







Effect of Chromium Picolinate and Chromium Nanoparticles Added to Low- or High-Fat Diets on Chromium Biodistribution and the Blood Level of Selected Minerals in Rats

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Key words: minerals, chromium, nanoparticles, high-fat diet

The metabolism of chromium (Cr), calcium (Ca), phosphorus (P), iron (Fe), copper (Cu), and zinc (Zn) is interconnected, and their deficiency or excessive accumulation may lead to various disturbances, including anemia and diabetes. The current research was undertaken to determine whether low-fat or high-fat diets with the Cr(III) addition in the form of picolinate (CrPic) or nanoparticles (CrNPs) have an interactive effect on the retention and accumulation of this element in organs and the content of P, Ca, Fe, Cu and Zn in the blood plasma of rats. The experiment was performed using 48 outbred male Wistar rats fed a low-fat or high-fat semi-purified rat diet with dietary addition of chromium at a dose of 0.3 mg/kg body weight. The obtained results point to the paramount importance of the dietary Cr form on the excretion pattern of this microelement. It has been found that CrNPs were to a greater extent excreted from the rat's body *via* urine and feces in comparison to CrPic, as indicated by the values of the Cr retention index (44.4 vs. 65.9%, respectively). The additional dietary Cr, irrespective of its form and diet type, was not accumulated in the analyzed internal organs, *i.e.* brain, spleen, kidneys, liver, thigh bone, and thigh muscle. It should be stressed that dietary CrPic, unlike CrNPs, added to the high-fat diet adversely reduced plasma concentration of vital minerals in comparison to the levels observed in rats fed the low-fat diet, *i.e.* Zn (60.5 vs. 69.9 μM), Cu (13.6 vs. 15.7 μM), and P (1.12 vs. 1.30 μM). In turn, the CrNPs, but not CrPic, added to the high-fat diet decreased plasma Fe level (1.41 vs. 2.43 μM).

INTRODUCTION

The available literature provides ample evidence that trivalent chromium (Cr(III)) is an important micromineral actively involved in the metabolic fate of carbohydrates, lipids, and proteins [Dworzański *et al.*, 2020, 2021; Inanç *et al.*, 2006; Ognik *et al.*, 2021; Sahin *et al.*, 2011; Suksomboon *et al.*, 2014; Tuzcu *et al.*, 2011]. On the other hand, there are many unanswered questions about Cr(III) supplementation as there are also articles questioning its importance [Stearns, 2000]. Chromium's advantage is that its safety was confirmed by no detrimental health consequences of dietary inclusion of trivalent Cr in animal experiments [Pechova & Pavlata, 2007]. In the released opinion of the European Food Safety Authority (EFSA), Cr(III) should not be considered as an essential nutrient [EFSA, 2014], but it is currently deemed a stock supplementary ingredient.

The intestinal tract is the main route of Cr(III) entering the internal tissues. In rodents, its most active absorption processes occur in the jejunum; while its absorption is less effective in the ileum and duodenum [Pechova & Pavlata, 2007]. Chromium is absorbed from the intestine to a little extent, with the absorption rate ranging from 0.4% to 2.0%. The retention rate of Cr from inorganic and organic species has been reported at less than 3% for the former and ten times higher for the latter [Lyons, 1994]. It should be stressed that the literature *in vivo* data describing the Cr accumulation in internal tissues and organs is relatively scarce [Anderson *et al.*, 1996; Chang *et al.*, 1992; Lindemann *et al.*, 2008; Şahin *et al.*, 2001; Staniek *et al.*, 2013].

High blood insulin level was shown as a diminishing factor in regard to Cr cycled in the body as chromium is incorporated to insulin-dependent cells *via* the low molecular weight chromium-binding substance (LMWCr) [Davis, 1997].

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Submitted: 30 May 2022

Accepted: 30 June 2022

Published on-line: 27 July 2022



Król *et al.* [2014] found that the administration of a high-fat diet caused elevated insulin levels in rat serum, regardless of Cr dietary application (applied doses of 10 or 50 mg Cr propionate per kg diet). It is worth noting that hepatic Cr accumulation may be enhanced under conditions of insulin-related disturbances. Studies on Cr distribution in rat tissues have demonstrated its highest content in the kidneys [Prescha *et al.*, 2014]. Previous studies addressing the impact of dietary Cr on tissue levels of other mineral components have shown a significant interaction of Cr with Fe [Anderson *et al.*, 1996; Lindemann *et al.*, 2008; Yoshida *et al.*, 2010]. Dietary Cr(III) has been reported to affect the metabolism of other macro- and micro-elements, for instance increased Zn level and diminished that of Cu [Vlizlo *et al.*, 2014]. The maintenance of the right, balanced physiological status of trace minerals, *i.e.* Fe, Zn, Cu, and Cr, seems to be of paramount importance. Undoubtedly, the metabolic fate of Fe, Cr, Cu, and Zn is mutually dependent and the body's mineral status and may greatly affect the occurrence of several physiological and metabolic disturbances, *e.g.* diabetes, anemia, or insulin-resistance.

The current research was undertaken to determine whether low-fat or high-fat diets with the addition of Cr in the form of chromium picolinate or nanoparticles have an interactive effect on the retention and accumulation of this element in organs and the contents of other minerals (P, Ca, Fe, Cu and Zn) in the blood plasma of rats.

MATERIAL AND METHODS

Chromium used in experiments

Chromium in the two forms was used as the additive to rat diets: chromium nanoparticles (CrNPs) and chromium picolinate (CrPic). CrNPs powder (with spherical morphology) was characterized by high purity (999 g/kg), large surface area (6–8 m²/g), bulk density of 0.15 g/mL, and true density of 8.9 g/mL and was acquired in SkySpring Nanomaterials Inc. (Houston, TX, USA). CrPic was purchased in Merck Co. (Darmstadt, Germany). Its purity was also high, >980 g/kg.

Animals

The experiment was performed using 48 outbred male Wistar rats (Cmdb:Wi CMDb) fed a low-fat or high-fat semi-purified rat diet (LFD and HFD, respectively) with dietary addition of chromium nanoparticles or chromium picolinate. The study schema consisted of two periods, *i.e.* initial and experimental, each of 9 weeks. During the initial 9-wk period, the rats aged 6-wk were fed a HFD. After the initial period, the high-fat rats were randomly divided into 4 groups with $n=12$ per group and fed, for the subsequent 9-wk, the following dietary treatments: the LFD-CrPic group was fed a standard low-fat diet supplemented with chromium picolinate; the LFD-CrNPs group – a standard low-fat diet supplemented with chromium nanoparticles; the HFD-CrPic group – a high-fat diet supplemented with chromium picolinate; and the HFD-CrNPs group – a high-fat diet supplemented with nanoparticle chromium (Table 1). The rats were administered chromium in a daily dose of 0.3 mg/kg of body weight (BW) proposed by the EFSA NDA Panel [2014] as a highly safe dose without any harmful side-effects. In order

TABLE 1. Composition of low-fat diet (LFD) and high-fat diet (HFD) with chromium picolinate (CrPic) and chromium nanoparticles (CrNPs) used in the experiment (g/kg).

	LFD-CrPic	HFD-CrPic	LFD-CrNPs	HFD-CrNPs
Casein ¹	148	148	148	148
DL-Methionine	2	2	2	2
Cellulose ²	80	30	80	30
Choline chloride	2	2	2	2
Cholesterol	3	3	3	3
Vitamin mix ³	10	10	10	10
Mineral mix ⁴	35	35	35	35
Maize starch ⁵	640	520	640	520
Rapeseed oil	80 (with Cr-Pic) ⁶	80 (with Cr-Pic) ⁶	80 (with Cr-NP) ⁶	80 (with Cr-NP) ⁶
Lard	0	170	0	170

¹Casein preparation: crude protein 89.7 g/100 g, crude fat 0.3 g/100 g, ash 2.0 g/100 g, and water 8.0 g/100 g.

² α -Cellulose (SIGMA, Poznan, Poland), main source of dietary fibre.

³AIN-93G-VM [Reeves, 1997], mix (g/kg): 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamine-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B₁₂ (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-*rac*- α -tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-*trans*-retinyl palmitate, 500,000 IU/g), 0.25 vitamin D₃ (cholecalciferol, 400,000 IU/g), 0.075 vitamin K₁ (phyloquinone), 974.655 powdered sucrose.

⁴Mineral mix, mix (g/kg): 357 calcium carbonate anhydrous, 196 dipotassium phosphate, 70.78 potassium citrate, 74 sodium chloride, 46.6 potassium sulfate, 24 magnesium oxide, 18 microelement mixture, 213.62 starch (to 1 kg). Microelement mixture, mix (g/kg): 31 iron (III) citrate (16.7% Fe), 4.5 zinc carbonate (56% Zn), 23.4 manganese (II) carbonate (44.4% Mn), copper carbonate (55.5% Cu), 0.04 potassium iodide, citric acid to 100 g.

⁵Maize starch preparation: crude protein 0.6 g/100 g, crude fat 0.9 g/100 g, ash 0.2 g/100 g, total dietary fibre 0 g/100 g, and water 8.8 g/100 g.

⁶The dosage of CrPic or CrNPs: 0.3 mg/kg body weight.

to maintain a safe and comparable regimen while preparing the diets, both sources of Cr were added along with rapeseed oil and not in the mineral mix.

The *in vivo* protocol including all manipulations planned to be performed on living rats was in compliance with regulations and ordinances in force in Poland, and Directive 2010/63/EU for animal use in research and education. The application for conducting *in vivo* experiment was accepted by the National Ethics Committee for Animal Experiments (Approval No. 73/2021).

Throughout the study, all animals were kept individually in stainless steel cages in an animal laboratory room with proper for the rodents inside temperature (22±1°C), relative humidity 60±5%, a 12-h day-night regimen, and an air exchange rate of 15 changes per 60 min. The rats were thoroughly scrutinized by the well-trained staff and the veterinarian towards a proper behaviour and health status. All humane endpoints in animal research mentioned in Directive 2010/63/EU were tailored strictly in the present study. The superordinate and the veterinarian were informed daily in the case of any sign of rat distress or pain. For 18 weeks (9-wk of the initial

and 9-wk of the experimental period), the animals were provided with free access to tap water and daily amount of semi-purified diets in order to keep the experimental dosage of additional dietary chromium. The diets constructed from well-known ingredients were stored at 4°C in plastic tubes for food and perishables during all the feeding period. The experimental dietary treatments for rats were constructed according to the rules described by Reeves [1997] and approved by the American Institute of Nutrition. The diets were based on casein as the main protein source, rapeseed oil as a fat source (oil was coupled with lard in the high-fat diets), and maize starch as a carbohydrate source. The experimental calculations and analyses of biological animal material were performed individually for each rat.

On day 28, the rats were relocated to the balance cages in order to conduct the Cr balance trial which enabled calculations of the Cr digestibility and retention (utilization) coefficients. The construction of the cages (TECNIPLAST S.p.A., Buguggiate, Italy) facilitates an exact collection of feces and urine excreted by an animal. The balance test consisted of a preliminary period lasting 10 days as well as a relevant balance 5-day period during which all excreted feces and urinated liquid were collected to special tubes kept then in the fridge environment. During the relevant balance days, the amounts of ingested diets and drank water were also recorded for each rat. All collected samples, *i.e.* fecal, urinal, dietary, and water ones, were analyzed for Cr content as described below.

The Cr digestibility was expressed as a percentage of the mineral ingested:

$$\text{Cr digestibility (\%)} = \frac{\text{Cr intake} - \text{Cr excreted in feces}}{\text{Cr intake}} \times 100 \quad (1)$$

The Cr retention (utilization) was calculated as follows:

$$\text{Cr retention (\%)} = \frac{\text{Cr intake} - \text{Cr excreted in feces and urine}}{\text{Cr intake}} \times 100 \quad (2)$$

During the entire feeding experiment, the rats were individually monitored for body weight and diet consumed. Before the termination of the rats, they were deprived of feed for 12 h but still had full access to tap water. On termination day, the animals were anesthetized with the mixture of ketamine (Ke) and xylazine (X) in 0.9% NaCl (Ke, 100 mg/kg BW; X, 10 mg/kg BW) according to the anaesthesia and euthanasia guidelines for laboratory rodents. The unconscious (a painless state) rats were then laparotomized, and their blood was collected from *caudal vena cava* into heparinized tubes in order to obtain blood plasma *via* centrifugation (350×g, 10 min, 4°C). Plasma samples were kept frozen at -70°C until assayed. After blood collection, the rats were euthanized by cervical dislocation in order to confirm the death. The selected internal organs and tissues (liver, kidneys, brain, spleen, thigh muscle, thigh bones) were cut off, weighed if needed, frozen in liquid nitrogen (-196°C), and stored in the low-temperature freezers at -70°C.

Mineral analysis

The concentration of minerals (Cr, P, Ca, Mg, Fe, Cu, and Zn) in the blood plasma samples (0.25 mL) and Cr content in feces, urine, water, diet, and tissue samples

(0.5 g, 0.25 mL, 0.25 mL, 0.5 g and 0.5 g, respectively) were determined by flame atomic absorption spectrometry (FAAS) with the aid of a Perkin-Elmer M 5000 atomic absorption spectrometer coupled with an HGA 500 graphite furnace (PerkinElmer Life and Analytical Sciences Co., Shelton, CT, USA). The furnace program was applied to Cr determination in diets, feces, and tissues with a wavelength of 357.9 nm, drying at 110°C, ashing at 1200°C, atomization and cleaning at 2700°C. In the case of the remaining minerals, the microwave oven (Milestone MLS 1200 MEGA, Milestone, Italy) digestion with HNO₃ was applied at three subsequent periods (5 min each) with the heating power of 210, 420, and 560 W, respectively. The FAAS instrument setting and conditions followed the manufacturers' recommendations. The calibration procedure was done with the aid of the Certipure multi-element standard solution (Merck KGaA, Darmstadt, Germany). The air-acetylene oxidizing flame was used in the FAAS procedure, whereas a D₂ lamp was used for background corrections in the case of Cr, Ca, Fe and Zn determination.

Statistical analysis

In tables, the results are presented as means with standard error of the mean (SEM), the latter calculated as standard deviations (SD) divided by square root of the number of rats ($n=48$). The two-way ANOVA was applied in order to assess p -values for the two main effects, namely the dietary Cr form (Cr; CrPic and CrNPs) and the diet type (D; LFD and HFD) as well as for the occurrence of a significant interaction between the two main factors (Cr×D). In the case of a significant Cr×D interaction ($p<0.05$), the post-hoc Duncan's test was applied to assess statistical differences among all four experimental groups. The two-way ANOVA requires the normal distribution of samples, so the data were checked for normality *via* the Shapiro-Wilk test. The STATISTICA ver. 12.0 software (StatSoft Corp., Krakow, Poland) was used in the analysis.

RESULTS AND DISCUSSION

Long-term use of an ill-balanced diet leads to many metabolic disorders, including obesity, caused by chronic consumption of a diet rich in saturated fats [Nascimento *et al.*, 2013; Orhan *et al.*, 2019; Żary-Sikorska *et al.*, 2021]. An increased level of dietary fat may additionally disturb the mineral homeostasis in the body [Meli *et al.*, 2013; Qi *et al.*, 2020; Stepniowska *et al.*, 2022]. The present research showed that the total level of Cr in the HFD was higher than in the LFD, regardless of Cr form ($p<0.001$; Table 2). In turn, the calculated intake of Cr during the balance test was higher in rats fed the HFD supplemented with CrNPs than in those fed the LFD (see the significant Cr×D interaction). The aforementioned results may be partly ascribed to the formation of adducts composed of Cr nanoparticles and high levels of saturated fatty acids in the HFD treatment [Muller *et al.*, 2017]. Irrespective of diet type, higher urinary Cr excretion was noted when dietary chromium was in the CrNPs form in comparison to that added as CrPic ($p=0.001$). Two-way ANOVA showed the Cr×D interaction also for fecal Cr excretion ($p<0.001$), total Cr excretion ($p<0.001$), Cr digestibility ($p<0.001$),

TABLE 2. Chromium excretion patterns in the digestibility and retention test in rats fed experimental diets.

	Cr content of diet (mg/kg)	Cr intake from diet (mg/5 d)	Excretion of Cr in feces (mg/5 d)	Excretion of Cr in urine (mg/5 d)	Total Cr excretion (mg/5 d)	Cr digestibility (%)	Cr retention (%)
LFD-CrPic	5.55	0.475 ^{ab}	0.097 ^b	0.011	0.108 ^b	79.6 ^a	77.2 ^a
HFD-CrPic	6.60	0.513 ^a	0.224 ^a	0.009	0.233 ^a	56.3 ^b	54.5 ^b
LFD-CrNPs	5.30	0.442 ^b	0.260 ^a	0.015	0.276 ^a	40.9 ^c	37.5 ^c
HFD-CrNPs	6.90	0.498 ^a	0.227 ^a	0.012	0.239 ^a	53.6 ^b	51.3 ^b
SEM	0.22	0.017	0.012	0.001	0.012	2.6	2.2
Diet type (D)							
LFD	4.16 ^b	0.351	0.138	0.011	0.150	59.0	55.3
HFD	5.60 ^a	0.422	0.174	0.008	0.182	60.9	59.0
Cr addition (Cr)							
Cr-Pic	6.08	0.494	0.160	0.010 ^b	0.170	67.9	65.9
Cr-NPs	6.10	0.470	0.244	0.014 ^a	0.257	47.3	44.4
<i>p</i> -Value							
D effect	<0.001	<0.001	0.008	0.078	0.016	0.542	0.254
Cr effect	0.101	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Cr×D interaction	0.102	<0.001	<0.001	0.924	<0.001	<0.001	<0.001

The LFD-CrPic group was fed a standard low-fat diet with supplementation of chromium picolinate; the HFD-CrPic group was subjected to a high-fat diet with chromium picolinate supplementation; the LFD-CrNPs group was fed a standard low-fat diet with supplementation of chromium nanoparticles; the HFD-CrNPs group was fed a high-fat diet with nanoparticle chromium supplementation. The amount of chromium administered to each rat was 0.3 mg/kg body weight. SEM, standard error of the mean ($n=48$). ^{ab}Mean values within a column with unlike superscript letters differ significantly ($p<0.05$); differences among the groups (LFD-CrPic, HFD-CrPic, LFD-CrNPs, HFD-CrNPs) are indicated with superscripts only in the case of a statistically significant interaction Cr×D ($p<0.05$). The Cr intake from the diet was the same as total Cr intake (tap water provided to rats contained no Cr).

and Cr retention ($p<0.001$). The nature of the Cr×D interaction for fecal and total Cr excretion was in significantly lower amounts of Cr removed from the body, mainly *via* the fecal route, in the rats administered LFD-CrPic as compared to the remaining three dietary treatments ($p<0.05$ vs. HFD-CrPic, LFD-CrNPs, and HFD-CrNPs). The Cr×D interaction showed that the highest percentage of Cr digestibility and retention indices followed the dietary treatments with LFD-CrPic, while the lowest values of these indices were noted in the rats fed LFD-CrNPs (in both cases $p<0.05$ vs. all other groups). It has been reported that Cr nanoparticles are better absorbed from the gastrointestinal tract than their organic counterparts [Stepniowska *et al.*, 2022]. Indeed, in the present study, CrNPs were well absorbed in the intestine but at the same time that Cr form was not efficiently retained in the body's tissues and organs, as indicated by considerably diminished Cr retention (utilization) percentage. That process was clearly depicted under the standard low-fat dietary treatment by the highest and lowest retention values for CrPic and CrNPs, respectively. The addition of chromium in the form of CrPic to the high-fat diet resulted in decreased digestibility and retention of Cr in the rats, which corroborates our previous findings on the effect of dietary high-fat environment on the mineral utilization in the body [Stepniowska *et al.*, 2022]. Chromium picolinate has been shown to exhibit hydrophobic properties and, therefore, to be easily absorbed from the gastrointestinal tract in comparison to other forms

of Cr, nicotinate or chloride, available in popular mineral supplements [DiSilvestro & Dy, 2007]. Moreover, it has been found relatively stable in human gastric juice for over three hours, requiring a high concentration of acid (0.1 M) to break the bonds between Cr and picolinic acid [Lamson & Plaza, 2002]. The relatively high stability of chromium picolinate may also partly explain the lesser excretion of dietary CrPic from the rat's body as compared to the novel dietary form of chromium, *i.e.* CrNPs. In the present experiment, regardless of diet type, a greater urinal Cr excretion followed the dietary treatment with CrNPs vs. rats administered dietary CrPic (Table 2). Wang *et al.* [2012], who administered Cr in the form of CrPic or CrNPs at a dietary dose of 200 $\mu\text{g}/\text{kg}$ to pigs, did not observe different fecal and urinal excretion pattern between those two forms of chromium. In turn, Stepniowska *et al.* [2022] noted a similar amount of chromium excreted with urine in rats fed diets supplemented with different chromium forms, *i.e.* picolinate, chromium-methionine, and as nanoparticles. At the same time, the rats fed diets with Cr nanoparticles excreted considerably more Cr in comparison to the animals subjected to dietary treatments with chromium picolinate or chromium-methionine.

The dietary treatments tested, *i.e.* HFD or LFD with the addition of Cr in the form of CrNPs or CrPic, did not differ among each other with respect to the Cr accumulation in the brain, spleen, kidney, liver, bones, and muscle of rats (Table 3). Jamal *et al.* [1991] observed that Cr was easily

TABLE 3. Chromium content in selected tissues of rats fed experimental diets ($\mu\text{g/g}$).

	Brain	Spleen	Kidney	Liver	Bones	Muscle
LFD-CrPic	0.133	0.563	1.03	0.438	0.311	0.169
HFD-CrPic	0.131	0.560	1.03	0.448	0.310	0.166
LFD-CrNPs	0.133	0.564	1.03	0.439	0.313	0.168
HFD-CrNPs	0.130	0.562	1.04	0.439	0.311	0.169
SEM	0.001	0.002	0.004	0.002	0.001	0.001
Diet type (D)						
LFD	0.132	0.564	1.03	0.440	0.312	0.169
HFD	0.130	0.560	1.03	0.443	0.310	0.167
Cr addition (Cr)						
Cr-Pic	0.132	0.561	1.03	0.443	0.310	0.167
Cr-NPs	0.131	0.563	1.03	0.439	0.312	0.169
<i>p</i> -Value						
D effect	0.219	0.376	0.870	0.540	0.106	0.331
Cr effect	0.950	0.938	0.917	0.718	0.499	0.670
Cr×D interaction	0.938	0.965	0.525	0.584	0.491	0.235

The LFD-CrPic group was fed a standard low-fat diet with supplementation of chromium picolinate; the HFD-CrPic group was subjected to a high-fat diet with chromium picolinate supplementation; the LFD-CrNPs group was fed a standard low-fat diet with supplementation of chromium nanoparticles; the HFD-CrNPs group was fed a high-fat diet with nanoparticle chromium supplementation. The amount of chromium administered to each rat was 0.3 mg/kg body weight. SEM, standard error of the mean ($n=48$).

accumulated in the kidneys of chickens fed diets containing potassium chromate. In our previous study on Wistar rats subjected to dietary treatments with nano-copper, those small nanoparticles were accumulated in the brain tissue, while such an effect was not observed in the case of CuCO_3 added to a mineral mixture [Ognik *et al.*, 2020]. An interesting observation was made by Staniek [2019] in female rats fed diets containing low (1 mg/kg), medium (50 mg/kg) and high (500 mg/kg) contents of chromium(III) without (recommended level 45 mg/kg) or with dietary excess (180 mg/kg) of iron. The author scrutinized the effects of the aforementioned treatments on Cu and Zn status in laboratory animals and found decreased Zn contents in the liver, spleen, and kidneys followed Cr(III) addition, especially when Cr(III) was provided at higher dosages. It was quite surprising that there was no interaction between the dietary chromium and the excessive supply of iron on the Cu and Zn status in the rat organism. The two-way ANOVA showed that, irrespective of the dietary additional Cr form, the HFD treatment resulted in a decrease in blood plasma Ca concentration ($p<0.05$ vs. LFD; Table 4). A significant Cr×D interaction was observed with respect to the plasma levels of Zn ($p=0.008$), Cu ($p=0.001$), P ($p=0.024$), and Fe ($p=0.014$). In the case of plasma Zn, Cu, and P concentrations, the nature of the interaction was that the LFD-CrPic group excelled significantly the HFD-CrPic one and such differences were not observed between both dietary CrNPs counterparts. Apart from the aforementioned differences between LFD-CrPic and HFD-CrPic groups, the latter animals had additionally a significantly

lower plasma Cu level than both CrNPs groups and a lower plasma P level than the LFD-CrNPs rats. The Cr×D effect showed the lowest plasma Fe concentration in the rats administered HFD-CrNPs in comparison to all other groups.

It has been reported that different dietary doses and forms of additional chromium may further affect the retention and tissue distribution of other macro- and microelements [Chang *et al.*, 1992; Dogukan *et al.*, 2009; Staniek *et al.*, 2013]. It should be noted that those effects of dietary chromium might be modulated by the ingested diet type. Indeed, in the present study, the HFD considerably diminished the plasma Ca level compared to LFD. It has been reported that long-term consumption of diets dense in energy derived from fats resulted in reduced gastrointestinal Ca absorption by creating hardly soluble Ca soaps in the intestinal contents [Wang *et al.*, 2016]. In addition, the ingestion of a high-fat diet may reduce the expression of genes related to calcium transport, *i.e.* calbindin-D9K, plasma membrane calcium ATPase (PMCA1b), Na-Ca exchanger [Xiao *et al.*, 2010]. High-fat diets, which are typically high in saturated fatty acids, have been shown to negatively affect bone mineral density in growing rats [Macri *et al.*, 2012]. McCarty [1995] observed that depressed excretion of calcium and hydroxyproline in older (postmenopausal) women followed the supplementary consumption of chromium in the form of picolinate and that this effect might be the cause for elevated bone resorption processes in those patients. The body's calcium management is closely related to the metabolism of phosphorus. The present experiment showed that blood plasma P concentration was substantially ($p<0.05$)

TABLE 4. Concentration of selected minerals in blood plasma of rats fed experimental diets.

	Zn (μM)	Cu (μM)	Mg (mM)	Ca (mM)	P (mM)	Fe (μM)
LFD-CrPic	69.9 ^a	15.7 ^a	0.549	2.26	1.30 ^a	2.73 ^a
HFD-CrPic	60.5 ^b	13.6 ^b	0.558	2.31	1.12 ^b	2.86 ^a
LFD-CrNPs	65.1 ^{ab}	16.0 ^a	0.604	2.32	1.35 ^a	2.43 ^a
HFD-CrNPs	64.4 ^{ab}	16.2 ^a	0.561	2.17	1.17 ^{ab}	1.41 ^b
SEM	1.2	0.6	0.007	0.03	0.02	0.11
Diet type (D)						
LFD	63.7	16.9	0.563	2.33 ^a	1.26	2.83
HFD	63.1	18.6	0.550	2.21 ^b	1.16	2.19
Cr addition (Cr)						
Cr-Pic	65.2	14.7	0.554	2.29	1.21	2.80
Cr-NPs	64.8	16.1	0.583	2.25	1.26	1.92
<i>p</i> -Value						
D effect	0.785	0.085	0.364	0.026	0.024	<0.001
Cr effect	0.136	<0.001	0.121	0.825	0.249	<0.001
Cr×D interaction	0.008	0.001	0.298	0.064	0.024	0.014

The LFD-CrPic group was fed a standard low-fat diet with supplementation of chromium picolinate; the HFD-CrPic group was subjected to a high-fat diet with chromium picolinate supplementation; the LFD-CrNPs group was fed a standard low-fat diet with supplementation of chromium nanoparticles; the HFD-CrNPs group was fed a high-fat diet with nanoparticle chromium supplementation. The amount of chromium administered to each rat was 0.3 mg/kg body weight. SEM, standard error of the mean ($n=48$). ^{a,b}Mean values within a column with unlike superscript letters differ significantly ($p<0.05$); differences among the groups (LFD-CrPic, HFD-CrPic, LFD-CrNPs, HFD-CrNPs) are indicated with superscripts only in the case of a statistically significant interaction Cr×D ($p<0.05$).

decreased in the rats fed the high-fat diet supplemented with CrPic as compared not only to the rats fed the LFD-CrPic but also to those administered LFD-CrNPs. Our recent research on growing rats revealed blood depletion of Ca, P, Mg, and Zn under the treatment with a diet rich in saturated fats (lard) [Stepniowska *et al.*, 2022]. Interestingly, among three sources of additional chromium (chromium picolinate, chromium methionine, chromium nanoparticles) analyzed in that study, the greatest depletion effect of blood P concentration was upon Cr picolinate and the lowest one (insignificant compared to the control non-supplemented group) when the chromium-methionine was applied. The aforementioned effect should be ascribed to the lower consumption of a high-energy diet and elevated P fecal excretion in rats fed HFD [Stepniowska *et al.*, 2022]. Nevertheless, additional research is needed in order to explain the exact mechanism of that phenomenon. Our previous work on growing chickens revealed that Cr picolinate added to a diet at 3 mg/kg, but not at 6 mg/kg dietary dose, significantly reduced blood plasma P concentration as compared to the control non-supplemented group [Stepniowska *et al.*, 2020].

It has been reported that, when ingested, minerals (Cr and Fe) contest over transferrin as a transport protein in the metabolic pathways [Quarles *et al.*, 2010]. A study by Sun *et al.* [1999] showed that the form of dietary Cr seemed to be of paramount importance in its final effects on Fe metabolism. In that study, the dietary addition of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$ had no effect on the hepatic

content of Fe in rats, while the dietary LMWCr did otherwise and caused a drop in liver Fe content. The mutual action of Cr and Fe in the body as well as their metabolism might be affected by different agents, *e.g.* the physiological state of tested animals as well as the physical and chemical structure of Cr added to a diet. In our research, a considerable decrease in blood plasma Fe concentration followed the dietary application of CrNPs to HFD, and that effect was noted compared to all other three dietary treatments. It is well known that the blood Fe concentration largely depends on its absorption rate in the duodenum and that this element is absorbed into enterocytes mainly by divalent metal transporter 1 (DMT1) [Szabo *et al.*, 2021]. According to Jiang *et al.* [2018], DMT1 levels might be reduced significantly in the duodenum of rats following the feeding regimen on diets rich in saturated fats, pointing at DMT1 as the factor reducing duodenal iron absorption. In our very recent work, the rats receiving dietary chromium nanoparticles had a higher iron blood concentration compared to the animals administered chromium in the picolinate form, irrespective of low- or high-fat dietary regimen [Stepniowska *et al.*, 2022]. The difference in Fe blood concentration in relation to the present work was probably due to the fact that the rats in the previous study were younger and not subjected to the preliminary high-fat dietary feeding period. In addition, in the present study, a higher dietary intake of Cr was reported in the rats from the HFD-CrNPs group than in those from the LFD-CrNPs group (Table 4). Iron and chromium(III) share the transportation route to

the tissues *via* a similar vehicle, namely transferrin protein consisting of two C and N lobes. The binding of Fe(III) to the former lobe is about 20 times higher than to the latter one. Deng *et al.* [2016] observed that Cr(III) distracted two Fe(III) binding sites of apotransferrin. It may be speculated that in the case of relatively high dietary supplementation of Cr, as in the present experiment, the binding sites for Fe in transportation and gripping proteins might be taken by chromium. Such possibilities of the diminishing effects of dietary chromium on the potency of transferrin and apotransferrin to capture Fe(III) have been proposed by other authors [Quarles *et al.*, 2011; Staniek & Krejpcio, 2017; Vincent & Love, 2012]. Considering blood Ca concentration, its chronically low level is considered as a potent detrimental factor leading to impeded muscle contractility [Moe, 2005], while a decreased Fe pool in the body may impair the redox status and erythropoiesis intensification [Robach *et al.*, 2007]. The available literature shows some discrepancies with regard to mutual iron and chromium competition, like in the case of transporting proteins [Quarles *et al.*, 2010, 2011; Vincent & Love 2012] or possible synergistic effect of those minerals against anemia [Angelova *et al.*, 2014]. But there is no doubt that the level of iron in the internal tissues, including blood, plays the main/important role in the metabolic fate of other trace elements, *e.g.* Zn and Cu. Our research showed that feeding rats with the HFD-CrPic compared to LFD-CrPic resulted in decreased levels of Cu and Zn in their blood plasma and that this effect was not dependent on the Fe blood concentration (Table 4). According to de Luis *et al.* [2013], obese patients suffer serum zinc depletion in comparison to the consumers with normal BW mass. A recent study on mild-diabetic rats showed that dietary addition of chromium (0.75 mg/kg BW) as Cr(III)-glycinate or Cr(III)-picolinate redressed the Cu/Zn balance in blood and selected tissues, *i.e.* kidneys and heart [Król *et al.*, 2020].

Experiments conducted on laboratory animals with undisturbed [Anderson *et al.*, 1996; Chang *et al.*, 1992; Zha *et al.*, 2007] and disturbed metabolism [Król & Krejpcio, 2010] have shown that kidneys are very sensitive organs to Cr dietary supplementation. In the present study, there were no side-effects in kidneys followed the dietary treatments with 0.3 mg/kg BW of Cr in the form of picolinate or nanoparticles (Table 3). It should be stressed that a simple comparison between different research could lead to invalid conclusions, and thus a deep insight in the subject must be done before the final claim would be drawn. It is not possible to determine the actual metabolic action of dietary chromium without considering differences in Cr administration time, dose, and form, and health condition in consumers (animals and humans). It should be noted that our study found no interactive effect of feeding rats with a low-fat or high-fat diet with the addition of CrPic or CrNPs chromium at a dose of 0.3 mg/kg BW on the accumulation of this element in internal organs. The results achieved with other type of nanoparticles presented by our research team showed that, in comparison to the commonly used CuCO₃, dietary copper nanoparticles to a greater extent were absorbed from the rat intestine, heavily accumulated in the brain tissue, and at the higher dose (6.5 vs. 3.25 mg/kg) caused damages to the rat liver [Cholewińska *et al.*, 2018]. There is no doubt that more research is needed

regarding dietary nanoparticle use in humans and caution is suggested when providing to public pros about a “new promising” source of trace elements in our diet.

CONCLUSIONS

Irrespective of the diet type, *i.e.* high-fat or low-fat, an enhanced excretion of chromium from the rat's body followed the dietary addition of CrNPs as compared to CrPic. It is worth noting that both sources of additional dietary chromium did not cause its accumulation in the analyzed internal organs, *e.g.* brain, spleen, liver, thigh bone, and kidneys. It should be stressed that both CrPic and CrNPs negatively modulated the mineral status of blood plasma when added to the high-fat diet, namely: the dietary CrPic reduced Zn, Cu and P concentrations while CrNPs diminished plasma Fe level.

RESEARCH FUNDING

This work was supported by the National Science Centre, Grant No. 2020/39/B/NZ9/00674.

CONFLICT OF INTERESTS

The authors have no competing interests.

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