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ORIGINAL PAPER

Effect of chromium(III) glycinate and picolinate supplementation on antioxidant status and calcium, magnesium and phosphorus levels in diabetic rats*

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Abstract

Diabetes mellitus is a metabolic disease that changes carbohydrate and lipid levels as well as affecting the body's mineral balance. One of the elements with a potential hypoglycaemic effect is Cr(III). This model study includes assessment of the effect of a new complex – chromium(III) glycinate (Cr(III)Gly) – and the reference compound – chromium(III) picolinate (Cr(III)Pic) – on Ca, Mg, P levels and the antioxidant status of diabetic rats. The study was conducted on 40 male Wistar rats. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 55 mg kg^{-1} bw). Subsequently, the rats were fed the AIN-93M diet supplemented with Cr(III) complexes at 10 mg Cr kg^{-1} of diet for seven weeks. It was shown that diabetes induced by STZ injection led to changes in the distribution between these elements in liver, heart, spleen and decreased the serum total antioxidant status. Supplementation with Cr(III) complexes partially normalised the Mg level in the heart as well as the P level in the liver and spleen of diabetic rats. Additionally, Cr(III)Pic increased the Ca/P ratio in kidneys. On the other hand, Cr(III)Gly reduced the Ca/P ratio in the heart. In summary, Cr(III) compounds affected the levels of Mg and P in diabetic rats, but did not influence the antioxidant status.

Keywords: chromium supplementation, calcium, magnesium, phosphorus, antioxidant status, diabetes

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INTRODUCTION

Diabetes mellitus is a disease that entails carbohydrate and lipid metabolism disorders. Chronic hyperglycaemia increases oxidative stress, which leads to further changes, including severe complications. Therefore, agents that improve insulin sensitivity and exhibit hypoglycaemic effects may prove to be important in delaying the complications of diabetes. In this context, Cr(III) compounds represent a potentially preferred solution. According to many animal studies, trivalent chromium improves insulin sensitivity (Król, Krejpcio 2011, Orhan et al.2018). On the other hand, the results of research involving humans are not so promising. In the literature, both inorganic (chloride, sulfate) and organic (picolinate, malonate, propionate, methionine, yeast enriched) forms have been analysed. They are characterised by different bioavailability, which may determine their biological activity. The compounds tested to date have a hypoglycaemic effect, improve insulin sensitivity and normalise oxidative stress in animal studies. Complexes of chromium(III) with glycine are relatively little known. The previous work of the authors, which constitutes a part of this experiment, includes the assessment of the hypoglycaemic potential of this substance and demonstrates that Cr(III)Gly reduced glycaemia, the concentration of triacylglycerols, as well as the Cu/Zn ratios in the serum and heart (Król et al. 2020).

Supplementation with minerals may affect their content in the body by interacting with other elements at the stage of absorption, transport or excretion. It is well-known that minerals can influence each other when one of them is administered in excess or insufficient amount. Furthermore, hyperglycaemia, impaired insulin sensitivity and oxidative stress change mineral distribution in the body. During the course of diabetes, it is possible to observe changes in Ca, Mg and P metabolism. Data on variations in the Ca content in diabetes are not consistent. According to some authors, the serum Ca levels increased, while others report that this level was constant or decreased (Skalnaya et. al. 2017, Zhang et al. 2017). The association between decreased serum Mg and diabetes was observed in a few studies (Wei et al. 2016, Zhang et al. 2017). Both of these macronutrients play important roles in the maintenance of glucose homeostasis. Calcium is known to be essential for insulin secretion. The glucose-dependent release of insulin is a process regulated by the intracellular level of Ca in the B cells of the pancreas (Wollheim, Sharp 1981). Several studies have shown a positive association between elevated serum Ca levels and an increased risk of developing type 2 diabetes in different populations (Becerra-Tomas et al. 2014, Suh et al. 2017). Also, a negative correlation of serum Ca and Mg levels with serum glucose and HbA1c was observed in postmenopausal women with DM2 (Skalnaya et. al. 2017) and prediabetes subjects (Yadev et al. 2017). An inverse association between increased Ca and P urinary excretion and chronic kidney disease progression was noticed (Duan et al. 2021).

In recent years, much attention has been paid to the link between glucose homeostasis and bone health. Previous studies have demonstrated that poor glycaemic control leads to an increase in urinary Ca excretion with simultaneous stimulation of parathyroid hormone secretion, which can cause skeletal changes and increase the risk of fractures (Cipriani et al. 2020). Compounds exhibiting hypoglycaemic activity can prevent these unfavourable changes. The influence of some hypoglycaemic drugs on bone metabolism has also been noticed. For example, sulphonylureas can stimulate the bone formation process, while thiazolidinediones have the opposite effect of reducing bone density and increasing the risk of fractures. With regard to metformin and insulin, the results vary between preclinical and clinical models (Cipriani et al. 2020). It was found that insulin therapy can preserve bone architecture and elasticity in diabetic rats, confirming the anabolic effect of this hormone (Bertolin et al. 2017).

The rationale for assessing the effect of Cr(III) supplementation on the metabolism of Ca, Mg and P may result from its influence on glycaemia, insulin, oxidative stress and mutual interactions between elements. Therefore, the authors of this study decided to assess whether Cr(III) glycinate and Cr(III) picolinate affect the Ca, Mg and P levels as well as oxidative stress parameters in diabetic rats.

MATERIAL AND METHODS

Animals

All the procedures used in this study were accepted by the Animal Bioethics Committee in Poznań, Poland (Approval #47/2010).

Male Wistar rats (n=40, six weeks old) were purchased from the licensed laboratory called Animal Breeding Centre at the Poznań University of Medical Sciences (Poznań, Poland). Throughout the experiment, rats were kept individually in cages under controlled temperature (21±2°C), and humidity (55-60%) with an artificial 12 h/12 h day/night cycle. They were maintained at an animal care facility.

Induction of diabetes

After a 7-day adaptation period, rats were intraperitoneally injected with STZ (55 mg kg^{-1} b.w.) in citric buffer (pH = 4.4) to induce diabetes, while rats in the control group received only buffer. Three days after injection, blood drops were obtained from the tail vein to measure the blood glucose concentration (Optium Medisense glucometer, Abbott Co.). After injection of STZ, all animals exhibited elevated fasting blood glucose levels (>9 mmol l^{-1}) and were classified as "diabetic" (D). Animals were divided into four groups: C – control group (n=10), D – diabetic control (n=10), D + Cr(III)Gly – dia-

betic rats fed a diet supplemented with chromium(III) glycinate at the dose of 10 mg Cr(III) kg^{-1} of diet (n=10), D + Cr(III)Pic – diabetic rats fed a diet supplemented with chromium(III) picolinate at the dose of 10 mg Cr(III) kg^{-1} of diet (n=10).

Diets

Rats were fed semi-synthetic diets composed according to the recommendations issued by the American Institute of Nutrition (AIN-93M) – Reeves (1997). The diets consisted of casein (14%), soybean oil (4%), wheat starch (62.32%), sucrose (10%), potato starch (5%), L-cysteine (0.3%), vitamin mix AIN-93M (1%) and mineral mix AIN-93M (3.5%). Two mineral mixes were enriched with either Cr(III)Gly (chemical formula $[Cr_3O(NH_2CH_2CO_2)_6(H_2O)_3]$ + + $NO_3 \cdot H_2O$, the theoretical content of Cr 25.7%, synthesised in the laboratory of the Department of Product Ecology, Poznań University of Economics) or Cr(III)Pic (Nutrition 21, Inc. NY, USA) to a level of 10 mg elemental Cr kg^{-1} of diet. The reliability of the chemical form and stability of Cr(III)Gly was previously reported in our work (Wieloch et al. 2008). Rats received these diets and distilled water *ad libitum* for seven weeks. The food intake was monitored daily. The content of minerals in diets is given in Table 1.

Table 1

The content of Cr, Ca, Mg and P in diets used in experiment

Index	Experimental group			
	C	D	D+Cr(III)Gly	D+Cr(III)Pic
Cr (mg kg^{-1})*	1.336±0.321^A	1.441±0.239^A	11.747±0.452^B	11.899±0.531^B
Ca (g kg^{-1})	5.258±0.431	5.066±0.225	5.072±0.407	5.198±0.095
Mg (g kg^{-1})	0.530±0.035	0.532±0.054	0.539±0.021	0.542±0.011
P (g kg^{-1})	3.123±0.109	3.267±0.215	3.345±0.372	3.237±0.274

Data are expressed as mean ± standard deviation (SD).
* Data previously published in Król et al. (2020).

Data collection

After 16 h fasting at the end of the experiment, rats were anaesthetised with an intraperitoneal injection of thiopental (40 mg kg^{-1} b.w.) and dissected to collect blood from the aorta and remove inner organs (liver, kidneys, spleen, heart, left femur) for appropriate biochemical tests. Blood was collected in test tubes and left to clot for 30 minutes. The tubes were then centrifuged at 4,000 g for 10 minutes. The serum and organs obtained were stored at -80°C until the analysis.

Determination of glucose, insulin, oxidative stress parameters and mineral content

The serum glucose and insulin concentration were determined by the hexokinase and radioimmunological methods, respectively. Serum total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) were measured with commercial kits (Cayman Chemical, USA). The liver homogenates were prepared according to manufacturer recommendation (0.1 g of sample + 0.9 cm^3 of phosphate buffer, pH 7.0). The activity of the enzymes (SOD and CAT) in the tissue were expressed on 1 g of protein. The protein contents were determined with the Bradford reagent (Sigma-Aldrich, Saint-Louis, Mo, USA). The absorbance was read on a microplate reader (Asys UVM340, Biochrom, UK).

For mineral analysis, about 1 g of tissue samples or 0.5 ml of serum were weighed, transferred to a Teflon vessel and quenched with nitric acid (65% GR ISO, Merck, Germany). The dishes were placed in a microwave digestion oven (Mars-5, CEM, USA) and mineralised. The samples were subsequently quantitatively transferred to volumetric flasks. Immediately before the determinations, the samples were diluted with a 0.5% $LaCl_3$ solution (Merck, Germany). The assessment of Ca and Mg contents was performed by means of flame atomic absorption spectrometry using an AAS-3 apparatus (Carl-Zeiss Jena, Germany). The phosphorus level was determined by the Eiconogen colorimetric method using a Nanocolor UV/VIS spectrophotometer (Macherey-Nagel MN, Germany).

The accuracy of this evaluation method was verified using two reference materials: bovine liver (NIST®-SRM 1577c) and Human Assayed Multi-Sera level 2 (HUM ASY Control 2, No. HS2611, Randox Laboratories, Crumlin, UK). The recovery values were in the ranges of 90 to 110%.

Statistical analysis

One-way analysis of variance and the Fisher's least significant difference (LSD) test were used to show the significance of the differences between groups. The significance level was assumed at $p<0.05$. The calculations were made using Statistica 13.1 software (Statsoft, USA).

RESULTS AND DISCUSSION

The study revealed no significant differences in the Ca, Mg and P intake in rats from all experimental groups (Table 2). Diabetes caused an increase in the blood glucose levels, and both Cr(III) compounds reduced its level to some extent, with Cr(III)Gly having a stronger hypoglycemic effect (Table 3). The serum insulin concentration was unchanged. In the study, a disturbed redox balance in diabetic rats was evidenced by a decrease in the serum

Table 2

Daily Cr, Ca, Mg and P intake by rats (mg)

Index	Experimental group			
	C	D	D+Cr(III)Gly	D+Cr(III)Pic
Cr*	0.027±0.001^{A}	0.033±0.006^{A}	0.236±0.010^{B}	0.248±0.017^{B}
Ca	106.7±5.51	116.0±22.60	101.7±4.42	108.2±7.60
Mg	10.76±0.56	12.18±2.37	10.80±0.47	11.29±0.79
P	63.35±3.72	74.82±14.58	67.08±2.91	67.35±4.73

Data are expressed as mean ± standard deviation (SD).
* Data previously published in Król et al. (2020).

Table 3

Serum glucose and insulin concentration and TAC, SOD, CAT activity in serum and liver of rats

Index	Experimental group			
	C	D	D+Cr(III)Gly	D+Cr(III)Pic
Serum glucose (mmol dm^{-3})	7.045±0.303^{A}	9.529±0.960^{B}	7.525±0.915^{A}	8.069±0.938^{AB}
Serum insulin (pmol dm^{-3})	276.0±57.0	144.0±81.0	93.0±37.5	228.0±151.5
Serum TAC (mmol Trolox dm^{-3})	3.762±0.899^{B}	2.567±0.839^{A}	2.575±1.251^{A}	2.383±0.333^{A}
Serum SOD (U cm^{-3})	7.003±2.207	5.293±1.397	5.645±2.696	5.474±1.680
Liver SOD (U mg^{-1} protein)	3.045±0.865	2.756±0.836	2.859±0.568	2.748±0.547
Serum CAT (nmol min^{-1} cm^{-3})	17.03±5.171	14.14±6.358	17.925±10.56	21.28±7.160
Liver CAT (nmol min^{-1} mg^{-1} protein)	225.3±34.56	203.8±24.03	204.47±32.85	203.8±36.38

Data are expressed as mean ± standard deviation (SD). The values in the same line which are not marked with the same superscript differ significantly.

TAC, and a slight although not significant decrease in the serum and liver SOD and CAT activity. Cr(III) compounds did not affect these indices, which may indicate no effect of these compounds in the tested dose on the intensity of free radical generation in these tissues.

The largest amounts of Ca are deposited in bones (approx. 99%). Insignificant amounts are also present in the intra- and extracellular fluids. Calcium homeostasis is strictly regulated and depends mainly on the dietary supply, intestinal absorption, renal reabsorption and bone resorption/formation. Low serum Ca levels cause parathyroid glands to secrete PTH, which in turn

stimulates osteoclasts to release Ca from bone, increased renal reabsorption, and increased intestinal absorption. The permeability of the small intestine also depends on dietary supply. With low supply, the vast majority of Ca is absorbed in the duodenum by active transport with the transient receptor potential vanilloid type 6 channel (TRPV6) – Wongdee et al. (2017). The factors responsible for the impaired absorption of calcium in the intestines in diabetes are not fully understood. This phenomenon may result from decreased insulin levels or other hormonal changes, as these alternations have been found to correlate with decreased serum levels of vitamin D metabolites. Moreover, the oxidative stress occurring in diabetic rats reportedly increased the permeability of the duodenum (Rivoira et al. 2015). Studies on rats with diabetes induced by STZ injection showed a lower concentration of Ca in the plasma (Rivoira et al. 2015). Similarly, a slightly decreased serum Ca was observed in the present study (Table 4). Hyperglycaemia can

Table 4

The content of Ca, Mg and P in serum and tissues of rats

Index	Experimental group			
	C	D	D+Cr(III)Gly	D+Cr(III)Pic
Ca				
Serum (mmol dm^{-3})	2.851 ± 0.914^{B}	2.090 ± 0.964^{AB}	2.028 ± 0.693^{AB}	1.821 ± 0.470^{A}
Liver (mg kg^{-1} d.m.)	42.13±16.46	65.40±17.00	64.89±19.88	65.99± 22.51
Kidney (mg kg^{-1} d.m.)	149.02±26.97	128.11±32.10	141.44±23.03	151.84±34.26
Heart (mg kg^{-1} d.m.)	221.25±56.12	185.67±65.63	150.59±37.25	225.74±55.85
Spleen (mg kg^{-1} d.m.)	150.6±60.31	163.0±51.97	146.5±51.71	137.4±55.35
Femur (g kg^{-1} d.m.)	151.1±11.49	139.3±13.81	145.5±51.71	147.7±17.54
Mg				
Serum (mmol dm^{-3})	0.737±0.234	0.496±0.183	0.654±0.068	0.488±0.152
Liver (mg kg^{-1} d.m.)	818.5±143.3	831.5±59.63	934.4± 113.8	893.4± 64.73
Kidney (mg kg^{-1} d.m.)	849.2±30.30	856.0±54.10	871.7± 48.16	888.0± 68.72
Heart (mg kg^{-1} d.m.)	889.4 ± 52.72^{B}	693.9 ± 111.4^{A}	835.5 ± 96.84^{B}	820.1 ± 129.49^{AB}
Spleen (mg kg^{-1} d.m.)	764.2±107.3	884.8±119.0	853.3± 56.6	797.6± 45.99
Femur (g kg^{-1} d.m.)	3.208±0.560	2.949±0.335	3.155±0.462	3.103±0.649
P				
Serum (mmol dm^{-3})	1.054±0.309	1.192±0.256	1.240±0.307	1.308±0.474
Liver (mg kg^{-1} d.m.)	11.201 ± 1.942^{A}	13.168 ± 1.819^{B}	12.007 ± 1.54^{AB}	12.698 ± 0.940^{AB}
Kidney (mg kg^{-1} d.m.)	8.093 ± 1.121^{B}	7.392 ± 1.617^{AB}	5.713 ± 1.083^{A}	5.912 ± 1.387^{A}
Heart (mg kg^{-1} d.m.)	11.064±0.553	10.585±0.961	10.544±1.076	10.844±1.110
Spleen (mg kg^{-1} d.m.)	12.220 ± 1.476^{A}	14.157 ± 1.356^{B}	13.690 ± 1.060^{AB}	13.506 ± 0.977^{AB}
Femur (g kg^{-1} d.m.)	100.10±6.56	103.21±11.21	102.18±5.706	101.86±6.734

Data are expressed as mean ± standard deviation (SD). The values in the same line which are not marked with the same superscript differ significantly.

also cause increased kidney Ca uptake and hypercalciuria (Wongdee et al. 2017). In the present study, there was no change in the Ca level in the kidneys; however there was an insignificant increase in Ca in liver content of diabetic rats. Similarly, Presley et al. found increased content of this element in the liver and intestines and lower in the brain in Goto-Kakizaki diabetic rats (Presley et al. 2017). It should be emphasised that in an animal model with STZ-induced diabetes mellitus, the initial decrease in intestinal Ca absorption over time (after 60 days) returned to values comparable to the healthy group. This homeostatic mechanism is accompanied by changes in the expression of transporters (mainly TRVP6) of this element (Rivoira et al. 2015).

In turn, Mg, as mentioned, is an important antioxidant and a cofactor of enzymes involved in glucose metabolism. Its deficiency is often associated with type 2 diabetes, impaired glucose tolerance and metabolic syndrome (Mooren 2015). Metabolic disorders accompanying diabetes may interfere with its absorption and increase the excretion of this element in the urine (Simmons et al. 2010). This is confirmed by numerous studies, which have demonstrated a significantly lower concentration of Mg in individuals suffering from type 2 diabetes (Khan 2015, Doddigarla et al. 2016, Fang et al. 2016, Yadav et al. 2017). Similarly, a lower Mg level was observed in clinical type 1 diabetics (Lin et al. 2016). In some studies, the inverse association between lower serum Mg levels and heart failure, atrial fibrillation and diabetic complication (e.g. chronic kidney disease, diabetic retinopathy) was reported (Oost et. al. 2021). In this study, diabetic rats exhibited decreased levels of Mg in the heart (Table 4). One of the reasons may be the loss of this element in urine, as previous studies have shown that increased diuresis may be the cause of mineral depletion. This may be a consequence of hyperglycaemia and kidney damage (Gomez et al. 2019). The tested Cr(III) compounds, especially Cr(III)Gly, normalised these changes to some extent.

The effect of Cr(III) compounds on Ca and Mg metabolism is not often subjected to studies. Previous research on healthy rats did not show that supplementation of Cr(III) at a dose of 5 mg kg^{-1} of the diet influenced Mg management parameters (Krejpcio et al. 2009). In another study on rats fed a high-fructose diet, the authors of this article found that Cr(III) propionate supplementation at doses of 10 and 50 mg kg^{-1} of diet significantly increased hepatic Mg contents simultaneously failing to affect Ca levels (Król, Krejpcio 2013). In a different study, the authors compared the influence of Cr(III) compounds (CrPic, CrProp, CrCl3 administered at a dose of 1 mg kg^{-1} b.w.) on the mineral content in genetic models of obesity and diabetes (Staniek et al. 2013). In lean control rats, CrPic and CrProp increased the hepatic Ca content, yet this effect was not observed when Cr(III) was supplemented to rats in the form of $CrCl_3$. However, in healthy female rats fed diets supplemented with higher doses of CrProp (100-1,000 mg kg^{-1} of diet), diminished contents of Ca in the liver were found to depend on the applied dose (Staniek, Krejpcio 2017).

The relationship between Cr(III) supplementation and bone health has rarely been studied. The work by Kong et al. (2019) showed that the effects of Cr (80 μg kg^{-1} b.w. in the form of $CrCl_3$) supplementation are similar to those induced by a low oestrogen dose, and that Cr(III) can modify bone mineral density in ovariectomised rats. Other studies suggest that Cr(III) may have a negative effect on bone health. It was found that $CrCl_3$ at doses of 12.5 and 25 mg kg^{-1} of feed significantly decreased Ca concentration in the femur and fibula in broiler chickens, and the effect was dose-dependent (Saeed et al. 2017). Bieńko et al. (2017) observed that supplementation of Cr(III) in the form of sulphate (doses of 104, 156 and 208 μg Cr kg^{-1} b.w.) reduces skeleton density and strength in healthy male rats. In the present study Cr(III) complexes did not influence on femur weight (data not shown) and contents of Ca, Mg and P in the bone (Table 4).

Both minerals (Ca and Mg) have similar physical and chemical reactivity, oxidation states and charge/mass ratios, thus potentially one element can mimic another, exhibiting antagonistic interactions or interfering with each other in biochemical processes (Presley et. al. 2017). The importance of an appropriate balance between Ca and Mg is relevant in terms of oxidative stress, which often occurs in the course of diabetes. It has been observed that in a state of Mg deficiency, calcium channels are blocked. This results in the influx of this element from extracellular sources and the release of Ca^{2+} from intracellular stores, which leads to the increase of intracellular concentrations of Ca^{2+} and oxidative stress (Nielsen 2018). Since the antagonistic relationship between Ca and Mg has been demonstrated, an appropriate cellular balance between these cations is essential. Therefore, the Ca/Mg ratio has clinical implications. Song et al. (2007) study has shown that the serum Ca/Mg ratio correlates with the spine mineral density. In another study, lower serum Mg and higher Ca/Mg ratios significantly increase the risk of all-cause and CVD mortality (Li et al. 2020). In this study, we did not observe significant influence of Cr(III) compounds on the Ca/Mg ratio in serum and most tissues. The only exception was a decreased value of the ratio in the heart of rats fed diets supplemented with Cr(III)Gly (Table 5).

The level of P in the body is regulated primarily by the intestines, kidneys, parathyroid glands and bones. The main deposit of this element lies in bones, where it occurs in the form of hydroxyapatite. The homeostasis of this element is hormonally regulated by parathormone (PTH), 1,25-D and fibroblast growth factor 23 (FGF23). The kidneys are the main organ responsible for P homeostasis, and when the function of this organ is disturbed, its serum level begins to increase. These changes can lead to parathyroid hyperplasia and, finally, to bone disease (Nadkarni and Uribarri 2014). Observational studies also show that in diabetics, the daily amount of Ca and P excreted in the urine decreases along with the progression of the disease. This may indicate that increased urinary Ca and P excretion protects against the development of chronic kidney disease (Duan et al. 2021).

Table 5

Molar Ca/Mg and Ca/P ratios in serum and tissues of rats

Index	Experimental group			
	C	D	D+Cr(III)Gly	D+Cr(III)Pic
Ca/Mg ratio				
Serum	3.876±0.478	4.138±1.416	3.241±1.253	3.461±1.096
Liver	0.031±0.010	0.047±0.013	0.042±0.014	0.043±0.015
Kidney	0.106±0.021	0.091±0.022	0.097±0.014	0.102±0.020
Heart	0.150 ± 0.042^{AB}	0.159 ± 0.046^{A}	0.111 ± 0.027^{B}	0.166 ± 0.035^{A}
Spleen	0.120±0.053	0.110±0.031	0.103±0.036	0.105±0.041
Femur	28.71±3.656	28.710±4.467	27.86±6.241	29.36±5.291
Ca/P ratio				
Serum	2.989±1.522	1.946±1.241	1.844±0.980	1.607±0.737
Liver	0.003±0.001	0.004±0.001	0.004±0.001	0.005±0.003
Kidney	0.015 ± 0.003^{AB}	0.014 ± 0.003^{A}	0.019 ± 0.003^{AB}	0.021 ± 0.006^{B}
Heart	0.015±0.003	0.014±0.005	0.011±0.002	0.016±0.003
Spleen	0.010±0.005	0.009±0.002	0.008±0.003	0.008±0.003
Femur	1.172±0.098	1.052±0.116	1.105±1.165	1.124±0.110

Data are expressed as mean ± standard deviation (SD). The values in the same line which are not marked with the same superscript differ significantly.

In this study, diabetes had only a minor effect on P levels in the body, and no changes were observed in most tissues and serum (Table 4). The only exception was the higher content of this element in the liver and spleen (by 18% and 16%, respectively). Similar changes, especially in the spleen, were also reported in other studies (Takita et al. 2004). Considering the influence of Cr(III) compounds on the homeostasis of this element, a decreased renal P level was found in both groups supplemented with Cr(III) compounds (CrGly and CrPic).

The data on the influence of Cr(III) compounds on P metabolism is insufficient. To the best of the authors' knowledge, to date, the effect of different Cr(III) compounds has only been investigated in the study by Stępniowska et al. (2020). The authors found that dietary supplementation of CrPic increased P levels in the liver, ileum and skin of chicken. However, this compound did not influence intestinal P absorption *ex vivo*. A negative effect of the supplementation consisted in the reduction of P in the femur, which may be dangerous due to lower deposits of this element in tissue and its influence on the function of some enzymes. In another study, lower Ca and P serum levels were observed in rats fed a high-fat diet supplemented with CrPic and Cr-NP, as well as increased plasma Mg after CrPic supplementation (Stępniowska et al. 2022*a*). In subsequent research, CrPic added to a high-fat diet lowered plasma P levels in rats (Stępniowska et al. 2022*b*).

Perhaps the differences in the observed results may stem from the fact that the aforementioned study used healthy animals, while in the present study the authors had induced diabetes in the rats subjected to tests.

As in the case of Ca and Mg, also Ca and P can influence each other. The serum Ca/P ratio can be a useful indicator of vascular calcification, and correlates inversely with incidence of ischemic heart disease (Jung et al. 2022). In the present study Cr(III)Pic increased the Ca/P ratio in the kidney, however the differences in the serum and other tissues were insignificant (Table 5).

The limitation of this research is the fact that the authors did not assess the amount of Ca, Mg and P excreted in the urine, nor did they analyse hormones and proteins responsible for the homeostasis of these elements in the body (e.g. PTH, osteocalcin). Such tests would shed more light on the mechanism of action of Cr(III) on bone health in diabetes. The reported effects of Cr(III) probably resulted from the influence of these supplements on hormonal factors (e.g. insulin), which may regulate the homeostasis of Ca, Mg and P.

In this study, the authors applied a dose of 10 mg Cr kg^{-1} of diet, which had already been used in previous studies (Król, Krejpcio 2010, 2011). It should be emphasised that this dose is high and according to pharmacological data, it corresponds to the level of – 0.121mg kg^{-1} b.w. in humans (Nair, Jacob 2016). It is also almost impossible to reach this dose as the highest supplementary dose ingested by humans was 1 mg per day.

CONCLUSIONS

Chromium(III) complexes improved Mg levels in the heart and, to some extent, restored the level of P in the liver and spleen. Additionally, Cr(III)Pic increased the Ca/P ratio in the kidney, while Cr(III)Gly lowered the Ca/Mg ratio in the heart. Neither Cr(III)Gly nor Cr(III)Pic affected the antioxidant status of serum and the liver. The data obtained in this experiment may indicate some role of these complexes in cardiometabolic health rather than an impact on bone health.

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