The Effects of Supplementary Cr₃ (Chromium(III) Propionate Complex) on the Mineral Status in Healthy Female Rats

Halina Staniek¹ & Zbigniew Krejpcio¹

Abstract

More and more people use food supplements for various reasons, e.g. to prevent mineral deficiency and diseases (e.g. osteoporosis, diabetes, anaemia). Supplements containing Cr(III) are purchased primarily for weight loss and antidiabetic effects. The aim of this study was to evaluate the effects of supplementary Cr₃ {chromium(III) propionate complex, \([\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)\text{H}_2\text{O}_3]\text{NO}_3)} on the mineral status in female Wistar rats. The study was carried out on 30 female Wistar rats, divided into five groups (six animals in each): a control group and test groups fed Cr₃ supplemented diets with 100, 200, 500 and 1000 mg Cr · kg⁻¹ diet (equivalent to 10, 20, 50 and 100 mg Cr · kg⁻¹ body mass (b.m.) per day) given as Cr₃ for 4 weeks. Supplementary Cr₃ increased the Cr content in tissues in a dose-dependent manner. High dietary doses of Cr₃, 20 and 100 mg Cr · kg⁻¹ b.m., increased the Cu content in the liver and spleen as well as the Zn content in the kidneys but decreased the liver Ca content. Doses of 50–100 mg Cr · kg⁻¹ b.m. decreased the serum Fe concentration and the Fe content in the liver and kidneys. Supplementation with Cr₃ at doses of 10 and 100 mg Cr · kg⁻¹ b.m. did not affect the Mg content in the rats’ tissues. In conclusion, high dietary doses of Cr₃ (10 and 100 mg Cr · kg⁻¹ b.m.) given for 4 weeks affected the mineral status of Fe, Zn, Cu and Ca in the tissues of healthy female Wistar rats.

Keywords

Chromium(III) propionate · Rats · Mineral status

Introduction

In recent years, chromium has been one of the most investigated dietary minerals. Chromium is a trace mineral that has received much attention as a dietary supplement because good dietary sources of chromium are scarce and the intake is usually low. Chromium(III) deficiency may contribute to carbohydrate metabolism disorder [1].

Many trials proved the positive effect of supplementary chromium(III) on fasting plasma glucose, lipid variables, especially in diabetic subjects [2–4]. For this reason, trivalent chromium has been postulated to be necessary for insulin efficacy in regulating the metabolism of carbohydrates, lipids and protein [5]. A number of chromium compounds can be considered as a perspective for metabolic syndrome treatment [6].

In 2002, the Food and Nutrition Board of the US National Academy of Science set the Adequate Intake (AI) of chromium at 25 μg · day⁻¹ for adult women and 35 μg · day⁻¹ for men [8], which was lower than the previous recommended dietary intake of 50–200 μg per day. Recently, the EFSA panel found no evidence of beneficial effects associated with chromium intake in healthy subjects and concluded that setting the AI for chromium was not appropriate [5].

In general, the oral intake of chromium has low toxicity partially due to its poor absorption (about 0.5–2.0%). However, different Cr(III) compounds have diverse rates of absorption [9]. Organic Cr(III) forms have greater bioavailability than inorganic ones. It is well known that the mineral intake at high doses has antagonistic effects on other elements [9].
Chromium is one of the best-selling mineral supplements in the USA [10]. Trivalent chromium, the form found in food and dietary supplements, is considered to be safe. Many organic chromium complexes, including chromium picolinate [Cr(Pic)3], chromium nicotinate (NCB) [11, 12], chromium histidinate (CrHis) [13, 14], chromium complex of D-phenylalanine [Cr(D-Phe)3] [15, 16], chromium propionate complex (Cr3) [4, 17–19] and chromium glycinate complex (CrGly) [19] have been synthesised and demonstrated to be biologically effective. Different coordinate ligands of these organic chromium complexes exhibited different bioactive compounds [20].

For humans, a typical Cr intake is 20–45 μg per day in the diet [21], while doses of supplements may contain 200–1000 μg Cr(III) [1, 22]. These doses correspond to daily body weight-adjusted doses of 0.29–0.64 μg Cr(III) · kg⁻¹ body mass (in the diet) and 2.86–14.3 μg Cr(III) · kg⁻¹ b.m. (in supplements) in an individual with an average weight of 70 kg [23].

Few studies have been designed to evaluate the effects of trivalent Cr supplementation on the content of Cr and other minerals in animal tissues. Chromium is distributed to various tissues of the body but appears to be most concentrated in the kidneys, liver, and muscles [24]. Dietary Cr supplementation at high doses can potentially affect the mineral status due to possible interactions with other macro- and microelements at absorption, transport, metabolism, excretion and other levels [25]. In this case, the high supply of Cr(III) can affect the metabolism of other minerals in healthy rats. Therefore, the aim of this study was to evaluate the effects of high doses of chromium (III) complex with propionic acid, so-called Cr3 (100–1000 mg Cr · kg⁻¹ diet, equivalent to 10, 20, 50 and 100 mg Cr/kg b.m. per day) on the mineral status in healthy female rats.

Material and Methods

Test Chemicals

The chromium(III) complex with propionic acid in the form of nitrate salt (chemical formula [Cr3O(O2CCH2CH3)6(H2O)3]NO3 (Cr3) was synthesised in a laboratory at the Department of Product Ecology, Poznań University of Economics, Poland, according to the method described by Earnshaw et al. [26]. The Cr3 was found to contain 21% of elemental Cr, determined by the AAS method (spectrometer AAS-3 with BC correction, Zeiss, Germany).

Animals and Diets

Thirty 10-week-old female Wistar rats were obtained from the Department of Toxicology, Poznań University of Medical Sciences, Poland. The animals were housed in single cages, at controlled temperature, photoperiod and air humidity (19–22 °C, 12-h light/dark cycle, 55–60% of ambient air humidity). After 5-day adaptation to laboratory conditions, the rats were divided into five equal groups (the control group and groups treated with Cr3—six animals in each group, equal body weight of 180 g). All the groups were fed a commercial diet for maintenance of adult rodents (Labofeed H) enriched with 0, 100, 200, 500 and 1000 mg Cr(III)/kg of diet (ca. 0, 10, 20, 50 and 100 mg Cr/kg b.m. per day) given as [Cr3O(O2CCH2CH3)6(H2O)3]NO3 for 4 weeks. Table 1 shows the composition of the basic Labofeed H diet. The Cr content in the basic diet (the control group) was 0.5 ± 0.06 mg · kg⁻¹, while in the supplemented diets it was 107.5 ± 6.5 mg · kg⁻¹ (A); 224.8 ± 32.4 mg · kg⁻¹ (B); 535.5 ± 26.22 mg · kg⁻¹ (C) and 1049.5 ± 17.6 mg · kg⁻¹ (D), respectively. The diets were stored at 4 °C. The rats were allowed free access to feed and distilled water throughout the whole experiment.

The feed intake was measured daily, while body weight gains were monitored weekly. At the end of the experiment, after 12-h starvation, the rats were euthanised by intraperitoneal injection of thiopental (40 mg · kg⁻¹ body mass). Blood was collected into tubes; tissue samples (liver, kidneys, heart, spleen, pancreas, ovaries) were collected, weighed and frozen. The experimental protocol was approved by the Local Bioethical Commission in Poznań (No. 12/2005).

Laboratory Analyses

The serum Fe concentration was determined with the colorimetric method by means of 2,4,6-tri(2-pyridylo)-5-triazine.

Diet and tissue samples for mineral analyses were digested with concentrated 65% spectra pure HNO3 (Merck) in a Microwave Digestion System (MARS-5, CEM, USA).

The concentration of copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg) and calcium (Ca) in mineralised samples was determined with the flame atomic absorption spectrometry method F-AAS (Zeiss AAS-3, with BC, Germany), while the concentration of Cr was measured using a graphite furnace atomic absorption spectrometer GF-AAS (AAS EA 5, with BC, Jenoptic, Germany). The accuracy of Cu, Zn, Fe, Mg and Ca measurements was assured by simultaneous analysis of certified reference material (Pig Kidney BCR No. 186, Brussels), while the analysis of Cr was controlled using certified reference material (Virginia Tobacco Leaves CTA-VTL-2, Poland) (Table 2). The mean recoveries of certified levels (expressed as percentage of mean certified values) were as follows: Cu—103%, Zn—101%, Fe—97%, Mg—104%, Ca—103% and Cr—102%.
Statistical Analyses

The data were presented as mean ± SEM. The results were analysed using one-way analysis of variance (ANOVA/ MANOVA) and the Tukey’s test to determine significant differences (p < 0.05). All calculations were done using Statistica ver. 7.0 software (StatSoft, Tulsa, USA).

Results

Figure 1 and Table 3 show the effects of Cr3 supplementation on the tissular content of Cr, Cu, Zn, Fe, Mg and Ca in healthy female rats. As expected, supplementary Cr3 increased the liver and kidney Cr levels in a dose-dependent manner (Fig. 1a). The addition of Cr(III) to the diet at a dose of 100 mg of Cr· kg\(^{-1}\) did not significantly increase the liver Cr content (3.91 ± 0.24 vs. 2.96 ± 0.18 μg· g\(^{-1}\) dry mass). However, in comparison with the control group (2.96 ± 0.18 μg· g\(^{-1}\) d.m.), the doses of 200, 500 and 1000 mg of Cr(III)· kg\(^{-1}\) significantly increased the liver Cr level, by 78% (5.27 ± 0.23 μg· g\(^{-1}\) d.m.), 242% (10.13 ± 0.21 μg· g\(^{-1}\) d.m.) and 504% (17.88 ± 0.93 μg· g\(^{-1}\) d.m.), respectively. Supplementary Cr3 at doses of 100 to 1000 mg· Cr· kg\(^{-1}\) had no effect on the kidney Fe concentration. However, the dose of 200 mg Cr(III)· kg\(^{-1}\) significantly increased the liver Cu level by 16% (27.15 ± 0.70 μg· g\(^{-1}\) d.m.) as compared with the control rats (23.42 ± 0.70 μg· g\(^{-1}\) d.m.). High doses of Cr(III) (500 and 1000 mg· kg\(^{-1}\)) increased the spleen Cu content by 48% (14.90 ± 0.52 μg· g\(^{-1}\) d.m.) and 53% (15.43 ± 0.57 μg· g\(^{-1}\) d.m.), respectively vs. control group (10.10 ± 0.70 μg· g\(^{-1}\) d.m.) (Table 3).

Supplementary Cr3 did not affect the liver and spleen Zn contents. However, in comparison with the control group (110.1 ± 4.9 μg· g\(^{-1}\) d.m.), the diets supplemented with 500 and 1000 mg Cr(III)· kg\(^{-1}\) significantly increased the kidney Zn level, by 29% (141.7 ± 5.5 μg· g\(^{-1}\) d.m.) and 21% (132.8 ± 3.6 μg· g\(^{-1}\) d.m.), respectively. The effect of supplementary Cr3 on Fe metabolism was assessed on the basis of morphological and haematological blood indices, such as haemoglobin concentration (Hb), haematocrit ratio (HCT), the number of erythrocytes in the blood (RBC), mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) (the data were presented in our previous paper [27]), serum Fe concentration and tissular Fe content (liver, kidney and spleen) (Table 3). The haematological indices under analysis in the rats supplemented with Cr3 were not significantly different from the control rats [27]. However, the highest dose of Cr 1000 mg kg\(^{-1}\) (100 mg· kg\(^{-1}\) b.m.) decreased the serum Fe concentration by 28%. As far as the tissular Fe levels are concerned, the Cr doses of 500 and 1000 mg· kg\(^{-1}\) significantly reduced the liver Fe content by 20% (1002 ± 58 μg· g\(^{-1}\) d.m.) and 21% (1020 ± 54 μg· g\(^{-1}\) d.m.), respectively, and the

| Element | Number of samples (n) | Certified value (μg· g\(^{-1}\)) | Analytical value (μg· g\(^{-1}\)) | Method accuracy [% certified value] |
|---------|----------------------|----------------------------------|-----------------------------------|-----------------------------------|
| Ca      | 6                    | 295 ± 2                          | 291.13 ± 7.46                    | 98.7                              |
| Mg      | 6                    | 830 ± 8                          | 843.80 ± 11.25                   | 101.7                             |
| Fe      | 6                    | 299 ± 2                          | 295.96 ± 11.53                   | 99.0                              |
| Zn      | 6                    | 128 ± 3                          | 123.60 ± 1.52                    | 96.6                              |
| Cu      | 6                    | 31.9 ± 0.4                       | 31.87 ± 0.20                     | 99.9                              |
| Cr      | 6                    | 1.87 ± 0.16                      | 1.81 ± 0.14                      | 96.9                              |

Table 2 The accuracy of the method of determination of elements (mean ± SD)
kidney Fe level by 18% (402 ± 19 μg·g⁻¹·d.m.) and 24% (375 ± 13 μg·g⁻¹·d.m.), respectively. No effects on the spleen Fe content were noted.

There were no significant differences in the tissular Mg levels of healthy female rats fed diets with Cr3 at doses of 100 to 1000 mg of Cr·kg⁻¹.

Fig. 1 Chromium contents in tissues after Cr3 supplementation at doses 100, 200, 500 and 1000 mg Cr·kg⁻¹·d.m. for 4 weeks in female Wistar rats, a liver, b kidney, c spleen, (microgram per gram dry mass). Different letter superscripts indicate statistically significant differences at p < 0.05. Control group, A—group supplemented with 100 mg Cr(III)·kg⁻¹·diet, B—group supplemented with 200 mg Cr(III)·kg⁻¹·diet, C—group supplemented with 500 mg Cr(III)·kg⁻¹·diet and D—group supplemented with 1000 mg Cr(III)·kg⁻¹·diet.
The kidney and spleen Ca levels in the groups supplemented with Cr3 were not different than in the control group. However, supplementary Cr3 decreased the liver Ca level in a dose-dependent manner. The addition of Cr(III) at a dose of 100 mg·kg⁻¹ to the diet had no effect. However, in comparison with the control group (49.8 ± 1.4 μg·g⁻¹·d.m.), the doses of 200, 500 and 1000 mg·kg⁻¹ significantly increased the liver Ca level, by 49, 59 and 68%, respectively.

### Discussion

Our previous studies showed that Cr3 was a relatively safe compound [18, 28, 29].

Publications concerning Cr are related to the transport, distribution and bioactivities of this element from various Cr compounds in different biological models.

The effects of the nutritional supplement Cr3 on healthy rats and rat models of insulin resistance and type 1 and 2 diabetes have been examined [4, 17, 27, 30–34]. Some reports have shown that chromium(III) has beneficial effects for the organism with disturbances of glucose and lipid metabolism [4, 17, 31]. However, data from the experiments conducted in healthy individuals quite often explicitly show lack of any favourable impact of Cr(III) on carbohydrates/lipid metabolism [27, 32–34]. Our study [27] showed no effect of Cr3 at doses 100–1000 mg of Cr(III)·kg⁻¹ diet on serum glucose, total cholesterol, LDL-cholesterol, and HDL-cholesterol concentration (Table 4).

Bennett et al. [34] found that Cr3 at doses of 1, 5 and 10 mg Cr·kg⁻¹ lowered plasma insulin, leptin and triglycerides concentrations but had no effect on plasma HDL, LDL and total cholesterol after 10 weeks of treatment in male Sprague-Dawley rats. Healthy Sprague-Dawley rats treated daily with...
20 μg·kg⁻¹ body mass as Cr3 intravenously for 12 weeks had lower blood plasma insulin, total cholesterol, LDL, HDL and triglycerides but not glucose levels [31]. The data obtained by Herring et al. [32] strongly suggest that long-term (15-month) Cr3 supplementation does not significantly affect metabolic responses in blood glucose concentration to glucose and insulin in male Wistar rats consuming a normal diet or high-fat, high-carbohydrate cafeteria-style diet. Also Król et al. [33] confirmed that supplementary Cr3, given in the dosages 0.6 and 3 mg·kg⁻¹ b.m. for 8 weeks, did not affect serum glucose, insulin and HOMA-IR index and serum lipid indices, except TAG (tended to decrease) in rats fed high-fat diet.

Unfortunately, the molecular mechanism by which chromium affects glucose and lipid metabolism is still unclear. This turn leads to the hypothesis that chromium ion supplementation has been beneficial only in disorders of glucose and lipid metabolism [35]. Most of the available literature exploring the effects of chromium supplementation in rats have been short-term studies [4, 25, 27–29, 36, 37]. Few have looked at the effect of long-term chromium supplementation [17, 31, 38, 39]. For the other roles of chromium in the body, we should use this supplement in a reasonable manner, being aware its possible side-effects.

However, there is little data on the influence of Cr on the mineral status in healthy individuals, particularly at pharmacological levels. Dietary supplementation with high doses of Cr(III) may disorder the status of other elements.

The accumulation of Cr in the liver and kidneys of rats receiving supplementary Cr3 was observed, but the results depended on the chemical form and dose of Cr. The Cr content in the liver, kidneys and spleen was found to increase in a manner dependent on the supply of Cr(III) in the diet. Other authors found similar relationships between the intake of Cr(III) and its concentration in the liver and kidney tissues in normal rats [40–42], in rats with diabetes mellitus type 1 and 2 [17, 30], in pig [43] and in quails [44].

Lindemann et al. [45] demonstrated differences between Cr sources in Cr concentrations in various tissues. In a study conducted by Yoshida et al. [37], the different effects of Cr on Cr concentration in the liver, kidney and femur were the result of the dietary Cr level (1, 10, 100 μg·g⁻¹ diet) than chemical form (CrPic vs. CrCl3). However, the greatest Cr concentration was found in kidneys, which was also confirmed in our study. Wang et al. [46] also reported that supplementary Cr at a dose of 200 μg·kg⁻¹ from CrNano and CrPic for 40 days increased the blood Cr level, Cr content in the liver, kidneys and heart as well as faeces and urine in finishing pigs. Different results were obtained by Clodfelder et al. [4], who found that 24-week supplementation with Cr3 at doses ranging from 250 to 1000 μg·Cr·kg⁻¹ diet did not increase the content of Cr in the liver and kidneys in healthy rats or the animals with type 2 diabetes. It is assumed that the absorption and utilisation of Cr depend on its status in the gastrointestinal tract [46].

Some authors [47–49] indicated that prolonged supplementation with Cr(III) compounds may have negative impact on the metabolism of Fe due to the fact that Cr and Fe are bound with the same protein—transferrin. Human serum transferrin (Tf) is the iron transport protein responsible for delivering iron and a variety of other metals to cells [50]. This protein consists of two almost identical lobes, referred to as the C-lobe and the N-lobe, which can bind one metal ion each [51–53]. It was found that Fe³⁺ binding to the C-lobe is approximately 20 times stronger than Fe³⁺ binding to the N-lobe. Trivalent chromium (Cr³⁺) same for iron (Fe³⁺) typically binds to the C-lobe first, followed by loading into the N-lobe. Under normal conditions, only approximately 30% of the potential Fe³⁺ binding sites in Tf are occupied, leaving the unoccupied binding sites in either the C-lobe or the N-lobe, or both, to potentially bind other metal ions [51, 52]. Cr³⁺, Cu²⁺, Zn²⁺, Al³⁺, Ga³⁺, Ni²⁺ and Ti⁴⁺ are known to bind to Tf, and these metal ions could either compete with Fe³⁺ for Tf coordination or bind to the unoccupied lobes of Tf [54]. Cr³⁺ preferentially binds to the C-lobe of Tf, suggesting that it has the potential to compete with Fe³⁺ for that binding pocket. When saturation of transferrin with iron increases to over 50%, iron competes with chromium binding, affecting its transport [55]. The relationship between iron and chromium metabolism needs to be further investigated. It is not yet clear if chromium decreases iron absorption or if it is also involved in the downregulation of iron absorption [55]. It is also possible that exposure to high doses of Cr causes Cr³⁺ to bind to Tf and interferes with normal iron uptake, thus affecting Fe metabolism [1].

In our study, the blood morphological indices remained unchanged, which indicates that Cr3 did not affect erythropoiesis [27]. A lower Fe level in the serum and tissue stores (in the liver and kidneys) was noted, but these changes occurred only after the application of very high doses of Cr(III) (50 and 100 mg Cr(III)·kg⁻¹ b.m.). These results correspond to the study by Anderson et al. [47], who found a reduced Fe level in the tissues of rats supplemented with CrCl3. Also, Ani et al. [48] reported reduced transferrin saturation and tissue stores of Fe as well as lower haemoglobin and haematocrit index in rats fed a diet with a high dose of Cr(III).

Sun et al. [17] found that Cr3 administered to normal Sprague-Dawley (SD) rats at a dose of 20 μg·kg⁻¹ b.m. for 24 weeks did not have a significant effect on the liver and kidney Fe contents. However, they observed higher liver Fe
level and lower kidney Fe concentrations than their controls in Zucker obese (ZKO) rats [17]. Also, Love et al. [56] observed that the dietary Cr (16–2000 μg· kg⁻¹ diet) given for 23 weeks had no effect on the blood iron level in Zucker lean (ZKL) rats. Similarly, Clodfelder et al. [4] did not observe adverse effects of Cr3 on the Fe status when given to healthy rats or to the animals with type 2 diabetes for 24 weeks as an aqueous solution at doses of 250–1000 μg of Cr· kg⁻¹ b.m. In contrast, Cr3 supplementary in doses 10 and 50 mg· kg⁻¹ diet for 8 weeks increased kidney Fe and spleen Cu contents but did not affect Zn status in rats fed with high-fat diet [33]. The studies by Shara et al. [38, 39] demonstrated that long-term supplementation with complex Cr(III) with niacin did not affect the metabolism of Fe in the rat (as assessed by the content of Fe in the serum, TIBC, RBC, haemoglobin and selected indicators of blood morphology). Prescha et al. [36] reported that supplementary Cr in a diet enriched with cellulose and/or pectin led to higher Cr and Fe contents in the femurs but did not change the Fe content in the liver, kidneys and muscles in male Buffaló rats.

Research on humans supplemented with Cr(III) at doses of 200–1000 μg· day⁻¹ did not confirm significant effects on the Fe status. Campbell et al. [57] showed that 3-month supplementation of males aged 60–70 years with Cr(Pic)₃ at an amount of 924 μg of Cr· day⁻¹ had no effect on the Fe status. Similarly, Lukaski et al. [58] did not observe any changes in haemoglobin, haematocrit, serum Fe content, TIBC or transferrin saturation in 83 premenopausal women supplemented with Cr(Pic)₃ at a dose of 200 μg of Cr· day⁻¹.

Volpe et al. [59] found that 12-week supplementation with Cr(Pic)₃ at a dose of 400 μg· day⁻¹ combined with the exercise programme did not affect Fe or Zn levels in the serum of women with moderate obesity.

Anderson et al. [43] studied the effect of Cr(Pic)₃ supplementation on the tissular levels of Fe, Zn and Cu in pigs. They found that Cr did not affect the level of these elements in the liver and heart but caused an increase in the Fe content and decrease in Zn and Cu contents in the kidneys.

Pechova et al. [60] observed that the addition of Cr(III) in the form of chromium yeast (5 mg· day⁻¹ at the initial period of the experiment and increased to 8 mg· day⁻¹ after 136 days) increased the Cu content, reduced the Mg and P levels in the serum, but had no effect on the plasma Zn concentration in young bulls.

Amatya et al. [61] conducted a study on broilers fed diets supplemented with 200 μg· kg⁻¹ in the form of CrCl₃ and yeast chromium for 21 and 35 days. They found that the Cr level in the liver was lower than in the control group, the Cu content increased in the blood serum and liver, while the Fe and Mn content decreased in the liver.

Débski et al. [62] reported an increase in the Cr and Cu content, but no change in the Zn level in the livers of hens fed a diet enriched with chromium yeast (0.5 mg· kg⁻¹ dry mass) for 2 months.

Sahin et al. [44] showed that supplementary Cr(Pic)₃ (200, 400, 800, 1200 μg· kg⁻¹) increased the Cr and Zn content but decreased the Cu level in the serum, liver, kidneys and muscles. The concentrations of Fe and Mg did not change in these tissues as dietary chromium supplementation was increased in the Japanese quail. Krejpcio et al. [63] found that supplementation with Cr3 (5 mg· kg⁻¹ of diet) did not affect Mg levels in Wistar rats. Król et al. [64] showed that Cr3 supplementation disturb mineral homeostasis in the rats’ organs fed high-fructose diet. Cr3 increased Mg, Cu and Cr levels, although it did not influence tissular Ca, Fe and Zn contents, given for 4 weeks in doses 1 and 5 mg· kg⁻¹ b.w. per day.

The addition of Cr(III) to the fibre-free diet and to the diets with cellulose or pectin did not change the Zn, Mg and P contents in the femur and Cr, Fe, and Zn levels in the muscles in rats [36]. However, the addition of pectin or cellulose to the diets, especially with Cr, increased the Zn content in the liver and kidneys and changed the Mg and Ca levels in these tissues [36].

Dorukan et al. [13] reported that supplementation with chromium histidinate (CrHis) increased the serum, liver, kidney Cr and Zn contents but decreased the Cu levels both in diabetic and non-diabetic rats. However, the serum, liver and kidney Fe concentrations were unchanged. In our previous

---

**Table 4**  The effect high dietary doses of Cr3 on blood glucose concentration and lipid profile indices in female rat (mean ± SEM) [27]

| Index                        | Control (1 mg· kg⁻¹) | A (100 mg· kg⁻¹) | B (200 mg· kg⁻¹) | C (500 mg· kg⁻¹) | D (1000 mg· kg⁻¹) |
|------------------------------|----------------------|------------------|------------------|------------------|------------------|
| Glucose concentration (mg dl⁻¹) | 105.7 ± 4.9          | 98.7 ± 8.6       | 98.8 ± 4.1       | 92.5 ± 2.5       | 94.8 ± 3.9       |
| Total-cholesterol concentration (mg dl⁻¹) | 61.5 ± 8.4          | 72.2 ± 2.8       | 67.0 ± 5.5       | 63.3 ± 4.6       | 64.3 ± 2.6       |
| LDL-cholesterol concentration (mg dl⁻¹) | 4.67 ± 0.49         | 5.17 ± 0.54      | 5.17 ± 0.54      | 5.00 ± 0.26      | 5.17 ± 0.87      |
| HDL-cholesterol concentration (mg dl⁻¹) | 39.8 ± 3.6          | 41.5 ± 1.3       | 39.8 ± 1.8       | 37.2 ± 3.5       | 38.8 ± 1.0       |
| TAG-triglycerides concentration (mg dl⁻¹) | 30.7 ± 1.9ab        | 32.2 ± 2.8ab     | 37.0 ± 3.0b      | 25.2 ± 2.4a      | 25.3 ± 2.6a      |

The values in the same row that do not share the same superscript letter are significantly different (P < 0.05).

*Control* control group, *A* group supplemented with 100 mg Cr(III)· kg⁻¹ diet, *B* group supplemented with 200 mg Cr(III)· kg⁻¹ diet, *C* group supplemented with 500 mg Cr(III)· kg⁻¹ diet, *D* group supplemented with 1000 mg Cr(III)· kg⁻¹ diet.
The role of Cr in the Ca of bone metabolism is unclear. CrPic₃ has been found to reduce the urinary excretion of hydroxyproline and Ca in postmenopausal women, presumably indicating a reduced rate of bone resorption [65]. Evans et al. [65] observed reduced Ca excretion, an increased dehydroepiandrosterone level (DHEA) and reduced hydroxyproline to creatinine ratio in the urine of postmenopausal women after 60-day supplementation with Cr(Pic)₃ at a dose of 200 μg·day⁻¹, suggesting that Cr(III) could effectively prevent osteoporosis.

In other studies, the liver Ca content in lean rats was significantly increased by Cr₃ and CrPicon when administered at 1 mg Cr·kg⁻¹·b.m., but the Mg level was not affected [25]. Prescha et al. [36] showed that the liver Ca content was very sensitive to supplemental Cr when given together with pectin and cellulose to Buffalo rats.

This study showed that high doses of supplementary Cr₃ (100–1000 mg Cr(III)·kg⁻¹·diet) reduced the Ca content in the rat liver in a dose-dependent manner. The research by Sankaramanivela et al. [66] revealed that the exposure of male Wistar rats to K₂Cr₂O₇ at a dose of 0.5 mg·kg⁻¹·b.m. for 5 days increased the Ca content in the femur and cranial vault but reduced the activity of ALP and TRAP (resistant acid phosphatase tartrate), which the authors explained with low Ca resorption activity from the bone tissue.

The results of this experiment suggest that supplementary doses of Cr₃ (100–1000 mg Cr·kg⁻¹·diet; eq. ~10–100 mg Cr·kg⁻¹·b.m.) given for 4 weeks did not affect the Mg status but influenced the Cr, Fe, Zn, Cu and Ca levels in healthy female Wistar rats.

In conclusion, high dietary Cr₃ supplementation may affect the mineral balance in rat tissues.

**Compliance with Ethical Standards** The experimental protocol was approved by the Local Bioethical Commission in Poznań (No. 12/2005).

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**References**

1. Cefalu WT, Hu FB (2004) Role of chromium in human health and in diabetes. Diabetes Care 27:2741–2751. doi:10.2337/diacare.27.11.2741
2. Tang H, Xiao Q, Xu H, Zang Y (2015) Hypoglycemic activity and acute oral toxicity of chromium methionine complexes in mice. J Trace Elem Med Biol 29:136–144. doi:10.1016/j.jtemb.2014.07.001
3. Sharma S, Agrawal RP, Choudhary M, Jain S, Goyal S, Agarwal V (2011) Beneficial effect of chromium supplementation on glucose, HbA1C and lipid variables in individuals with newly onset type-2 diabetes. J Trace Elem Med Biol 25:149–153. doi:10.1016/j.jtemb.2011.03.003
4. Clodfelder BJ, Gullick BM, Lukaski HC, Neegers Y, Vincent JB (2005) Oral administration of the biomimetic [Cr₃(O₂CCH₂CH₃)₃(H₂O)]⁶⁺ increases insulin sensitivity and improves blood plasma variables in healthy and type 2 diabetic rats. J Biol Inorg Chem 10:119–130. doi:10.1007/s00775-004-0618-0
5. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2014) Scientific opinion on dietary reference values for chromium: dietary reference values for chromium. EFSA J 12(10):3845. doi: 10.2903/j.efsa.2014.3845
6. Tinkov AA, Popova EV, Polyakov SA, Kwan OV, Salny AV, Nikonov AA (2015) Adipose tissue chromium and vanadium disbalance in high-fat fed Wistar rats. J Trace Elem Med Biol 29:176–181. doi:10.1016/j.jtemb.2014.07.006
7. Vincent JB, Love ST (2012a) The need for combined inorganic, biochemical, and nutritional studies of chromium (III). Chemistry & Biodiversity 9:2923–1941. doi:10.1002/cbdv.201100440
8. Institute of Medicine (U.S.) (2002) Dietary reference intakes for: vitamin a, vitamin K, arsenic, boron, chromium, copper, iodine, iron, molybdenum, nickel, silicon, vanadium and zinc. In: A report of the panel of micronutrients, subcommittee on upper reference levels of nutrient and interpretations and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes. National Academy of Press, Washington DC.
9. Sirirat N, Lu J-H, Hung AT-Y, Chen S-Y, Lien T-F (2012) Effects of different levels of nanoparticles chromium picolinate supplementaion on growth performance, mineral retention, and immune responses in broiler chickens. J Agricul Sci 4(12):48–58. doi:10.1016/j.jinorgbio.2005.07.017
10. Laschinsky N, Kottwitz K, Freund B, Dresow B, Fischer R, Nielsen P (2012) Bioavailability of chromium (III)—supplements in rats and humans. Biometals 25:1051–1060. doi:10.1007/s10525-012-9571-5
11. Shara M, Yasin T, Kincaid AE, Limpach AL, Bartz J, Brennan KA, Chatterjee A, Bagchi M, Stohs SJ, Bagchi D (2005a) Safety and toxicological evaluation of a novel niacin-bound chromium (III) complex. J Inorg Biochem 99:2161–2183. doi:10.1016/j.jinorgbio.2005.07.017
12. Shara M, Kincaid AE, Limpach AL, Sandstrom R, Barrett L, Norton N, Bramble JD, Yasin T, Tran J, Chatterjee A, Bagchi M, Bagchi D (2007a) Long-term safety evaluation of a novel oxygen-coordinated niacin-bound chromium (III) complex. J Inorg Biochem 101:1059–1069. doi:10.1016/j.jinorgbio.2007.03.015
13. Dogukan A, Sahin N, Tuzcu M, Juturu V, Onderci M, Komorowski J, Sahin K (2009) The effects of chromium histidinate on mineral status of serum and tissue in fat-fed and streptozotocin-treated type II diabetic rats. Biol Trace Elem Res 131:124–132. doi:10.1007/s12001-009-8251-8
14. Tuzcu M, Sahin N, Orhan C, Ali Agca C, Akdmir F, Tuzcu Z, Komorowski J, Sahin K (2011) Impact of chromium histidinate on high fat diet induced obesity in rats. Nutrition & Matabolism 8(28):1–8. doi:10.1186/1743-7075-8-28
15. Yang X, Palanichamy K, Onkto AC, Rao MN, Fang CX, Ren J, Sreejayan N (2005) A newly synthetic chromium complex-chromium(phenylalanine) improves insulin responsiveness and reduces whole body glucose tolerance. FEBS Lett 579(6):1458–1464. doi:10.1016/j.febslet.2005.01.049
16. Yang X, Li SY, Dong F, Ren J, Sreejayan N (2006) Insulin-sensitizing and cholesterol-lowering effects of chromium (\(\text{Cr}_{\text{III}}\)-glycinate), J Inorg Biochem 100:1187–1193. doi:10.1016/j.jinorgbio.2006.01.039

17. Sun Y, Clodfelder BJ, Shute AA, Irvin T, Vincent JB (2002) The biomimetic \([\text{Cr}_3\text{O} (\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^{+}\) decreases plasma insulin, cholesterol and triglycerides in healthy and type II diabetic rats but not type I diabetic rats. J Biol Inorg Chem 7:852–862. doi:10.1007/s00775-002-0366-y

18. Staniek H, Krejpcio Z, Iwanik K (2010a) Evaluation of the acute oral toxicity class of trinuclear complex in rat. Food Chem Toxicol 48(3):859–864. doi:10.1016/j.fct.2009.12.022

19. Staniek H, Krejpcio Z, Iwanik K, Szymusiak H, Wieczorek D (2011) Evaluation of the acute oral toxicity class of trinuclear chromium (III) glycinate complex in rat. Biol Trace Elem Res 143(3):1564–1575. doi:10.1007/s111-011-8959-3

20. Li F, Wu X, Zou Y, Zhao T, Feng W, Yang L (2012) Chromium (III) propionate complex supplementation on pregnancy outcome and maternal and foetal mineral status in rat. Food Chem Toxicol 50:1623–1631. doi:10.1016/j.fct.2012.02.012

21. Institute of Medicine (2001) Dietary references intake for vitamin a, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc, National Academy Press, Washington, DC ISBN 10: 0-309-07279-4, DOI:10.17226/10026

22. Komorowski JR, Greengerg D, Juturu V (2008) Chromium picolinate does not produce chromosome damage. Toxicol in Vitro 22:232–234. doi:10.1016/j.tiv.2007.12.007

23. Stout MD, Nyska A, Collins BJ, Witt KL, Kissling GE, Malarkey DE, Hooth MJ (2009) Chronic toxicity and carcinogenicity studies of chromium picolinate monohydrate administered in feed to F344/N rats and B6C3F1 mice for 2 years. Food Chem Toxicol 47(4):729–733. doi:10.1016/j.fct.2009.01.006

24. Hepburn DD, Vincent JB (2003) Tissue and subcellular distribution of chromium picolinate with time after entering the bloodstream. J Inorg Biochem 94:86–93. doi:10.1016/s0162-0134(02)00623-2

25. Staniek H, Rhodes NR, Di Bona KR, Deng G, Love ST, Pledger LA, Blount J, Gomberg E, Grappe F, Cernosek C, Peoples B, Rasco JF, Krejpcio Z, Vincent JB (2013) Comparison of tissue metal concentrations in Zucker lean, Zucker obese, and Zucker diabetic fatty (III) propionate complex supplementation on pregnancy outcome and maternal and foetal mineral status in rat. Food Chem Toxicol 50:1623–1631. doi:10.1016/j.fct.2012.02.012

26. Earnshaw A, Figgis BN, Lewis J (1966) Chemistry of polynuclear compounds. Part VI. Magnetic properties of trinuclear chromium and iron carboxylates. J Chem Soc A:1656–1663

27. Staniek H, Krejpcio Z, Wieczorek D (2016) The effects of high chromium concentrations in Zucker lean, Zucker obese, and Zucker diabetic fatty rats. J Biol Inorg Chem 48:3–8. doi:10.1016/s01062-0134(02)00382-3

28. Staniek H, Kostrzewska-Poczekaj M, Arndt M, Szyfter K, Krejpcio Z (2005) Genotoxic assessment of chromium (III) propionate complex in the rat model using the comet assay. Food Chem Toxicol 43(4):859–864. doi:10.1016/j.fct.2005.07.017

29. Lindemann MD, Cromwell GL, Monegque HJ, Purser KW (2008) Effects of chromium source on tissue chromium concentrations and body mass in control and somatotropin-treated pigs are associated with increased chromium in adult rats from the bloodstream to the urine. J Biol Inorg Chem 15(3):419–430. doi:10.1007/s00775-005-0647-3

30. Wang M-Q, Li H, He Y-D, Wang C, Tao W-J, Du Y-J (2012) Efficacy of dietary chromium(III) supplementation on tissue chromium deposition in finishing pigs. Biol Trace Elem Res 148:316–321. doi:10.1007/s111-011-9369-x

31. Anderson RA, Bryden NA, Polansky MN, Gaulscht K (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rat. J Trace Elem Exp Med 9:11–25. doi:10.1002/jtme.10103

32. Bennett R, Adams B, Fretch A, Neggars Y, Vincent JB (2006) High-dose chromium (III) supplementation has no effects on body mass and composition while altering plasma hormone and triglycerides concentrations. Biol Trace Elem Res 113:53–66. doi:10.1385/BTER:113:1:53

33. Król E, Krejpcio Z, Iwanik K (2014) Supplementary chromium(III) propionate complex does not protect against insulin resistance in high-fat-fed diet. J Biol Trace Elem Res 157:147–155. doi:10.1007/s12011-013-9877-3

34. Shara M, Yasin T, Kincaid AE, Limpach AL, Sandstrom R, Barrett L, Norton N, Bramble JD, Yasmin T, Tran J, Chatterjee A, Bagchi M, Bagchi D (2005a) Safety and toxicological evaluation of a novel niacin-bound chromium (III) complex. J Inorg Biochem 99:2141–2183. doi:10.1016/j.jinorgbio.2005.07.017

35. Yoshida M, Hatakeyama M, Kosai K, Sashiyama T, Fukunaga K (2010) Tissue accumulation and urinary excretion of chromium in rats fed diet containing graded levels of chromium chloride or chromium picolinate. J Toxicol Sci 34(4):485–491. doi:10.2131/jts.2010-2109

36. Anderson RA, Bryden NA, Evock-Clover CM, Steele NC (1997) Beneficial effects of chromium on glucose and lipid variables in Japanese quails. J Trace Elem Exp Med 15:13–16. doi:10.1002/jtme.10103

37. Lindemann MD, Cromwell GL, Monegque HJ, Purser KW (2008) Effect of chromium source on tissue concentration of chromium in pigs. J Anim Sci 86:2971–2978. doi:10.2527/jas.2008-0888

38. Wang M-Q, Li H, He Y-D, Wang C, Tao W-J, Du Y-J (2012) Efficacy of dietary chromium(III) supplementation on tissue chromium deposition in finishing pigs. Biol Trace Elem Res 148:316–321. doi:10.1007/s111-011-9369-x

39. Anderson RA, Bryden NA, Polansky MN, Gaulscht K (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rat. J Trace Elem Exp Med 9:11–25. doi:
Ani M, Moshtaghie AA (1992) The effect of chromium on parameters related to iron metabolism. Biol Trace Elem Res 32:57–64 PMID: 137807

Preuss HG, Anderson RA (1998) Chromium uptake: examining recent literature 1997-1998. Curr Opin Clin Nutr Metab Care 1(6):509–512 PMID: 10565402

Chua AC, Graham RM, Trinder D, Olynyk JK (2007) The regulation of cellular iron metabolism. Crit Rev Clin Lab Sci 44:413–459. doi:10.1080/10408360701428257

Quarles CD, Brumaghim JL, Marcus RK (2010) Instrumental comparison of the determination of Cr3+ uptake by human transferrin. Metallomics 2:792–799. doi:10.1039/c0mt00032a

Quarles CD, Marcus RK, Brumaghim JL (2011) Competitive binding of Fe3+, Cr3+, and Ni2+ to transferrin. J Biol Inorg Chem 16:913–921. doi:10.1007/s00775-011-0792-9

Vincent JB, Love S (2012b) The binding and transport of alternative metals by transferring. BBA 1820:362–378. doi:10.1016/j.bbagen.2011.07.003

Tinoco AD, Valentine AM (2005) Ti (IV) binds to human serum transferrin more tightly than does Fe (III). J Am Chem Soc 127:11218–11219. doi:10.1021/ja052768v

Terpiłowska S, Siwicki AK (2012) The influence of chromium and iron on interleukin-1x and interleukin-6 concentration in vitro and in vivo. Centr Eur J Immunol 37(2):106–109 file:///C:/Users/user/Downloads/CEJIOI_Art_18635-10%20(1).pdf

Love ST, Di Bona KR, Shinha SH, McAdory D, Skinner BR, Rasco JF, Vincent JB (2013) Urinary chromium excretion in response to an insulin challenge is not a biomarker for chromium status. Biol Trace Elem Res 152:57–65. doi:10.1007/s12011-012-9594-3

Campbell WW, Beard JL, Joseph LJ, Evans WJ (1997) Chromium picolinate supplementation and resistive training by older men: effects on iron-status and hematologic indexes. Am J Clin Nutr 66(4):944–949 PMID: 9322572

Lukasi HC, Siders WA, Penland JG (2007) Chromium picolinate supplementation in women: effects on body weight, composition and iron status. Nutrition 23:187–195. doi:10.1016/j.nut.2006.12.001

Volpe SL, Huang HW, Larpadisorn K, Lesser II (2001) Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. J Am Coll Nutr 20(4):293–306 PMID: 11506057

Pečová A, Íilek J, Šindelar M, Pavlata L (2002) Effects of chromium supplementation on growth rate and metabolism in fattening bulls. Acta Vet Brno 71:535–541. doi:10.2754/avb200271010009

Amatya JL, Haldar S, Ghosh TK (2004) Effects of chromium supplementation from inorganic and organic sources on nutrient utilization, mineral metabolism and meat quality in broiler chickens exposed to natural heat stress. Anim Sci 79:241–253. doi:10.1017/s135772980009010x

Dębski B, Zalewski W, Gralak MA, Kosla T (2004) Chromium-yeast supplementation of chicken broilers in an industrial farming system. J Trace Elem Med Biol 18(1):47–51. doi:10.1016/j.jtremb.2004.02.003

Krejpcio Z, Wójciak RW, Staniek H, Wisniewska J (2009) Effect of dietary supplementation with inulin-type fructans and chromium(III) on magnesium metabolic indices in rat. Zywosc Nauka Technologia Jakosc 4(65):175–182 (in polish) pttz.org/zyw/wyd/czas/2009,%204(65)/22_Krejpcio.pdf

Król E, Krejpcio Z (2013) Dietary chromium(III) propionate complex supplementation affects tissue mineral levels in rats fed high-fructose diet. J Elem 18(1):91–98. doi:10.5601/jelem.2013.18.1.07

Burckhardt P, Dawson-Hughes B, Heaney RP (2004) Aspects of osteoporosis, Second edn. Elsevier Academic Press, Amsterdam, Boston, pp 141–151 ISBN 0-12-14704-2

Sankaramanivel S, Jeyapriya R, Hemalatha D, Djody S, Arunakaran J, Srinivasan N (2006) Effect of chromium on vertebral, femur and calvaria of adult male rats. Hum Exp Med 25:311–318. doi:10.1191/0960327105ht627oa