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Chromium and iron content in duplicate meals at a university residence: daily intake and dialysability

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Abstract

The objective of the present study was to determine total Cr and Fe content and the corresponding mineral dialysable fraction in a total of sixty-three duplicate meals. Samples of breakfast, lunch and dinner were taken over twenty-one consecutive days at a female university residence in Granada (Spain). Cr content in the duplicate daily meals ranged from 98·50 to 120·80 μg, with a mean of 110·00 μg, and Fe levels ranged from 9·50 to 40·00 mg, with a mean content of 18·50 mg. The mean Cr and Fe dialysable fractions ranged from 0·50 to 1·50 % and from 7·75 to 11·80 %, respectively. Possible correlations with energy and other nutrient intakes were also evaluated. Adherence of the meals to the Mediterranean dietary patterns was tested, and these findings reveal that a balanced and varied diet based on a Mediterranean-style diet plan provides adequate levels and bioaccessibility of Cr and Fe for young women, which is especially important to avoid mineral deficiencies.

Key words: **Chromium: Iron: Duplicate meals: Dietary intake: Dialysability**

Cr and Fe are essential micronutrients for human health and play an important role in human metabolism. Cr is involved in lipid and carbohydrate metabolism, and the most frequent manifestation of Cr deficiency is hyperglycaemia. In addition, hyperglycaemia, neuropathy and weight loss have been observed in a classic case of Cr deficiency in patients on total parenteral nutrition, with these abnormalities being corrected with supplementation of Cr to the parenteral nutrition solution. This nutrient has also been associated with CVD and gene expression[1]. The US Institute of Medicine[2] estimates a Cr adequate intake of 25 μg/d for women aged 19–30 years. The toxicity of Cr(III), the chemical form present in foods, is low enough to provide a sufficient safety margin between usual consumed and harmful amounts, as humans cannot oxidise Cr(III) to potentially carcinogenic Cr(VI) compounds[1–3].

Fe is a component of a number of proteins, including enzymes and Hb. Almost two-thirds of Fe in the body is found in Hb present in circulating erythrocytes, and it is important for the transport of oxygen to tissues throughout the body for metabolism. Most of the remaining 15 % is in the myoglobin of muscle tissue and a variety of enzymes necessary for oxidative metabolism and many other functions in all cells. The estimated average requirement for women aged 19–30 years is 8·1 mg/d[2]. Fe deficiency is considered the most common single nutrient deficiency disease in the world, affecting most seriously women, children and adolescents[1]. This deficiency is produced by an unbalance between requirements and the quantity of the mineral that is ingested, absorbed and utilised. Considerable amounts of Fe must be provided by the diet in an available form, as the bioavailability of dietary Fe appears to be an important determinant of Fe status.

In general, estimates of the total element content in food and diets are insufficient, and its bioavailability also needs to be considered. Mineral bioavailability has gained increasing interest in the field of nutrition. *In vivo* studies are both expensive and laborious, and the possibility of measuring certain parameters during the experiments is often limited[4]. *In vitro* methods of simulated digestion are an alternative for calculating the percentage of the mineral that is transformed into absorbable forms in the digestive tract (bioaccessibility). These procedures are rapid, usually inexpensive, and they allow individual experimental variables to be easily controlled. The results are usually expressed as the dialysable fraction under given experimental conditions such as pH, enzyme addition and temperature[4,5].

The aim of the present study was to determine Cr and Fe content in duplicate meals, which represent the habitual diet served in a female university residence in Granada (Spain), in order to test their adequacy to dietary reference intakes. Moreover, an *in vitro* method that simulates gastrointestinal conditions by employing a dialysis membrane was used to determine the Cr and Fe dialysable fractions. Electrothermal

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atomic absorption spectrometry was used as an analytical technique.

Materials and methods

Reagents

Standard solutions of Cr and Fe (1·00 (SD 0·002) g) (Tritisol; Merck, Darmstadt, Germany) were used, diluted as necessary to obtain working standards. High-quality concentrated HNO_3 (65 %), perchloric acid (70 %) and vanadium pentaoxide (Merck) were used for sample mineralisation. Magnesium nitrate (Merck) was used as the chemical modifier for Cr determination. Ammonium molybdate (Merck) was used to precondition the furnace tubes. All reagents used were of analytical grade. Pepsin (Sigma P7000 porcine pancreas; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), pancreatin (Sigma P1500 porcine pancreas) and bile salts (Sigma B8756) were used to simulate gastric and intestinal digestion. A pepsin solution was prepared by dissolving 16 g of pepsin in 100 ml of 0·1 M-HCl; a pancreatin–bile extract mixture was prepared by dissolving 4 g of pancreatin and 25 g of bile extract in 1 litre of 0·1 M-$NaHCO_3$. NaOH (0·5 M) and HCl (6 M) were used to adjust pH. Dialysis tubing Spectra with a molecular mass cut-off of 10–12 kDa ('Visking size 3–20/32'; Medicell International, Liverpool, London, UK) was used. The dialysis tubing was freed of trace metal impurities by boiling in 2 % (w/v) $NaHCO_3$, 0·1 % (w/v) SDS and 0·01 M-EDTA disodium salt for 30 min, followed by thorough washing with bidistilled deionised water. It was then preserved in 20 % ethanol solution.

Apparatus

A Perkin-Elmer 1100B double-beam atomic absorption spectrometer equipped with deuterium arc background correction (Perkin-Elmer, Norwalk, CT, USA) and hollow Cr and Fe cathode lamps made by the same manufacturer were used. In addition, a Perkin-Elmer HGA-700 graphite furnace atomiser was used. Pyrolytically coated graphite tubes (ref. B013-5653) and pyrolytic graphite platforms (ref. B012-1092) were obtained from Perkin-Elmer. Ar of 99·999 % purity (Sociedad Española de Oxígeno, Barcelona, Spain) at 300 ml/min was used as the internal gas during all stages except atomisation, when the flow was stopped. Background-corrected integrated absorbance was used as the analytical signal. Furnace conditions were optimised on the basis of time–temperature assays (Table 1); these conditions obviated most matrix interferences and other sources of unspecific absorption. A Moulinex blender, model 327 (Moulinex, Bangnolet, France), of which different parts were Teflon-coated following Van Cauwenbergh *et al.*[6], was used for sample homogenisation. To determine the total Cr content in the diets, the samples were dried using a microwave oven (Moulinex) and mineralised in an acid digestion block (Selecta, Barcelona, Spain). A thermostatic Selecta water-bath (Selecta) and a Radiometer model 26 pH meter (Radiometer, Copenhagen N.V., Denmark) were used for the *in vitro* assays.

Material

In order to decrease the risk of contamination, the use of glassware was reduced to a minimum, and plastic (polypropylene) vessels and pipette tips were used. All materials were washed with HNO_3 and rinsed several times with bidistilled deionised water.

Sampling strategies

To determine the dietary intake of minerals and trace elements, study designs that include the collection and preparation of ready-for-consumption food are believed to produce the most realistic and reliable results[7–9]. Duplicate portion samples of sixty-three different meals, corresponding to twenty-one breakfast, twenty-one lunch and twenty-one dinner, were taken over twenty-one consecutive days at a female university residence in Granada (Spain) that provides full board to 165 students aged 18–24 years. These meals were the only food served in the residence, and no drinks other than milk, coffee or water were included. Due to the impossibility of quantifying water consumption, this was not included in the present study. Food samples were subjected to a simulated eating procedure using normal knives and forks. The food items were sliced, and the inedible parts discarded. The remaining parts were weighed and then homogenised in the blender.

Combined weight and estimated dietary records were completed in parallel with the duplicate diet collection. All the foods as well as the size of the portions were recorded. The food content of the diet was transformed into energy and nutrient values using the Spanish Food Composition Tables[10] and AYS44 Diet Analysis software, supplied by ASDE, S.A. (Valencia, Spain).

Table 1. Instrumental parameters for chromium and iron determination in duplicate meals by electrothermal atomic absorption spectrometry

Instrumental parameters	Cr	Fe
Wavelength (nm)	357·9	248·3
Slit width (nm)	0·7	0·2
Atomisation system	Stabilised temperature platform furnace	Wall tube atomisation system
Ashing temperature (°C)	1650	1400
Atomisation temperature (°C)	2500	2400
Injection volume (μl)	10	10
Matrix modifier	50 μg $Mg(NO_3)_2$	–

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Sample preparation

The dried and homogenised samples were mineralised in an acid digestion block. A detailed description of duplicate meals treatment before determining total Cr and Fe by electrothermal atomic absorption spectrometry is reported elsewhere[5].

In vitro *method for estimating chromium and iron dialysability*

The simulated gastrointestinal digestion procedure and the *in vitro* absorption estimation were carried out in accordance with previously reported methods[5,11]. The technique measures the Fe and Cr fraction dialysed from a sample under simulated gastrointestinal conditions and, therefore, available for absorption. Fe and Cr dialysability is expressed as the percentage of dialysed element in relation to the total content in the duplicate meal. Dialysis mineral percentages were calculated as follows: dialysis (%) = $100 \times D/C$, where D is the dialysed mineral content (μg/g sample) and C is the total mineral content (μg/g sample).

Statistical analysis

Interpretation of the data was performed using the statistical software package SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). Results are expressed as means and standard deviations. The normal distribution of variables and the homogeneity of variances were checked by the Kolmogorov–Smirnov and the Bartlett test, respectively. Comparisons were made using Student's t test when the variable fulfilled parametric conditions and the Kruskal–Wallis test when the conditions were non-parametric. Additionally, correlations by Pearson's or Spearman's test (for parametric and non-parametric conditions, respectively) and regression models were employed.

Results and discussion

Method validation

For method validation, the detection limit, sensitivity, precision and accuracy were tested for each element. The slopes of the aqueous and standard addition calibration graphs were compared. To check the similarity of slopes, Student's t test was applied; the results showed that the values were similar, with an absence of matrix effects (slope ratios approaching 1). Thus, calibration was performed using the aqueous standard, which greatly simplified the analysis. The reliability of the method was further corroborated by using a certified reference material obtained from the International Atomic Energy Analytical Quality Services of Vienna (IAEA-H-9 mixed human diet). The paired t test showed good agreement at a significance level of 0·05 %. The results are summarised in Table 2.

Chromium and iron levels in duplicate meals

Cr content in the analysed duplicate meals corresponding to each day ranged from 98·50 to 120·80 μg, with a mean of 110 μg (Table 3), which surpassed the adequate intake recommendations[2]. Mean dietary Cr intake was similar to that reported by García *et al.*[7] and Schuhmacher *et al.*[12] in duplicate diet studies of Spanish families, but these authors included water consumption. Cr intakes less than 110 μg/d have been observed in Finland[13], Belgium[6] and France[9]. Anderson *et al.*[14] reported data on Cr dietary intake from a duplicate plate technique of 23·1 (SD 2·9) and 38·8 (SD 6·5) μg/d for American women and men, respectively. On the other hand, higher intakes have been reported by Ysart *et al.*[15], who even showed a Cr intake of 340 μg/d for the general population from the UK and neither quantified drinking-water consumption. Additional data on Cr daily intake in different countries according to other authors are summarised in Table 4.

Table 2. Analytical characteristics for chromium and iron determination in convenience and fast foods (Mean values and standard deviations or ranges)

	Cr		Fe	
Analytical parameter	Mean/range	SD	Mean/range	SD
Detection limit* (pg)	4·0		6·0	
Sensitivity† (pg)	10·0		8·0	
Accuracy‡ (%)	98·80	1·20	98·70	0·75
Precision§ (RSD, %)	4·5–4·7		4·00–5·50	
Slope ratio blank/addition‖	0·999–1·010		0·996–1·010	
Against certified reference material (mixed human diet IAEA-H9)				
Measured value¶ (mg/kg)	0·148	0·015	2·29	0·10
Certified value¶ (mg/kg)	0·150	0·054	2·32	0·23
Accuracy (%)	98·67	0·05	98·70	0·05
Precision (RSD, %)	4·1–4·3		4·5–4·7	

* Calculated according to International Union of Pure and Applied Chemistry rules and corresponding to three times the standard deviation of the blank (n 10).
† Expressed as characteristic mass in pg/0·0044 absorbance units.
‡ Results from recovery assays of five randomly chosen samples.
§ Relative standard deviation (RSD) for ten replicate determinations in each of five samples.
‖ Application of the standard addition method of five randomly chosen samples.
¶ Means and standard deviations at 95 % CI about the mean (n 10), referred to dry weight.

Table 3. Total content and dialysable fraction of chromium and iron in daily duplicate meals from a female university residence
(Mean values and ranges, *n* 21)

	Total content in duplicate meals*		Dialysable fraction* (%)	
	Mean	Range	Mean	Range
Cr (μg)	110·00	98·50–120·80	0·78	0·50–1·50
Fe (mg)	18·50	9·50–40·00	9·80	7·75–11·80

* Data refer to the fresh weight of the edible portion.

In the present study, the highest Cr levels were observed in meals that include meat, a high content of spices and aromatic herbs and chocolate. In previous studies, we analysed several foods and beverages that are widely consumed in Spain, in order to estimate their possible contribution to the total dietary Cr intake. A high Cr presence in dairy products, meat, stimulant drinks and infusion (especially tea and coffee), whole cereals, brown sugar, spices and aromatic herbs was observed[7,16]. In accordance with Storelli[17], we observed that seafood consumption (except some cephalopods) does not represent an important contribution to daily dietary Cr intake.

Fe content in the analysed daily duplicate meals ranged from 9·50 to 40·00 mg, with a mean of 18·50 mg (Table 3). These values are similar to those reported by Velasco-Reynold *et al.*[18] for duplicate meals provided daily in hospitals in Granada, Spain (17·7 mg; range 9·51–42·00 mg). Our mean value of 18·50 mg/d is higher than the estimated average requirement of 8·1 mg/d proposed by the US Institute of Medicine[2] and is similar to the Fe intake estimated in other Spanish epidemiological studies, as reported by Fernández-Morales *et al.*[19] in female adolescents (16·63–17·21 mg/d), although higher than that reported by the enKid study in women aged 18–24 years (12·9 mg/d)[8]. Women from countries such as Germany[20], The Netherlands[21] and Norway[22] present Fe intake levels of about 10–11 mg/d, while the lowest values are found in Denmark, where women aged 19–24 years have an Fe intake of 8·5 mg/d[23]. Additional data are summarised in Table 5.

The highest levels of Fe in the analysed duplicate meals appeared in meals that include liver terrine, blood sausage, meat, dry fruits or chocolate; in general, food of animal origin, such as meat, fish and their products, would be primary sources of Fe. In any case, the analysed meals do not supply Fe levels close to the recommended upper limit (45 mg/d)[2].

The intake of a given element may be related to that of other nutrients, particularly minerals and vitamins[1,2]. In addition, Van Cauwenbergh *et al.*[6] suggested that Cr intake may increase in parallel to energy intake. We observed a significantly positive and linear correlation among total Cr levels and energy intake, and carbohydrate, protein, Zn, Fe, Mg, K, Na, Ca and nicotinic acid content (Table 6), similar to the relationships reported by García *et al.*[7]. Positive correlations were observed between total Fe levels and carbohydrate, protein, different amino acids, P, Se, I, K, vitamin E and nicotinic acid content. In agreement with Velasco-Reynold *et al.*[18], the present results show that the total Fe supplied in meals is directly related to its macronutrient content.

We consider that the implementation of a total diet study offers the advantage of providing more realistic exposure data since foods are analysed 'as-consumed'. In addition, it

Table 4. Chromium daily dietary intake in different countries according to other authors

Population group	Location	Sampling technique	Cr (μg/d)	Reference
Young men	Finland	2 × 24 h Recall	40	Räsänen *et al.*[13]
Young women	Finland	2 × 24 h Recall	27	Räsänen *et al.*[13]
Adult women	USA	Duplicate plates	23·1	Anderson *et al.*[14]
Adult men	USA	Duplicate plates	38·8	Anderson *et al.*[14]
Families	Spain	Duplicate diet	129	Schuhmacher *et al.*[12]
University hospital	Belgium	Duplicate portion	20·1–51·0	Van Cauwenberg *et al.*[6]
Military quarters	Belgium	Duplicate portion	30·8–57·9	Van Cauwenberg *et al.*[6]
Military academy	Belgium	Duplicate portion	89·1–125·2	Van Cauwenberg *et al.*[6]
Families	Poland	Daily food diet	77–228	Marzec[29]
General population	UK	Household consumption	340	Ysart *et al.*[15]
School children	Yugoslavia	1 d Record	1·02–2·43	Pavlovic[30]
Students living outside the home	Southern Spain	Duplicate diet	82·88	García *et al.*[7]
Families	Southern Spain	Duplicate diet	112·63	García *et al.*[7]
Military personnel	Southern Spain	Duplicate diet	121·60	García *et al.*[7]
Students in a university residence	Southern Spain	Duplicate diet	41·59	García *et al.*[7]
General population	Greece	Household budget survey	143	Bratakos *et al.*[31]
General population	France	Duplicate portion	62	Noël *et al.*[32]
Adults	Poland	Duplicate portion	60–90	Marzec[33]
General population	Tarragona, Spain	24 h Recall	88·3	Bocio *et al.*[34]
Adults	France	Duplicate diet	77	Leblanc *et al.*[35]
Elderly people	France	Food records	40·23	Roussel *et al.*[9]
General population	Catalonia, Spain	Foodstuffs purchased	57·5	Martí-Cid *et al.*[36]
Young women	Finland	Food record	31	Kumpulainen[37]
Different groups	Spain	Food consumption	99	Moreiras & Cuadrado[38]
Adult women	USA	Duplicate diet	25	Anderson & Kozlovsky[39]
Adult men	USA	Duplicate diet	33	Anderson & Kozlovsky[39]
Adult males	USA	Duplicate diet	36·7–36·9	Offenbacher *et al.*[40]

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Table 5. Iron daily dietary intake in young women from different countries according to other authors

Age (years)	Location	Sampling technique	Fe (mg/d)	Reference
16–24	UK	Weighed record	11·8	Gregory *et al.*[41]
18–24	Germany	7 d Protocols	11·6	Heseker *et al.*[20]
19–22	The Netherlands	2 d Record	10·6	Van Dokkum *et al.*[21]
18	Norway	FFQ	11·4	Frost-Anderson *et al.*[22]
18–34	Hungary	3 × 24 h Records	10·8	Biró *et al.*[42]
16–20*	Sweden	Diet history	11	Larsson & Johansson[43]
16–20†	Sweden	Diet history	14	Larsson & Johansson[43]
18–24	Austria	24 h Recall	13·1	Elmadfa & Weichselbaum[23]
19–24	Denmark	7 d Record, personal interview	8·5	Elmadfa & Weichselbaum[23]
19–24	Germany	Household budget survey	12·2	Elmadfa & Weichselbaum[23]
18–29	Portugal	4 d Record semi-quantitative, FFQ	18·0	Elmadfa & Weichselbaum[23]
19–24	UK	7 d Record	10·0	Elmadfa & Weichselbaum[23]
18–24	Spain	24 h Recall, FFQ	12·4	Serra-Majem *et al.*[8]

*Vegans.
†Omnivores.

provides a good tool for identifying the population or age groups that are most exposed (children, the elderly, etc.) and identifies the main food or food groups contributing to the exposure when food sampling is based on an individual approach. Thus, food consumption is monitored, and useful trends are identified for orienting food safety programmes.

In vitro *chromium and iron dialysable fraction*

In vitro simulation of the digestive process and the subsequent quantification of the concentrations of the soluble and/or dialysable compounds in the gastrointestinal medium (bioaccessibility determination) represent one of the most effective methods of identifying an efficient source of a nutrient in a food product. *In vitro* methods are routinely used to estimate the bioaccessible amounts of essential elements in diets. Table 3 shows the results of the Cr and Fe dialysable fraction after *in vitro* simulation of the digestion process in the duplicate meal samples. These results express the element fraction, as a percentage, that would be available for absorption by intestinal cells (bioaccessible Cr and Fe).

The Cr dialysable fraction in the analysed daily meals ranged from 0·50 to 1·50 %. García *et al.*[11] obtained similar results (0·4–1·6 %) using an *in vitro* method for a total dietary intake ranging from 16 to 117 μg/d. These authors reported that Cr bioaccessibility is higher for low levels of daily dietary intake (< 40 μg/d) than for levels of 40–80 μg/d; for high levels (> 80 μg/d), there was an increase in the dialysable fraction. This influence of the total Cr content in the analysed meals on the Cr dialysable fractions has also been observed. Paustenbach *et al.*[24] reported values of 2 % in drinking-water, and Cabrera-Vique & Bouzas[5] found 0·38–1·05 % in convenience and fast foods.

In the present study, wide variability in the Fe dialysable fraction was observed, with levels ranging from 7·75 to 11·80 % (Table 3). A similar variability was also reported by

Table 6. Statistically significant correlations between total chromium and iron and other nutrients in the analysed duplicate meals

	Cr		Fe	
	Pearson's correlation coefficient	*P*	Pearson's correlation coefficient	*P*
Carbohydrate	0·219	< 0·05	0·490	< 0·01
Protein	0·229	< 0·05	0·398	< 0·05
Zn	0·353	< 0·01	–	–
Fe	0·279	< 0·01	–	–
Mg	0·355	< 0·01	–	–
Ca	0·357	< 0·01	–	–
P	–	–	0·385	< 0·01
Se	–	–	0·310	0·05
I	–	–	0·423	< 0·05
Na	0·280	< 0·01	–	–
K	0·308	< 0·01	0·395	< 0·05
Vitamin E	–	–	0·380	< 0·05
Nicotinic acid	0·270	< 0·01	0·460	< 0·01
Thiamin	–	–	0·420	< 0·01
Met	–	–	0·410	< 0·01
Cys	–	–	0·380	< 0·05
Lys	–	–	0·420	< 0·01
Phe	–	–	0·450	< 0·01
Arg	–	–	0·450	< 0·01
Tyr	–	–	0·425	< 0·01
His	–	–	0·395	< 0·01

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Velasco-Reynold *et al.*[18] in hospital duplicate diets (4·81 (SD 3·25) %) and by Cámara *et al.*[25] in Spanish school meals (0·23–19·0 %). We observed that a high animal protein and ascorbic acid content in the meals significantly enhanced the Fe dialysable fraction. This fact has been reported previously, as has the finding that animal proteins can counteract the phytate and tanin inhibitor effect[18]. The mean Fe dialysable fraction estimated in the present study (9·80 %) was similar to the Fe *in vitro* availability in diets using Caco-2 cells, as reported by Mesías *et al.*[26]. In Western countries, the Fe absorbed fraction is about 10 %[18,26].

Adherence of the analysed diets to the Mediterranean diet

The beneficial health effects of the Mediterranean diet are commonly attributed to the association of regular physical activity with a complex combination of dietary characteristics. This has been corroborated by numerous epidemiological and experimental nutrition studies[26,27]. In order to determine the adherence of a diet to the Mediterranean diet patterns, Serra-Majem *et al.*[28] developed the Mediterranean Diet Quality Index, based on principles sustaining Mediterranean dietary patterns as well as those that undermine it. The index ranges from 0 to 12 and is based on a sixteen-question test. On applying this test to the diet provided at the university residence, we obtained an index of 9, which represents optimal adherence to the Mediterranean diet. This is as expected in view of the high dietary content of legumes, cereals, fish, fresh fruits and vegetables, which is characteristic of the Mediterranean patterns. Taking into account this index and the analytical data obtained, we consider that a diet based on the Mediterranean-style plan supplies adequate amounts of Fe and Cr for young women. In the same way, Mesías *et al.*[27] reported the beneficial effect of Mediterranean dietary patterns on dietary Fe utilisation in male adolescents aged 11–14 years.

In summary, the present study shows that a balanced and varied diet based on the Mediterranean-style diet plan supplies adequate amounts and bioaccessibility of Fe and Cr for young women. The data obtained should also be useful for international comparison and enhance our knowledge of the nutritional value of these nutrients.

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