomization temperature (°C)	2500
Injection volume (μl)	10
Matrix modifier	50 μg $Mg(NO_3)_2$

- Students from out of town living outside the home in the city of Granada.

A total of 161 duplicate diets from twenty-three subjects were sampled for seven consecutive days between January and July of 1997. Each participant also recorded all foods and beverages consumed on a daily diet card. To determine the dietary intake of macro- and micronutrients, food intake was transformed into energy and nutrients by a computer program based on Wander's food composition tables (Jiménez *et al.* 1998).

Materials

To eliminate contamination by detergents and samples, glassware and polyethylene sample containers were washed with tap water after each use, soaked in 6 M HNO_3 (at least overnight), and rinsed several times with ultrapure water.

Sample preparation

The food was subjected to a simulated eating procedure using normal knives and forks. Different food items were sliced and inedible parts thrown away. The remaining parts were homogenized and the total amounts of food and beverages weighed. After further homogenization in a blender with Teflon-coated parts (Van Cauwenbergh *et al.* 1996), several aliquots of about 100 g were dried in a microwave oven (Moulinex FM-460, Moulinex, F-75008 Paris, France) under controlled temperature conditions (Cabrera *et al.* 1994*a*,*b*). About 0·4 g dried sample was put into a Pyrex tube and subjected to acid mineralization using a digestion block (Selecta). Samples were treated with 5 ml of 65 % (vol./vol.) nitric acid (Merck, D-64293 Darmstadt 1, Germany; suprapure) and a few micrograms V_2O_5 (Merck; analytical grade) as a catalyst, at 120°C for 90 min. Then the mixture was cooled, 1 ml of 70 % (vol./vol.) perchloric acid was added, and the mixture was heated at 120°C for 90 min. Solutions were left to cool to room temperature and diluted to a final volume of 25 ml with ultrapure water (18 MΩ·cm specific resistivity, Milli-Q system, Millipore, F-91191 Gifsur-Yvette, France). Cr in the resulting solutions was determined by electrothermal atomization atomic absorption spectrometry. Three portions of each sample were analysed. *Caution*: Perchloric acid should not be allowed to evaporate to dryness and should only be used in an appropriate fume hood.

Analytical procedure

We used a Perkin-Elmer 1100B double-beam atomic absorption spectrophotometer equipped with a deuterium background corrector (Perkin-Elmer, Norwalk, CT, USA) and a hollow Cr cathode lamp made by the same manufacturer. A Perkin-Elmer HGA-700 furnace with pyrolytically coated graphite tubes and a L'vov platform (Perkin-Elmer) were used. The samples were injected manually with a Pipetman micropipette. Argon of 99·999 % purity at 300 ml/min flow was used as the internal gas. The samples were atomised under the conditions shown in Table 1. Furnace conditions were optimized on the basis of time–temperature assays. Use of a L'vov platform improved the reproducibility of the results. Several matrix modifiers were tested, and $Mg(NO_3)_2$ (Merck; analytical grade) gave the best results. Argon flow was stopped during atomization to increase sensitivity; this did not alter the usable life of the tube. Analyses were done in peak area mode (integrated absorbance). Optimized assay conditions obviated most matrix interferences and other sources of unspecific absorption.

Analytical characteristics

The detection limit was calculated according to the rules of the International Union of Pure and Applied Chemistry (Long & Winefordner, 1983). Sensitivity and selectivity of the analytical conditions were evaluated. Precision was checked with ten determinations of five samples. Precision as interday reproducibility was tested by analysing five samples in 6 d. Accuracy was checked by recovery of known amounts of analyte added to five samples chosen at random. The results are summarized in Table 2. We also used a certified reference material of the International

Table 2. Analytical characteristics of the proposed method to determine Cr in diets by electrothermal atomization atomic absorption spectrometry

Detection limit[a] (pg)	Characteristic mass[b] (pg)	Recovery[c] (%)		Precision RSD (%)	Blank-to-sample slope ratio
		mean	SD		
1·0	3·0	99·00	2·8	5·0[d] 6·6[e]	0·985–1·080

RSD, relative standard deviation.
[a] Detection limit is analyte concentration corresponding to three times the SD of the blank for an injection volume of 10 μl.
[b] Characteristic mass in pg/0·0044 A·s.
[c] Results obtained from recovery assays of five samples.
[d] Relative SD for ten replicate determinations in each of five samples.
[e] Relative SD for six replicate determinations (interday) in each of five samples.

Table 3. Accuracy and precision of the proposed method against a standard reference material

(Cr values are mean and SD at a 95 % CI about the mean for ten samples)

Reference material	Cr values (μg/g dry wt) Certified Mean	SD	Determined Mean	SD	Accuracy (%)	Precision RSD (%)
IAEA-H-9 Mixed Human Diet	0·150	0·054	0·148	0·015	98·67	10·13

Table 4. Cr dietary intake (24 h periods for seven consecutive days)

Subject	Sex	Age (years)	Cr dietary intake (μg/d) Mean	Range
1	Woman	50–60	136·00	53·37–301·02
2	Man	60–70	137·40	38·50–272·90
3	Woman	50–60	77·56	18·77–169·80
4	Woman	20–30	99·66	18·66–190·90
5	Man	20–30	47·87	23·37–84·94
6	Woman	20–30	60·31	29·94–115·50
7	Man	20–30	43·73	17·94–64·25
8	Woman	20–30	49·34	14·14–84·16
9	Man	20–30	130·45	47·65–416·64
10	Man	20–30	112·70	35·86–146·04
11	Woman	30–40	130·51	49·25–193·80
12	Woman	20–30	27·41	3·65–127·60
13	Woman	60–70	165·78	83·87–236·63
14	Woman	20–30	75·18	44·97–110·40
15	Woman	50–60	28·52	24·75–35·39
16	Man	20–30	120·70	113·20–134·40
17	Man	20–30	45·11	19·30–87·93
18	Man	20–30	38·07	30·61–45·53
19	Woman	20–30	205·16	157·69–252·63
20	Woman	20–30	171·75	160·10–175·30
21	Woman	30–40	83·78	80·65–90·00
22	Woman	20–30	9·39	9·00–11·30
23	Woman	20–30	83·20	80·50–87·85

Table 5. Dietary intake of Cr in different Spanish populations

Population groups	Dietary intake (μg/day) Mean	Range
Families (*n* 6; males and females)	112·63	24·75–301·02
Students in university residence (*n* 6; females)	41·59	19·30–134·40
Students living outside the home (*n* 6; males and females)	82·88	3·65–252·63
Military personnel (*n* 5; males)	121·60	35·86–416·64

Atomic Energy Analytical Quality Services of Vienna (IAEA-H-9 Mixed Human Diet; Table 3).

The detection limit and sensitivity were suitable for the range of Cr concentrations encountered. Analytical precision and accuracy were acceptable (Horwitz *et al.* 1980, 1990). In the addition method for all samples, slope ratio values were close to 1 (Table 2), indicating that the standard addition method was unnecessary and consequently simplifying the analysis greatly.

Results and discussion

The mean daily Cr intake (24 h periods for seven consecutive days) in the diets we analysed ranged from 9·39 to 205·16 μg/d (Table 4). The mean daily Cr intake in the total samples we analysed was close to 100 μg/d, a value within the 50–200 μg/d range recommended by the National Research Council. Only five diets consumed by different persons (belonging to different population groups) supplied more than 200 μg/d.

The large variations in dietary Cr, even within a single subject during a 1-week period, illustrate the influence of composition of the diet, which may in turn be affected by the presence in some meals of Cr-rich foods. When we compared the different meals eaten by individuals, we found that higher daily Cr intakes were related to meat and dairy products. We also observed a high Cr intake in diets that contained foods made with whole wheat (bread, biscuits, noodles, etc.) and brown sugar. We determined Cr presence in food and beverages, and we concluded that the presence is higher in dairy products, meat, stimulant drinks and infusions (especially tea and coffee), and cereals and related products (Cabrera *et al.* 1996; Lendínez *et al.* 1998; Garcia *et al.* 1999*a*,*b*). The high consumption rate of these products might explain the high values of Cr intake for some individuals included in this study. In addition, other products such as spices and aromatic herbs that are widely consumed in the Spanish diet and in the Mediterranean diet, in general could contribute to dietary intake of this element. We determined the Cr presence in seventeen different spices and aromatic herbs; Cr concentrations ranged from not detectable to 1·42 μg/g (dry wt.) and Cr presence was detected in 95 % of samples (Garcia *et al.* 2000).

There were no statistically significant differences in Cr intake between population groups (P=0·05; Table 5). Mean daily Cr intake for women (93·57 μg/d) was higher than for men (84·54 μg/d). The highest values were found for subjects between 30 and 60 years of age. These findings are similar to the data reported in early studies in other countries (Table 6).

Descriptive statistics for daily Cr intake are shown in Table 7. Approximately 55 % of the daily Cr intakes were within the 50–200 μg/d range, and 90 % of the daily chromium Cr intakes were below 200 μg/d. Analysis of variance of the data showed statistically significant differences in mean Cr intakes between individuals (P=0·05). We then used the least significant difference test to identify three ranges of daily Cr intake: high (>104 μg/d), moderate (42–104 μg/d) and low (<42 μg/d). Statistical analyses were done using Statgraphic Statistical software (v. 5.0, Statgraphics, 1991).

Our findings showed that 55 % of the diets consumed by our subjects provided amounts of Cr within the range recommended by the National Research Council (1989). In terms of mean weekly intake, none of the participants in this study consumed more than 200 μg/d (only one subject consumed 205 μg/d), and only four subjects received significantly less than 50 μg/d. Some recent studies noted that the lowest intake considered adequate may be higher than the actual nutritional requirements, as some set the

Table 6. Daily dietary Cr intake in countries other than Spain

Population group	Location	Sampling technique	Cr (μg/d)	Reference
Adult women	Canada	Duplicate diet	47	Gibson *et al.* 1983
Adults	Belgium	Duplicate diet	240	Buchet *et al.* 1983
Males	Norway	Duplicate diet	29	Bibow & Salbu, 1986
Adults	Spain	Shopping basket	120	Farré & Lagarda, 1986
Families	Belgium	Duplicate portion	29–95	Brätter & Schramel, 1987
Males and females	Sweden	Duplicate diet	160	Abdulla *et al.* 1989
Fifteen subjects	Sweden	Duplicate portion	20	Jorhem *et al.* 1989
Industry employees	Spain	Duplicate diet	110–140	Barbera *et al.* 1989
Different groups	Brazil	Self-selected diets	85	Parr *et al.* 1990
Not stated	Sweden	Shopping basket	22	Becker & Kumpulainen, 1991
Women (23–28 years)	Finland	Food record	31	Kumpulainen, 1992
Different groups	Spain	Food consumption	99	Moreiras & Cuadrado, 1992
Families	Spain	Duplicate diet	129	Schuhmacher *et al.* 1993
Adults	USA	Duplicate diet	233	Anderson *et al.* 1993
Military Academy	Belgium	Duplicate portion	89·1–125·2	Van Cauwenbergh *et al.* 1996
University hopital	Belgium	Duplicate portion	20·1–51·0	Van Cauwenbergh *et al.* 1996
Military quarters	Belgium	Duplicate portion	30·8–57·9	Van Cauwenbergh *et al.* 1996
Different groups	Italy	Household food	146	Van Cauwenbergh *et al.* 1996
Adults	Japan	Duplicate diet	47	Van Cauwenbergh *et al.* 1996

Table 7. Summary of descriptive statistics from 161 duplicate diets, sampled for 7 consecutive days (with twenty-three subjects) using Pearson's correlation test

	Mean	SD	Minimum	Maximum	Percentiles		
					25	50	75
Cr intake	91·83	69·76	9·39	416·64	36·06	77·60	129·64
ln Cr intake	4·22	0·83	2·24	6·03	3·59	4·35	4·86

minimum daily amount at closer to 25 μg/d than 50 μg/d (Nielsen, 1994; Wood *et al.* 1995). In the United Kingdom the recommended daily intake is 25 μg for adults and 0·1–1·0 μg for children (Committee on Medical Aspects of Food Policy, 1991). However, Wood *et al.* (1995) consider the available data on Cr contents in different foods to be scarce and inadequate, and suggest that reanalysis is needed.

Farré & Lagarda (1986) noted that the possible causes of Cr deficiency include intestinal infection, metabolic disorders, and inadequate intake of the trace element because of low Cr content in the diet or the consumption of many highly processed foods. As noted by Mertz (1982), the well-known effect of Cr on three risk factors for coronary ischaemia (i.e. circulating insulin, glucose tolerance and serum lipoproteins) should stimulate further in-depth research on the influence of dietary Cr intake in humans.

Table 8. Correlation between the intake of Cr and other nutrients from the diets analysed

	Pearson correlation coefficient	Significance level
Carbohydrate	0·219	$P < 0·05$
Fat	−0·107	–
Protein	−0·219	$P < 0·05$
Zn	0·353	$P < 0·01$
Fe	0·279	$P < 0·01$
Mg	0·355	$P < 0·01$
K	0·308	$P < 0·01$
Na	0·278	$P < 0·01$
Ca	0·356	$P < 0·01$
Ascorbic acid	0·034	–
Folic acid	0·178	–
Nicotinic acid	0·277	$P < 0·01$

Several authors have noted that the intake of a given element can be directly or inversely related with the intake of other nutrients, particularly minerals and vitamins (Concon, 1988; Committee on Medical Aspects of Food Policy, 1991; Mertz, 1995). We looked for statistically significant relationships (Pearson correlation) between Cr intake and the intake of energy and macronutrients (Table 8). The results revealed that Cr intake was significantly related with energy, protein and carbohydrate intake, and with Zn, Fe, Mg, K, Na, Ca and nicotinic acid intake ($P < 0·01$).

Conclusion

The amounts of Cr in the normal diet consumed by our subjects in southern Spain varied widely. The variations were influenced by the types of foods and beverages consumed (typical of the Mediterranean diet). A varied, balanced diet supplies adequate amounts of Cr; however, because of the wide range of intakes and the limited capacity of the human body to absorb this element, further research will be needed to reliably establish recommended dietary intakes of Cr.

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