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Miguel Navarro. Determination of daily dietary intake of chromium by duplicate diet sampling: in vitro availability study. *Food Additives and Contaminants*, 2008, 25 (05), pp.604-610. 10.1080/02652030701678843 . hal-00577435

HAL Id: hal-00577435

<https://hal.science/hal-00577435>

Submitted on 17 Mar 2011

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Determination of daily dietary intake of chromium by duplicate diet sampling: in vitro availability study

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-114.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	03-Sep-2007
Complete List of Authors:	Navarro, Miguel; University of Granada, School of Pharmacy, Nutrition and Food Science
Methods/Techniques:	Duplicate diets, GFAAS, Health significance, Metals - bioavailability
Additives/Contaminants:	Metals, Metals - nutritional, Trace elements (nutritional), Trace elements (toxic)
Food Types:	Processed foods

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4 1 **Determination of daily dietary intake of chromium by duplicate**
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6 2 **diet sampling: *in vitro* availability study**
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Abstract

The intake of chromium was estimated using a duplicate diet sampling method of 108 meals (36 breakfasts, 36 lunches and 36 dinners) from the restaurant of the Hospital of Motril (SE Spain) corresponding to 36 consecutive days. The total and dialyzable Cr levels were measured by a validated electro thermal atomic absorption spectrometry (ETAAS) method. A mean Cr fraction of $26 \pm 12 \mu\text{g meal}^{-1}$ was found. The Cr supply from meals was directly and significantly ($p < 0.001$) correlated with their macronutrient (carbohydrates, fiber and protein) content. Cereals and by-products, legumes, dry fruit, meat, potatoes, dairy products and seafood are the primary sources of Cr. The mean Cr fraction dialyzed through the dialysis tubing was $1.2 \pm 1.1 \mu\text{g meal}^{-1}$ ($4.6 \pm 3.8\%$ as mean Cr dialysability). The Cr intakes found for breakfasts were significantly lower ($p < 0.001$). A correlation between the logarithmic data of total and dialyzable fraction of Cr in meals ($p = 0.020$) was found. The dialysis ratio enhancement and therefore bioavailability increased with the total Cr amount. The dialysed element content present in meals was significantly correlated with fibre, protein, Fe, Na, I, F, sodium, ascorbic acid and vitamin A levels ($p < 0.05$). At Fe contents in meals higher than $\cong 7.5 \text{ mg meal}^{-1}$ the net absorption of Cr decreased significantly. The mean Cr daily dietary intake (DDI) was $77 \pm 17 \mu\text{g day}^{-1}$ which indicates no adverse effects in relation to Cr nutrition (deficiency or toxicity) should occur in individuals from the area.

Keywords: Chromium, ETAAS, daily dietary intake, duplicate diet method, in vitro availability

Introduction

Chromium is an essential element that forms part of the glucose tolerance factor and helps to maintain normal glucose metabolism (Sola-Larrañaga and Navarro-Blasco 2006). Specifically Cr has been identified as a factor that enhances insulin activity (Goldhaber 2003). The National Institute of Health's programme reported that this element could act as an adjuvant in the therapy for the type 2 diabetes and impaired glucose tolerance (Vincent 2004). In humans submitted to total parenteral nutrition not supplemented with Cr, the addition of this element as CrCl_3 led to a decrease in the insulin needs and ameliorated the glucose tolerance (Jeejeebhoy et al. 1977). It seems that this element can have a biochemical function that would enhance the capacity of the insulin receptor to interact with its hormone. It has been observed from *in vitro* studies that the RNA synthesis increases by the Cr binding to DNA. This finding suggests that this element could exert a regulation of a molecule synthesis that would reinforce the insulin action and therefore as such would influence carbohydrate, lipid and protein metabolism (Anderson 2004; Navarro-Alarcon et al. 2005). Nevertheless, other researchers have indicated that individuals with type 2 diabetes did not obtain any benefit from yeast-based Cr supplements of $100 \mu\text{g Cr day}^{-1}$ (Trow et al. 2000). Schachter et al. (2001) also suggested that at a dosage range of $20\text{-}60 \mu\text{g kg}^{-1} \text{day}^{-1}$ the Cr picolinate caused no beneficial or harmful effects in insulin-treated diabetic dogs. Berner et al. (2004) also reported how one particular form of Cr^{3+} , the Cr picolinate (Chromax®) was determined to be safe for use as a nutrient supplement in foods consumed by human beings. Other studies have indicated the positive effect exerted by this mineral on the immune system (Moonsie and Mowat 1992). Other researchers have found that the intake of Cr supplements by malnourished children produced a significant enhancement of the growth rate (Gurson and Saner 1973). Similarly, others reported that organic Cr tended to improve growth performance of pigs (as Cr picolinate; Cho et al. 2000) or broiler chicks (as Cr picolinate or Cr yeast; Suksombat and Kanchanatawee 2005). Recently, the Cr has been used as an agent for increasing lean body mass and decreasing percent body fat (Kobla and Volpe 2000). Lately, due to the hypothesized anabolic capacity of Cr, an enhancement of the consumption of Cr supplements by sportsmen in order to rise or to keep their muscle mass (Crawford et al. 1999), has been observed. A beneficial effect of the Cr on lipid profiles, with a diminishment of the total cholesterol and low-density-lipoproteins (LDL) and triglyceride levels and a

1 concomitant rise in the high-density-lipoproteins (HDL), was also noticed (Navarro-Alarcon
2 et al. 2005).

3 The toxic effects produced by the Cr were directly related with industrial exposure
4 and associated with the different Cr⁺⁶ species carried by the air (Von Burg and Liu, 1993).
5 The main health effects related to Cr⁺⁶ are allergenic dermatitis, lesions in the skin and in the
6 nose wall as well as a higher lung cancer incidence (Von Burg and Liu 1993, Losi et al. 1994,
7 Rowbotham et al. 2000) associated with some occupational exposure scenarios. Contrarily,
8 the Cr⁺³ is an essential nutrient with a broad safety range and low toxicity (Rowbotham et al.
9 2000). Like the absorption of the Cr⁺³ is scarce, their oral intakes have to be very high in
10 order to rise damage levels.

11 Cr absorption depends of the elemental species and the content in food components of
12 meals (Stoecker, 1992). The fraction of the Cr⁺³ form usually absorbed in the bowel from
13 dietary intakes mainly by passive paracellular absorption is very low and usually ranges
14 between 0.5-2% (García et al. 2001a). Nevertheless, the absorption of Cr⁺⁶ species seems to
15 be until 6 times higher. Although the intestinal absorption mechanism for Cr has still not been
16 clearly identified, it seems that in addition to the passive route previously noted, active
17 mechanisms also exist (Navarro Alarcon et al. 2005).

18 The absorption (15-20%) and bioavailability of Cr is affected by dietary factors like
19 chemical form, meal composition, lumen intestinal content, interaction with other nutrients as
20 well as endogenous physiological factors (status of the intestinal mucosa, nutritional status of
21 individuals, homeostatic mechanisms, carrier proteins, etc.; Garcia et al., 2001a; Anderson
22 2004; Navarro Alarcon et al. 2005). On the other hand, the Cr present in meals interacts with
23 other nutrients that influence its net absorption. Specifically, the amino acids and starch
24 (Seaborn and Stoecker 1989, Anderson 2004), and the ascorbic acid (Offenbacher 1994)
25 facilitate the net Cr transport and absorption by forming soluble complexes. The
26 bioavailability of this is also element enhanced by Fe deficiency (Anderson 2004). This
27 finding could be due to problems of competence existing between both minerals by joint at
28 the same intestine absorption sites (Navarro Alarcon et al. 2005).

29 In the present study, the assessment of the Cr DDI by adult inhabitants from south eastern
30 Spain by one retrospective method, was performed. A duplicate portion sampling method of
31 108 duplicate meals (36 breakfasts, 36 lunches and 36 dinners) corresponding to 36
32 consecutive days from the restaurant of the Hospital of Motril (SE Spain), and analysis by an
33 electro thermal atomic absorption spectrometry (ETAAS) method, was carried out. Due to the

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3 1 low concentrations of Cr in food, body tissues and body fluids, careful and appropriate
4 analytical techniques are required for its measurement, as well as new standard reference
5 materials for accurate determinations (Anderson 2004). Consequently, a previous
6 development and optimization of a specific time-temperature program for the Cr
7 measurements by ETAAS was performed. The interaction of other nutrients present in
8 analyzed meals with Cr and their influence on the element absorption was also studied. In
9 order to determine the available Cr fraction an in vitro method that simulates the gastro
10 intestinal conditions with the employment of dialysis tubing, was used (Garcia e tal.2001a).
11 The determination of levels of several nutrients considered present in meals was carried out
12 by the employment of food composition tables compiled in the programme Dietsource 2.0
13 (Moreira Ingles 2000). The study of the interaction of the levels of these nutrients on the total
14 and dialyzable Cr contents in analyzed meals was also performed. Additionally, to assess the
15 magnitude of inadequate intakes of Cr (deficiency or excess and toxicity) we employed the
16 dietary adequate intakes (AIs) cut off points proposed by the USA Institute of Medicine
17 (IOM, 2002) (35 and $25 \mu\text{g day}^{-1}$ for male and female healthy adults older than 19 y).
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33 **Materials and methods**

34 *Apparatus*

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39 21 A Perkin-Elmer 1100B double-beam atomic absorption spectrophotometer equipped with a
40 deuterium background corrector (Perkin-Elmer, Norwalk CT, USA) and a Cr hollow cathode
41 lamp were used together an HGA-700 furnace with pyrolytically-coated graphite tubes and a
42 L'Vov platform. The samples were injected manually with a Pipetman micropipette. Argon
43 of 99.999% purity at a flow rate of 300 ml min^{-1} was used as the internal gas. A Stomacher
44 blender, model 400, of which different parts were Teflon-coated, and Teflon containers were
45 used for sample homogenization. A thermostatic multiplace digestion block (Selecta S.A.,
46 Barcelona, Spain) and a Radiometer model 26 pHmeter (Radiometer, Copenhagen N.V.,
47 Denmark) were used for in vitro assays.
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55 *Reagents*

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57 31 Commercially available standard solution of Cr ($1,000 \mu\text{g ml}^{-1}$; Tritisol, Merck, Darmstadt,
58 Germany) was used to prepare calibration graphs. Magnesium nitrate was used as chemical
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4 1 modifier. Nitric acid (65% v v⁻¹) and perchloric acid (70% v v⁻¹) were employed to mineralize
5 2 the diet samples.

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7 3 Pepsin (Sigma P7000, porcine pancreas, Sigma-Aldrich Chemie GmbH, Steinheim,
8 4 Germany), pancreatin porcine pancreas (Sigma p1500, Sigma Chemical co., St Louis, MO)
9 5 and bile salts (Sigma B8756) were used to simulate gastric and intestinal digestion. A pepsin
10 6 solution was prepared by dissolving 16 g pepsin in 100 ml of 0.1 M HCl. Pancreatin-bile
11 7 extract mixture was prepared by dissolving 4 g pancreatin and 25 g bile extract in 1 l of 0.1 M
12 8 NaHCO₃. The solutions of 0.5 M NaOH and 6 M HCl were employed to adjust the pH.

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14 9 Dialysis tubing Spectra with molecular mass cut-off (MMCO) of 12-14 kDa (Visking size 3-
15 10 20/32), Medicell International, London, UK) were used. The dialysis tubing was freed of
16 11 trace element impurities by boiling in a 2% w v⁻¹ NaHCO₃ 0.1 % w v⁻¹ sodium dodecyl
17 12 sulphate and 0.01 M ethylenediaminetetraacetic acid disodium salt (EDTA) for 30 min,
18 13 followed by thoroughly washing with double distilled deionized water, and preserved in 20%
19 14 ethanol solution.

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21 15 All solutions were prepared from analytical reagent grade reagents (Merck, Germany,
22 16 Suprapur). The water employed for preparing the standards for calibration and dilutions was
23 17 deionised double distilled water with a specific resistivity of 18 MΩ cm obtained by filtering
24 18 distilled water through a Milli-Q purifier (Millipore, Waters, Mildford, MA) immediately
25 19 before use.

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21 ***Duplicate portion sampling of meals and Cr analysis by ETAAS***

22 Duplicate portion samples of 108 different meals, corresponding to 36 breakfasts, 36 lunches
23 and 36 dinners, were taken over a 36-days consecutive period from May to June 2002 from
24 the restaurant of the Hospital of Motril. The dietician was instructed to collect duplicates of
25 the three meals consumed during each 24-hr period in 2-2.5 l previously cleaned polyethylene
26 containers. As a second instruction all food and beverage items corresponding to each menu
27 were recorded and weighed on balances available in the hospital. The containers were then
28 stored at -18°C. Meal samples were transported to the laboratory of the Department of
29 Nutrition and Food Science of the University of Granada. The analysed meals (n= 108) were
30 sliced and mixed in a blender (Stomacher 400), of which various parts were Teflon coated,
31 noting the menu weight. Aliquots of about 100 g of the mixed food were dried at temperature
32 ranged from 60-80°C. After drying, liquid content was calculated and dried samples were
33 homogenized in previously cleaned Teflon containers. About of 400 mg of the pulverized

1 meal sample was placed in a flask and subjected to an acid mineralization in a multiple place
2 digestion block by adding 5 ml of HNO₃ cc and heating at 60° C for 30 min. Another 1 ml of
3 4:1 HNO₃-HClO₄ (4:1) was added and heating continued at 120°C for 90 min. The digest was
4 then cooled and the resulting solution diluted to 25 ml with double distilled water. The
5 analytical solution obtained was suitable for the Cr ETAAS determination.

6 The Cr determination was directly performed in acidified samples adequately diluted
7 with 0.2% HNO₃ solution. The element was directly determined by the linear calibration
8 method. A sample volume of 10 µl was manually injected with a micropipette through a
9 graphite tube with L'Vov platform. The chemical modifier (10 µl) [0.5% Mg(NO₃)₂ (equivalent
10 to 40 µg Mg(NO₃)₂)] was also injected. The appropriate conditions for Cr measurements were
11 optimised by us on the basis of time-temperature studies using certified standards (CBR CRM
12 278R mussel tissue and CBR CRM 185R bovine liver). Peak area was used as the analytical
13 signal. Furnace conditions for Cr determination by ETAAS at 357.9 nm are summarised in table
14 I. The Cr hollow cathode lamp used at 18 mA and a spectral slit width of 0.7 nm was selected.
15 The samples were analysed in triplicate.

18 *Duplicate portion sampling of meals and estimation of nutrient intakes by food* 19 *composition tables*

20 The estimation of contents of several nutrients and energy in analysed meals was done by the
21 employment of food composition tables compiled in the programme (Dietsource version 2.0,
22 Novartis, Barcelona). It has allowed us to calculate their daily dietary intakes as well as that of
23 energy. These values have also been used to study the correlations existing among total and
24 dialyzable Cr levels and contents of other nutrients and energy in the analysed meals.
25 Unfortunately, these food composition tables do not include levels for Cr. Consequently, the
26 comparison of Cr DDI determined in the present method vs. that estimated using the food
27 composition tables compiled in the referred programme was not possible.

29 *Estimation of the absorbable fraction by the in vitro method*

30 The simulated gastrointestinal digestion procedure and the in vitro absorption estimation was
31 done according to a modification of the method of Garcia et al. (2001a), which was based in
32 other methods previously developed (Miller et al. 1981; Shen et al. 1996). To simulate the
33 gastric digestion, a portion of 5 g of homogenized sample was added to 40 ml of deionised

1 doubly distilled water and the pH was adjusted to 2.0 with 6 M HCl. After 15 min, we
2 determined again the pH adjusting again to 2 if it was necessary. Titratable acidity corresponded
3 to the number of equivalents of 0.5 NaOH required to titrate the amount of combined pepsin
4 digest pancreatin-bile extract mixture to pH= 7.5. For its determination, a 20 g aliquot of the
5 pepsin digest which 5.0 ml of the pancreatin-bile extract mixture were added employing 0.5 M
6 NaOH in the titration.

7 8 *Statistics*

9 For interpreting data the statistical package SPSS 12.0 for Windows program was employed.
10 Results are expressed as arithmetic mean and standard deviation. They were analysed in order
11 to evaluate the existence of statistical differences according to the meal (breakfast, lunch or
12 dinner) considered for the estimation of Cr intakes. The normal distribution of variables and
13 the homogeneity of variances were checked by the Kolmogorov-Smirnov and Bartlett's tests,
14 respectively. The comparisons were done using the Student's t-test when the variable fulfilled
15 parametric conditions and the Kruskal-Wallis test when conditions were non-parametric.
16 Additionally, correlations by the Pearson or Spearman's tests (for parametric and non-
17 parametric conditions, respectively), linear regression models and correlation of logarithmic
18 data were also used, to check the association between determined total and dialyzable Cr
19 levels from meals and among them and total levels present of other nutrients and energy
20 intake.

21 22 23 **Results and discussion**

24 25 *Analytical quality assurance*

26 The performance characteristics of the proposed method were determined following the
27 instructions indicated elsewhere (Delgado-Andrade et al. 2003). The detection limit and
28 characteristic mass were 187 ng g^{-1} and 3 pg , respectively. Mean recovery for spiked samples
29 was 100%. The mean Cr concentrations determined in the BCR CRM 278R and 185R ($0.78 \pm$
30 0.06 and $0.80 \pm 0.20 \text{ } \mu\text{g g}^{-1}$, respectively) were 0.80 ± 0.08 and $0.83 \pm 0.26 \text{ } \mu\text{g g}^{-1}$, respectively.
31 The relative standard deviation (n= 7) ranged from 5 to 12% (mean value= 7%) for the three
32 different samples for which between-day variability was determined.

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3 1 In the present study an ETAAS method has been developed for measuring total and dialysed
4 2 Cr levels in 108 meals. The results obtained for the standard reference materials show an
5 3 appropriate accuracy and precision of the program with the time-temperatures employed. The
6 4 detection limit and characteristic mass were suitable for the Cr concentrations found.
7 5 Additionally, the values obtained for the interday repeatability and percentage of recovery of
8 6 previously added samples with Cr show the adequacy of this technique for determining this
9 7 element in meals in order to check its total dietary intake as well as its dialyzable fraction.
10 8 Consequently, it could be used to assess the magnitude of inadequate intakes of Cr
11 9 (deficiency or excess and toxicity).
12 10

11 ***Total Cr and other nutrient intakes and energy levels from duplicate meals of the hospital***
12 ***restaurant***

13 The mean Cr intake determined by this method for the three groups of principal meals eaten
14 every day (breakfast, lunch and dinner) are given in table II. The Cr intakes found for
15 breakfasts were significantly lower than those determined for lunch and dinner ($p < 0.001$).
16 Lunch (43%) is the meal that contributes mainly to the 24-hr Cr DDI followed by dinner
17 (38%) and finally breakfast (19%). By using of the Pearson or Spearman's tests significant
18 correlations among Cr intake levels and supply of several nutrients and energy by the meals
19 analysed ($p < 0.05$, table III) were found. The corresponding correlation coefficients are also
20 collected in this table.

21 There was a positive and linear correlation between total Cr intake levels, and energy,
22 carbohydrates, fibre and protein and several amino acid supplied by the meals (table III).
23 Garcia et al. (2001a) also reported a statistically significant correlation between the presence
24 of Cr and the protein and carbohydrate content of the diet and energy intake. Our findings
25 establish that Cr supply by meals is directly related with macronutrient contents in them.
26 Consequently, food like cereals and by-products, legumes, dry fruits, meats, potatoes, dairy
27 products and seafood would be primary sources of Cr in the meals as other researchers had
28 previously noted (Haro et al. 2001, Garcia et al. 2001b, Santos et al. 2004, Anderson, 2004,
29 Leblanc et al. 2005, Navarro Alarcon et al. 2005). Bratakos et al. (2002) found that in
30 addition to meat and cereals the importance of vegetables as contributors to Cr dietary intake
31 from Greek foods. Consequently, the food with high carbohydrate and/or protein content
32 would also be a better source for Cr. Van Cauwenbergh et al. (1996) also suggested that Cr
33 intake may increase parallel to energy intake. Garcia et al. (2001b) also found a significant

1 correlation between the Cr presence and the protein and carbohydrate content in the diet and
2 the energy intake as we found in the present study.

3 As it can be checked in table II the mean Cr DDI for the 24-hr meals was $77 \pm 17 \mu\text{g day}^{-1}$
4 corresponding to the range 46-132 $\mu\text{g Cr day}^{-1}$ for the 36 consecutive days studied. All daily
5 menus of the restaurant of the hospital considered, supplied element levels higher to the daily
6 adequate intakes (AIs) established by the US IOM (IOM, 2002; 35 and 25 $\mu\text{g Cr day}^{-1}$ for 19-
7 50 y old men and women, respectively). Even when comparing with AIs indicated for the
8 physiological periods of pregnancy and lactation (AIs: 30 and 45 $\mu\text{g Cr day}^{-1}$ for 31-50 y old
9 women, respectively) the Cr supplied by all daily menus studied were higher than them. For
10 the Cr no upper levels (ULs) defined as the maximum level of daily Cr intake that is likely to
11 pose risk of adverse effects, have been noted by the IOM. Nevertheless, the US National
12 Research Council (NRC) established a daily intake recommended for Cr^{3+} (main form of Cr
13 presents in consumed food in meals with low toxic effects) between $\leq 50\text{-}200 \mu\text{g day}^{-1}$. In our
14 study the 24-hr daily menus that supplied the highest Cr DDI contributed with a 66% to the
15 upper level noted by the NRC. Consequently, no adverse effects in relation to Cr nutrition
16 (deficiency or toxicity) are present in the individuals that usually intake the meals of the
17 restaurant of the Motril Hospital (SE Spain), namely staff, users of ambulatory services and
18 patients' family.

19 The Cr DDIs previously reported for healthy adults by other researchers in different countries
20 ranged from 23 $\mu\text{g day}^{-1}$ in individuals from Rio de Janeiro (Brazil) (Santos et al. 2004) to
21 283 $\mu\text{g day}^{-1}$ in inhabitants from Ibaraki (Japan) (Shiraishi 2005). We can observe a high
22 variability among the range reported. The mean Cr DDI determined in our study ($77 \pm 17 \mu\text{g}$
23 day^{-1} , table II) is similar to that found by Leblanc et al. (2005) in French individuals aged ≥ 15
24 y ($77 \mu\text{g day}^{-1}$). Taking into consideration the three ranges of daily Cr intakes pointed out by
25 Garcia et al. (2001b) (high: $>104 \mu\text{g day}^{-1}$; moderate: 42-104 $\mu\text{g day}^{-1}$; and low: $<42 \mu\text{g day}^{-1}$),
26 almost all the daily menus evaluated by us correspond to moderate element intake levels.

28 ***Dialyzable Cr levels and Cr dialysabilities from duplicate meals of the hospital restaurant***

29 The interpretation of Cr intake is complicated and may indicate higher or lower level of
30 deficiency, "adequacy" and toxicity than intake alone. In this sense, the determination of the
31 element fraction that would be absorbed and transformed into a biologically active form in
32 the organism would be more informative than the measurement of the total mineral content

1 and supply from the daily diet alone. Consequently, in the present study we analyzed the
2 dialyzable Cr fraction from duplicate meals by an *in vitro* method that simulates the gastro
3 intestinal conditions because as a previous step to be absorbed each mineral has to be present
4 in a soluble form able to cross the intestinal mucosa. The mean Cr amount dialysed in the *in*
5 *vitro* study performed was $1.2 \pm 1.1 \mu\text{g meal}^{-1}$ (table IV) from a total mean content of $26 \mu\text{g}$
6 Cr meal^{-1} . The corresponding mean percentage of Cr dialysed from the total element content
7 of the meals (Cr dialysability) is 4.63% (table IV).

8 In the regression analysis a correlation between the logarithmic data of total and the
9 dialyzable fraction of Cr in the hospital meals ($p= 0.020$) was found (table III). Additionally,
10 dialyzable Cr levels were linearly correlated with the Cr dialysability of the meals considered
11 ($p= 0.000$, $r= 0.978$). The mean dialysed Cr determined was $1.2 \pm 1.1 \mu\text{g meal}^{-1}$ (table IV)
12 which corresponds to a Cr dialysability of $4.6 \pm 3.8\%$ in meals. Contrarily, Nielsen (1994)
13 collected data on different human studies indicating that when the Cr DDI was $10 \mu\text{g day}^{-1}$
14 the net absorption was 2%, which diminished with the rise of the Cr content. Mahan and
15 Arlin (1992) observed that when the Cr DDIs were higher than $240 \mu\text{g day}^{-1}$ the amount
16 absorbed was constant while the excretion increased proportionally with the element intake.
17 Anderson (2004) noted that with intakes $\geq 40 \mu\text{g day}^{-1}$, Cr absorption reaches and remains at a
18 plateau. The differences observed between these human studies and the *in vitro* study
19 performed by us are related with the endogenous physiological factors of individuals like the
20 status of their bowel mucosa, nutritional status, homeostatic mechanisms, carrier proteins,
21 etc. (Navarro Alarcon et al. 2005). In the present study we only determine the soluble Cr
22 fraction that forms complexes able to dialyze through the dialysis tubing and therefore to be
23 absorbed.

24 The statement that the bioavailability of a single element may depend on interactions with
25 other components, and both competitive and cooperative interdependence, has been reported
26 (Garcia et al. 2001a). In the present study significant correlations among the dialyzable Cr
27 fraction and the levels of other nutrients like protein, Fe, Na, I, F, ascorbic acid, fiber and
28 vitamin A ($p < 0.05$) were found. Specifically, it has been noted by others that the ascorbic
29 acid stimulates the Cr bioavailability (Seaborn and Stoecker, 1990; Offenbacher, 1994)
30 similarly to the result found by us. This finding could be related with the capacity of the
31 ascorbic acid to facilitate the formation of soluble complexes with Cr^{3+} and consequently to
32 stimulate its crossing through the dialysis tubing.

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1 In relation to Fe, Anderson (2004) reported that the deficiency in this element stimulates the
2 net absorption and bioavailability of Cr suggesting that this element shares some similarities
3 with the Fe absorption pathway. In our study a quadratic correlation ($p= 0.031$) between the
4 Fe and dialyzed Cr levels in meals, has been observed. This result reported that from 0 to
5 $\cong 7,5$ mg Fe meal⁻¹ the dialyzable Cr fraction enhances significantly with the Fe content of
6 meals. Nevertheless, at Fe contents higher than $\cong 7,5$ mg meal⁻¹ the net absorption decreased
7 significantly as was also reported by Anderson (2004).

8 We have also found that dialyzed Cr levels were correlated with fiber content of meals
9 by a logarithmic model ($p= 0.011$). Therefore, the possibility that fiber could facilitate the net
10 absorption of soluble complexes with Cr³⁺ through the dialysis tubing should be considered.
11 Anderson (2004) reported that oxalate increases Cr absorption.

12 Despite the results reported in the present study, future research leading to better
13 knowledge of Cr bioavailability from diet using *in vivo* studies performed in human beings is
14 needed. Although the *in vitro* studies of bioavailability can be useful for evaluating the
15 percentage of Cr that could be transformed into absorbable forms in the digestive tract and
16 the influence exerted by other nutrients of the diet, they do not exactly reproduce the
17 physiological conditions and are insufficient to enable a comprehensive evaluation of it.
18 Consequently, they should be combined with *in vivo* studies in order to reach a better
19 understanding of the bioavailability process in the human organism.

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1 Table I. Furnace conditions for Cr determination in duplicate meal samples by ETAAS technique

Step	Temperature (°C)	Time (s)	Argon flow (ml/min)
Injection	20	-	300
Ashing	150	60	300
Charring	1650	40	300
Atomization	2500	6	0
Cleaning	2650	3	300

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1 Table II. Mean intake and DDI of total Cr determined by the technique of duplicate portion sampling
 2 of meals (breakfast, lunch and dinner) and analysis by ETAAS

Meal	N	Total Cr (μg)		% of Cr DDI
		Range	Mean \pm SD	
Breakfast	36	8.2-26	15 \pm 7.0 ^a	19
Lunch	36	20-75	33 \pm 14	43
Dinner	36	17-45	29 \pm 8.4	38
24-hr meals (DDI)	36	46-132	77 \pm 17	100

3 ^a $p < 0.001$.

1 Table III. Significant correlation coefficients and significance levels among the total and dialyzable
 2 Cr levels of the analyzed hospital meals (n= 108) and other nutrients estimated from the food
 3 composition tables compiled in the statistics programme *Dietsource 2.0* (Novartis)

Cr form	Nutrient	<i>r</i>	Nutrient	<i>R</i>	Nutrient	<i>R</i>	
Total Cr	Energy	0.736 ^a	Vitamin E	0.375 ^c	Protein	0.725 ^a	
	Carbohydrates	0.732 ^a	Folate	0.411 ^b	Trp	0.453 ^b	
	Fiber	0.717 ^a	Vitamin B ₁₂	0.462 ^a	Thr	0.451 ^b	
	Fe	0.657 ^a	Monounsaturated acids	0.370 ^c	Ile	0.473 ^a	
	P	0.527 ^a	C16:1	0.301 ^c	Leu	0.448 ^b	
	K	0.502 ^a	C18.1	0.364 ^c	Lys	0.453 ^b	
	F	0.357 ^c	Polyunsaturated acids	0.342 ^c	Met	0.447 ^b	
	Ascorbic acid	0.373 ^c	C20:5	0.369 ^c	Phe	0.462 ^a	
	Niacin	0.519 ^a	C22:6	0.426 ^b	Val	0.462 ^a	
	Pyridoxine	0.553 ^a	Total cholesterol	0.362 ^c	Ethanol	0.351 ^c	
	Vitamin A	0.314 ^c					
	Dialyzable Cr	Na	0.437 ^b	Ascorbic acid	0.389 ^b	Vitamin A	0.414 ^b
		I	0.340 ^c	Fiber	0.375 ^c	Total Cr	0.345 ^c
F		0.300 ^c	Protein	0.423 ^c	Fe	0.390 ^c	

4 ^a*p* ≤ 0.001.

5 ^b*p* < 0.01.

6 ^c*p* < 0.05.

1 Table IV. Mean levels of dialyzable Cr and Cr dialysabilities determined by the technique of duplicate
 2 portion sampling of meals (breakfast, lunch and dinner) and analysis by ETAAS

Meal	<i>n</i>	Dialyzable Cr (μg)		Percentage of Cr dialysed (Cr dialysability)	
		Range	Mean \pm SD	Range	Mean \pm SD
Breakfast	36	0.001-1.1	0.535 \pm 0.405 ^a	0.006-8.0	3.6 \pm 2.6
Lunch	36	0.003-3.2	1.3 \pm 0.956	0.005-12	4.0 \pm 3.3
Dinner	36	0.001-4.2	1.8 \pm 1.3	0.006-16	6.3 \pm 4.9
Mean value	36	0.001-4.2	1.2 \pm 1.1	0.006-16	4.6 \pm 3.8

^a*p* < 0.001.