

## Food chromium content, dietary chromium intake and related biological variables in French free-living elderly

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Trivalent chromium ( $\text{Cr}^{3+}$ ) is an essential trace element involved in insulin function. Cr deficiencies result in decreased insulin sensitivity, glucose intolerance and an increased risk of diabetes. Cr status decreases with age suggesting that the elderly may be at high risk of Cr deficiency. This study aimed to provide information about the Cr content of foods in France and the Cr intake in French free-living elderly. We measured the food Cr content and daily Cr intake of freely chosen diets for 3 d in twelve French free-living elderly people and their Cr excretion and plasma hormonal related variables, leptin, insulin and cortisol. Considering the relationship between insulin resistance and oxidative stress, we also determined plasma thiobarbituric acid reactive substance, thiol groups and total and reduced glutathione. Although these subjects had well-balanced diets, their daily Cr intakes did not reach the French recommendations. The low Cr intakes were due to the low Cr density of the foods. We found a negative correlation between Cr intakes and insulin, BMI and leptin.

### Chromium intakes: Elderly: Food chromium content

Chromium ( $\text{Cr}^{3+}$ ) is an essential trace element functioning in glucose and insulin metabolism and is associated with age-related chronic diseases including diabetes and CVD<sup>1–3</sup>. Cr also acts in regulating corticosteroid metabolism<sup>4</sup>, is postulated to decrease losses in bone density and lean body mass<sup>5</sup> and protects against the loss of cognitive function in the elderly<sup>6</sup>. Moreover, Cr also acts both as an insulin potentiating factor and antioxidant<sup>7,8</sup> and could, therefore, counteract the increased oxidative stress associated with insulin resistance in the elderly.

Cr status declines with age<sup>9</sup> in relation to lower energy intake, change in food patterns, decreased absorption or increased Cr losses<sup>10</sup>. Marginal Cr deficiencies in this population may lead to an increased risk of the metabolic syndrome, diabetes, CVD, may go undetected and contribute to morbidity and even mortality. In the USA and Europe, few studies have measured Cr intakes in the general population and none has focused on the determination of food Cr content and intake in French elderly people. Therefore, the objective of this study was to provide missing data about the food Cr content and intakes in French aged free-living subjects and to investigate the possible relationships between the level of these intakes and the biological parameters involved in the glucose–insulin system and oxidative stress. We analysed the individual food Cr contents and daily Cr intakes from freely chosen diets for 3 d in French free-living elderly people and determined their

Cr excretion and plasma hormonal related variables including insulin, leptin and cortisol. In association with the relationship between insulin resistance and oxidative stress, we also measured plasma lipid peroxidation as thiobarbituric acid reactive substance, plasma protein oxidation as plasma thiol groups and blood total and reduced glutathione.

### Experimental methods

#### Patients

Twelve autonomous free-living volunteers (8 women and 4 men) aged 70 to 85 years participated in the study. They were recruited in Valence Hospital Geriatric Centre which has been chosen previously chosen as representative of the French elderly population for investigation in the EURONUT Seneca Study<sup>11</sup>. The inclusion criteria were defined as French people with usual dietary habits with no specific or regional intakes and representative of French dietary habits, no trace element and antioxidant supplementation 3 months before the study, BMI between 22 and 30, non smoking, <30 g/d alcohol consumption for men and <20 g/d for women, no severe pathologies (cancer, CVD, stroke), no surgery within 3 months of the study, no diabetes, creatinemia >30  $\mu\text{mol/l}$ , no alanine aminotransferase, aspartate

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aminotransferase and gamma-glutamyl transpeptidase greater than three times the physiological values.

To compare the biological data between older and younger subjects, blood and urine samples were also obtained from young healthy French subjects ( $n$  9, three males and six females), 25–35 years old, using the same criteria of inclusion and same methodology for determination of biological parameters. The Ethical Committee approved the study, and each volunteer signed an informed consent. Subjects were not compensated for their participation.

#### *Food collection and analyses*

Food records for three consecutive days were analysed and the Cr content of all food (breakfast, lunch, snacks, dinner and beverages) was determined. Beverages and foods were analysed as consumed. Food weight estimation was evaluated as eaten and by serving using the validated SUVIMAX French food evaluation guide<sup>12</sup>. Cr contents of 108 daily meals were analysed. Samples were collected in polyethylene containers, weighed and homogenised in a blender fitted with low-Cr steel blades to minimise Cr contamination<sup>13</sup>. A composite human diet sample, RM 8431, with a certified value of 102 (SD6) ng Cr/g (NIST, US Department of Commerce, Gaithersburg, MD), was used as an analytical check on the accuracy of the results. Under our conditions, we obtained a Cr concentration of 110 (SD9) ng/g. We also used NIST Bovine Muscle RM 8414 with a certified value of 71 (SD38) ng/g (our value was 62 (SD13) ng/g). Cr intakes by meal and then by day were calculated given the weight record of the different items. For each participant, values were expressed as the mean of 3-d Cr intake ( $\mu\text{g}/\text{d}$ ) with standard deviation.

#### *Analyses*

Urine samples were collected after a 12h overnight fast. Second morning void urine was collected using sterile polyethylene snap-lid containers (Fisher Scientific, Pittsburgh, PA, USA). Samples were kept on ice, and stored at  $-20^{\circ}\text{C}$  before analysis.

Blood was collected after a 12h overnight fast and centrifuged for 30 min at 2000g. After centrifugation, serum was stored at  $-20^{\circ}\text{C}$  before analysis.

#### *Urinary chromium*

Urinary Cr was analysed by graphite furnace atomic absorption using an AAnalyst 800, (Perkin-Elmer Corporation, Norwalk, CT, USA) using end capped graphite tubes<sup>14</sup>. Two pooled urine samples, whose Cr concentrations had been verified by four independent methods, were assayed at least twice daily to validate the analytical reliability of the urinary Cr concentrations<sup>15</sup>. Data were expressed as ng Cr/mg creatinine with standard deviation. Cr content of foods was analysed using the same instrument<sup>15</sup>.

#### *Related hormonal variables*

Cortisol, insulin and leptin were measured by ELISA (American Laboratory Products Co., Windham, NJ, USA).

#### *Oxidative stress parameters*

Lipid peroxidation was measured by thiobarbituric acid reactive substance concentrations in the plasma<sup>16</sup>. Protein oxidation was determined by measuring the concentration of plasma thiol groups<sup>17</sup>. Total and reduced glutathione levels were measured in plasma as previously described by Akerboom<sup>18</sup>. For this determination, within 20 min of venipuncture, whole blood samples were deproteinized by adding an aqueous solution of metaphosphoric acid (6%, w/v), then the mixture centrifuged at 2500g for 10 min at  $4^{\circ}\text{C}$ . The acidic protein-free supernatant fractions were stored at  $-80^{\circ}\text{C}$  until analysis.

#### **Statistics**

Statistical treatment of data was completed using Statistica Statistical Software, Paris, France, and SAS, SAS Institute, Cary, NC. Results are expressed as mean values with standard deviations. Biological variable mean comparisons between the groups were tested for significance by Student's  $t$  test. Statistical significance was set as  $P < 0.05$ . Pearson's correlation analyses were performed to establish the relationship between Cr intakes and key variables.

#### **Results**

##### *Cr content of foods and daily Cr intakes*

The Cr content of foods and beverages, freely chosen and consumed for three consecutive days, is shown in Table 1. With the exception of special food items such as dark chocolate, Parisian bread or some prepared foods, most of the foods contained Cr below  $100 \mu\text{g}/\text{kg}$ .

The mean energy intake was 7280 kJ/d (1742 kcal/d) for men and 6580 kJ/d (1575 kcal/d) for women. Expressed as percentage of the total energy intake, carbohydrates represented 43.8% in men and 46.5% in women, proteins as 17.1% in both men and women, and lipids as 39.1% in men and 37.6% in women. Mean Cr daily intakes were 40.2 (SD 13.9)  $\mu\text{g}$  (Table 2) without a significant difference due to gender or age ( $>$  or  $<$  75 years old). All the mean Cr individual intakes except one were below the French RDA for adults (60  $\mu\text{g}/\text{d}$ ) and 50% were below two-thirds of this RDA. Moreover, considering the recommendations of the French Geriatric Board for optimal Cr intake for people over 70 years<sup>19</sup>, which recommends 125  $\mu\text{g}/\text{d}$ , all the measured intakes were below two-thirds of this level and 50% below one-third of this level.

##### *Chromium excretion and related hormonal variables*

Urinary Cr excretion was similar in the older group compared with younger (0.15 (SD 0.05) v. 0.14 (SD 0.02) ng/mg creatinine; Table 3). When we analyzed the data for Cr concentration directly to minimize for changes in creatinine excretion related to ageing, there were also no significant differences between the groups. Fasting insulinemia was significantly higher in the elderly group than in younger subjects while, in the elderly, fasting glycemia remained in the physiological range (1.1 (SD 0.1) g/l). Plasma leptin and cortisol were not different between the two groups. We found a negative correlation between Cr intakes and BMI ( $r = -0.28$ ;  $P < 0.06$ ), fasting insulin ( $r = -0.56$ ;  $P < 0.04$ ) and leptin ( $r = -0.46$ ;  $P < 0.04$ ).

**Table 1.** Chromium content of food and beverages

	Cr content ng/ml	µg/serving
<b>Beverages</b>		
Tap water	0.09	0.02
Evian	0.07	0.01
Contrexeville	0.03	0.01
Vittel	0.07	0.01
Vernet	0.06	0.01
Volvic	0.33	0.07
Green tea with mint	1.20	0.24
China tea	0.83	0.00
Coffee	0.74	0.11
Lyophilized coffee	7.32	0.73
Herbal tea	0.45	0.05
Herbal tea (mint)	0.77	0.14
Chicory	1.04	0.21
Chicoree with milk	1.65	0.25
Grapefruit juice	19.60	2.94
Orange juice	1.06	0.21
Lemon juice	0.82	0.08
Juice 12 fruits	3.33	0.50
Red wine	16.44	2.47
Beer	1.29	0.32
Whisky	0.78	0.02
<b>Dairy products</b>		
Cottage cheese (20 % fat)	28.92	2.89
Yoghurt	1.48	0.15
Yoghurt (0 % fat)	3.69	0.37
Yoghurt with fruit	8.94	1.12
Vache qui rit	47.49	1.19
Edam	5.54	0.22
Swiss cheese	29.97	1.80
Camembert cheese	6.88	0.28
Brie cheese	3.65	0.11
St Nectaire cheese	6.32	0.19
St Marcellin cheese	1.51	0.07
Goat's cheese	2.04	0.06
Powdered milk	0.62	0.01
Milk (low fat)	0.54	0.14
Butter	5.78	0.10
Margarine	0.43	0.07
<b>Bread and cereals</b>		
Parisian bread	224.84	11.24
Farmhouse bread	91.34	3.65
Toasted bread	63.03	1.26
Melba toast	58.18	2.00
Sesame seed loaf	5.82	0.23
Six-cereal bread	30.58	1.53
<b>Fruits</b>		
Clementine	22.65	1.36
Orange	31.09	6.22
Apple	37.78	6.80
Kiwi	72.28	5.09
Strawberries	7.71	1.54
Pear	7.45	1.49
Banana	4.01	0.60
Nuts	0.40	0.20
Dry plums	55.71	1.39
Dry apricots	33.84	0.68
<b>Marmalades and chocolates</b>		
Dark chocolate	1428	14.28
White chocolate	7.81	0.08
Almond milk chocolate	7.77	0.08
Dark chocolate with almonds	74.49	0.75
Honey	18.19	0.36
Chestnut chocolate paste	62.11	1.24
Quince marmalade	6.00	0.09
Blackberry marmalade	19.10	0.40
Red currant marmalade	13.21	0.26
<b>Sweets and pastries</b>		
Stewed apple	13.98	2.77
Stewed apple and rhubarb	13.62	1.40

**Table 1.** *Continued*

	Cr content ng/ml	µg/serving
Chocolate mousse	131.30	13.13
Crème brûlée	5.87	0.59
Cereals plus chocolate	316.03	6.32
Apple pie	8.56	0.86
Cookies	7.31	0.73
Sweets (bonbons)	17.59	0.18
<b>Meat and fish</b>		
Pork sausage with tomatoes	27.95	5.59
Parisian ham	23.15	1.67
Pork rib	28.02	4.20
Veal rib	18.28	2.74
Beef steak	2403	2.88
Minced beef	4553	6.83
Grilled chicken	38.30	3.83
Crab sticks (surimi)	23.92	2.39
Coalfish	26.86	4.03
Fresh cod	14.71	1.47
Sea bass	29.73	5.95
Sole	39.53	3.95
Whiting	15.57	1.56
Boiled egg	2.93	0.29
<b>Vegetables</b>		
Leek	51.87	5.18
Celeriac	11.85	1.19
Green beans	20.52	2.05
Carrots (grated raw)	4.81	0.96
Artichoke	3.55	0.36
Tomatoes	4.00	0.49
Avocado	19.11	1.91
Green salad	49.03	4.90
Radish	26.06	2.61
Beetroot	6.37	0.64
Broccoli gratin	21.98	4.40
Vegetable soup	6.20	1.24
<b>Prepared foods</b>		
Spinach with cream	32.72	3.27
Pasta with spinach and cheese (French ravioli)	242.74	48.55
Endives with olive oil, nuts and ham	25.10	2.51
Endives with cream and cheese	7.43	0.74
Poultry cake (quenelle)	45.89	4.59
Leek and bacon pie	77.07	11.50
Boiled potatoes	51.02	10.20
Spinach beet with cream and cheese	172.17	34.00
Stewed tomatoes, egg plants, pepper in olive oil	45.54	11.39
Sorrel salmon pie	25.83	5.17
Sorrel soup	190.79	47.7
Green vegetables and rice	31.22	7.81
Onion soup with Swiss cheese	14.22	2.84
Tuna with tomatoes and rice	130.76	13.08
Quinoa (cereal)	16.50	1.65
Rabbit with carrots	59.11	5.91
Potatoes with cream and Swiss cheese	28.61	6.65
Boiled leek juice	1.26	0.38
Couscous	51.16	20.40
Ham, leek, and Swiss cheese	23.99	2.40
Chicken nugget with ham and cheese	96.67	9.67
Salmon pie	25.83	2.58
Cooked carrots with butter	40.43	8.09
Rice	12.41	1.24
Pasta with parmesan cheese	11.63	1.16
Omelet	56.36	5.64
Dry pork sausage	117.17	3.52
Mashed potatoes	104.54	10.45
Sauté potatoes	20.33	3.05
Pasta bolognese	35.98	7.20

### Oxidative stress parameters

There were no significant differences in oxidative stress parameters between the two groups except for plasma thiobarbituric acid reactive substance levels which were higher in older than in younger subjects ( $P < 0.05$ ) and weakly correlated ( $r = 0.11$ ) to insulin plasma levels (Table 4).

### Discussion

In this study, despite a well balanced composition of the diets and a fairly good energy intake the Cr nutritional density was very low, in relation to the low Cr content of the selected foods. More than 90% of the self-selected diets did not reach the French RDA for adults (60  $\mu\text{g}$  Cr/d), and all were

dramatically below the specific recommendations (125  $\mu\text{g}$  Cr/d) for people older than 70 years.

Only limited data on Cr intakes exist for comparison. Moreover, they have been mostly obtained from adults and very few from the elderly. In the USA, the reported Cr intakes in elderly people were lower than those measured in this study (25 to 37  $\mu\text{g}/\text{d}$ )<sup>20</sup>. In younger US adults (22–65 years), Cr intakes were still lower than those measured in the present work, at 23.1 (SD 2.9)  $\mu\text{g}/\text{d}$  for women and 38.8 (SD 6.5)  $\mu\text{g}/\text{d}$  for men<sup>21</sup>. In Europe, low levels of Cr intakes, similarly to our data, were found for most countries. In Finland, Sweden and Switzerland, the intakes were approximately 50  $\mu\text{g}/\text{d}$  or lower<sup>22</sup>. They have been evaluated at 53 (SD 31)  $\mu\text{g}/\text{d}$  in Belgium<sup>23</sup>, 60–90  $\mu\text{g}/\text{d}$  in Poland<sup>24</sup> and, in Germany, Cr recommendations were nowhere near reached in the daily diet

**Table 2.** Daily energy and chromium intakes and contributions of different meals

	Total (n 12)		Females (n 8)		Males (n 4)	
	Mean	SD	Mean	SD	Mean	SD
Energy intakes (kJ/d)	6930	630	6580	410	7280	960
(kcal/d)	1658	150	1575	98	1742	230
Mean Cr intake ( $\mu\text{g}/\text{d}$ )	40.23	13.85	42.74	14.67	35.18	10.88
Range Cr intake ( $\mu\text{g}/\text{d}$ )	27.14–68.97		29.44 – 68.97		27.14 – 44.04	
Cr ( $\mu\text{g}/1000$ kJ)	5.81		6.49		4.83	
( $\mu\text{g}/1000$ kcal)	24.26		27.14		20.20	
Lunch (Cr $\mu\text{g}/\text{d}$ )	19.30	11.01	21.40	12.44	15.33	6.25
Dinner (Cr $\mu\text{g}/\text{d}$ )	14.88	9.82	14.18	10.50	15.34	5.89
% < two-thirds RDA (adult French RDA)	50%		25%		75%	

**Table 3.** Urinary Cr excretion and related hormonal variables in elderly free-living French subjects compared with younger French subjects (Mean values and standard deviations)

	Elderly French subjects (n 12)		Younger French subjects (n 9)	
	Mean	SD	Mean	SD
Urinary Cr (ng/mg creatinine)	0.15	0.05	0.14	0.02
Plasma leptin (ng/ml)	104.3	122	196	86
Plasma insulin (mU/ml)	6.9*	1.7	3.6	1.9
Plasma cortisol ( $\mu\text{g}/\text{dl}$ )	16.0	3.7	29.8	14

Mean value was significantly different from the younger group: \* $P < 0.05$ .

**Table 4.** Oxidative stress parameters in elderly and younger subjects (Mean values and standard deviations)

	All together (n 12)		Females (n 8)		Males (n 4)		Younger subjects (n 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GSHt ( $\mu\text{M}/\text{l}$ )	963.33	126.87	939	116	1036	155	967	128
GSSG ( $\mu\text{M}/\text{l}$ )	13.66	4.61	12.94	5.01	16.90	1.61	12.34	5.78
GSH ( $\mu\text{M}/\text{l}$ )	876	290	925	117	765	526	912	189
Plasma SH groups ( $\mu\text{M}/\text{g}$ proteins)	5.12	0.80	5.03	0.40	5.55	1.40	6.12	0.50
Plasma TBARs ( $\mu\text{M}/\text{l}$ )	2.96*	0.29	3.01*	0.29	2.80	0.30	2.30	0.20

GSHt, total glutathione; GSSG, oxidised glutathione; GSH, reduced glutathione; SH, thiol; TBARs, thiobarbituric acid reactive substances. Mean values are significantly different from the younger group: \* $P < 0.05$ .

from nursing homes for the elderly<sup>25</sup>. Higher Cr intakes have been also reported as 96.4 µg/d in Canadian free-living women aged 66.2 years<sup>26</sup>, 143 µg/d in Greek adults<sup>27</sup>, and 129 µg/d<sup>28</sup> or 88.3 µg/d<sup>29</sup> in Spanish adults. In French young adults, 154 µg/d was measured using duplicate meals purchased from catering establishments<sup>30</sup> or 98 µg/d from food usually eaten in France<sup>31</sup>. Some of this variation might result from discrepancy in the average daily energy levels as well as methodological differences in the collection and analyses of the diets. Compared with the literature, our data are in the lower range of values reported in Europe for adults but higher than those measured in the USA. They confirm previous observations showing that it is practically impossible to reach the optimal intakes of Cr if the total energy intake remains below 2500 kcal/d (10450 kJ)<sup>32</sup>. Regarding biological parameters, Cr excretion was not different between the two groups and was similar to the values measured previously in French healthy post menopausal women<sup>33</sup> and in American elderly subjects<sup>34</sup>. Insulin plasma levels were higher in older than in younger subjects. The increased level of insulin with ageing is well documented<sup>35</sup> and the negative correlation between Cr intakes and insulin, underlines the role of Cr in insulin sensitivity.

In the presence of Cr, much lower amounts of insulin are required and the insulin sensitivity is improved<sup>2</sup>.

In this study, lipid peroxidation was significantly increased in older participants. In agreement with this observation, a positive correlation between age and plasma thiobarbituric acid reactive substance levels has been reported<sup>36</sup> and numerous studies have pointed out an increased lipid peroxidation in elderly subjects<sup>37</sup>. By decreasing high levels of insulin and preventing auto-oxidation of glucose, Cr may act as an indirect antioxidant. Recent interventional trials have reported the antioxidant effects of Cr supplementation in people with type 2 diabetes<sup>7,8</sup>. In contrast, plasma thiol groups, oxidation of which is an early determinant of oxidative stress<sup>38</sup>, were not significantly modified in older subjects while protein oxidation is generally reported to increase during ageing<sup>39</sup>.

The present study clearly shows that even though free-living elderly French subjects have good dietary habits and consume well-balanced diets, their daily Cr intakes are dramatically lower than the French recommendations. The analyses of the food Cr content demonstrate that low Cr intakes are due mainly to the low Cr density of foods. The potential needs of Cr supplementation in the elderly in France is still a matter of debate since some studies failed to demonstrate a beneficial effect of Cr supplementation in the elderly<sup>20,40</sup>. French RDA for Cr is similar for adults and older persons<sup>41</sup>. Specific recommended intakes for the elderly should move from avoiding deficiency status to preventing chronic diseases.

In conclusion, future research along these lines is needed to establish specific Cr requirements for the elderly especially as dietary patterns change to include less refined high-sugar and high-fat foods which are not only low in Cr but also enhance Cr losses<sup>14</sup>.

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