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DIETARY CHROMIUM(III) PROPIONATE COMPLEX SUPPLEMENTATION AFFECTS TISSUE MINERAL LEVELS IN RATS FED HIGH-FRUCTOSE DIET

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Abstract

Chromium(III) plays an important role in carbohydrate and lipid metabolism, thus supplements containing this element are broadly advertised as efficient agents improving blood glucose levels or even reducing body mass. However, their hypoglycemic potential depends on the chemical form, bioavailability, dosage and the duration of treatment. Chromium(III) supplementation is generally considered safe although some data point to interaction of this ion with other elements. Thus, the aim of this study was to evaluate the effect of chromium(III) supplementation on tissue mineral content in rats fed high-fructose diets. Nine-week old male Wistar rats were fed *ad libitum* with a standard diet (control) or a high-fructose diet to induce insulin resistance. Next, supplementary dosages of chromium(III) propionate complex (1.0 and 5.0 mg Cr kg^{-1} b.w. day^{-1}) were introduced and rats were fed those diets for 4 weeks.

It has been found that supplementary chromium(III) did not affect tissue calcium, iron and zinc levels, but significantly increased hepatic magnesium and copper level, while decreasing splenic copper level in rats fed high-fructose diets. Higher chromium(III) dosages increased content of this element in kidneys.

In conclusion, short-term supplementation of chromium(III) propionate complex affects mineral homeostasis in the tissues of rats fed high-fructose diets; however, the mechanisms of such interactions are only partially known.

Key words: chromium(III) propionate, supplementation, minerals, high-fructose diet.

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WPŁYW SUPLEMENTACJI DIETY PROPIONIANEM CHROMU(III) NA POZIOMY TKANKOWE PIERWIASTKÓW U SZCZURÓW KARMIONYCH DIETĄ WYSOKOFRUKTOZOWĄ

Abstrakt

Chrom(III) odgrywa znaczącą rolę w metabolizmie węglowodanów i lipidów, co sprawiło, że suplementy zawierające ten pierwiastek są szeroko reklamowane jako skuteczne środki poprawiające poziom glukozy we krwi, a nawet obniżające masę ciała. Aczkolwiek potencjał hipoglikemiczny suplementów chromu(III) zależy od ich formy chemicznej, biodostępności, dawki oraz czasu podawania. Ogólnie suplementacja chromem(III) uważana jest za bezpieczną, chociaż nieliczne dane wskazują na możliwość interakcji chromu(III) z innymi pierwiastkami. Z tego względu celem badań była ocena wpływu suplementacji Cr(III) na tkankowe poziomy pierwiastków u szczurów karmionych dietą wysokofruktozową. Szczury Wistar w wieku 9 tygodni karmiono *ad libitum* dietą kontrolną lub dietą wysokofruktozową w celu wywołania insulinooporności. Następnie do diet dodano dawki kompleksu chromu(III) z kwasem propionowym (1,0 i 5,0 mg Cr kg^{-1} m.c. na dzień) i karmiono szczury tymi dietami przez 4 tygodnie.

Stwierdzono, że dawki chromu(III) nie wpływały na tkankowe poziomy wapnia, żelaza i cynku, ale znacząco podwyższały poziom magnezu i miedzi w wątrobie, jednocześnie obniżając poziom miedzi w śledzionie u szczurów karmionych dietą wysokofruktozową. Ponadto wyższe dawki chromu(III) wpływały na wzrost zawartości tego pierwiastka w nerkach.

Podsumowując, krótkotrwała supplementacja kompleksem chromu(III) z kwasem propionowym wpływa na homeostazę pierwiastków u szczurów karmionych dietą wysokofruktozową, jednakże mechanizm tych interakcji jest poznany tylko częściowo.

Słowa kluczowe: propionian chromu(III), suplementacja, składniki mineralne, dieta wysokofruktozowa.

INTRODUCTION

Several minerals, owing to their function as cofactors of enzymatic processes involved in the metabolism of carbohydrates and lipids, play a crucial role in insulin resistance and diabetes. Some (zinc and magnesium) participate in insulin production, secretion and action on the cellular level (BARBAGALO et al. 2003, EMDIN et al. 1980). Also disturbances in carbohydrate metabolism, mainly high blood glucose level, can affect mineral indices in the body. Insulin resistance or diabetes affect absorption, excretion and tissue levels of some elements. One of these elements is chromium (III), which has been shown to play an important role in metabolic disorders associated with insulin resistance and hyperglycemia, including type 2 diabetes mellitus (VINCENT 2000). This role probably involves potentiation of insulin signaling and consequently various Cr(III) compounds have been introduced to dietary supplements for the treatment of diabetes and its complications. However, their hypoglycemic potential depends on the chemical form, bioavailability, dosage and duration of treatment.

The use of Cr(III) compounds as dietary supplements requires examination of its safety for animals and humans. In our latest publication (Staniek et al. 2010) we provided experimental evidence that the chromium(III) propionate complex (CrProp) is of low acute toxicity in rat. In another study a high-fructose diet was used to induce insulin resistance in laboratory animals. It was shown (Król, Krejpcio 2010) that supplemental CrProp given orally at dosages of 1 and 5 mg kg^{-1} b.w. day^{-1} for 8 weeks to Wistar rats fed high-fructose diets is able to ameliorate insulin resistance symptoms, at the same time not causing toxic effects. However, supplementary Cr(III), administered at excessive dosages or over a long period of time, can accumulate in internal organs and affect mineral homeostasis in animals and humans (Campbell et al. 1997, Lamson, Plaza 2002, Król, Krejpcio 2010, 2011).

The aim of this study was to evaluate the effect of 4-week CrProp supplementation on mineral homeostasis in rats fed high-fructose diets.

MATERIAL AND METHODS

The experiment was performed on 9-week old male Wistar rats (n=32) divided into 4 groups (8 animals each). The control group was fed *ad libitum* a standard AIN-93M diet (0.1 mg Cr kg^{-1} b.w. day^{-1}), while three groups were fed high-fructose diets (60% w/w) for 4 weeks to induce insulin resistance. Then supplementary Cr(III) (in the form of chromium(III) propionate, CrProp) dosages (1.0 and 5.0 mg Cr kg^{-1} b.w. day^{-1}) were introduced to those diets. Two groups of rats were fed those diets for another 4 weeks, while the other two with control and high-fructose diets with a standard Cr(III) level. After 4 weeks, at the end of study, rats were sacrificed to collect blood and internal organs for biochemical analyses. Organs were washed in saline, weighed and stored at -20ºC until analyzed. All the procedures used in this study were approved by the Animal Bioethics Committee of Poznan, Poland (Approval # 37/2007). Tissue samples were digested by wet mineralization in a microwave system (MARS-5, CEM). The content of Ca, Mg, Fe, Zn and Cu in the liver, kidneys and spleen was determined by the flame atomic absorption spectrometry method (AAS-3, Carl-Zeiss, Germany), while Cr content in organs was assayed using a graphite furnace AAS technique (AAS-5, Jenoptic). The accuracy of quantitative determinations of these elements was assured by simultaneous analysis of the certified reference material (Pig Kidney BCR® No 186, Brussels, fortified with Cr standard).

All results are presented as means ± standard deviation. Significance of differences of means was calculated using the one-way ANOVA and Tukey's tests. Means were considered statistically different at $p < 0.05$. All calculations were made using the Statistica (ver. 7.0) program (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISSCUSION

Insulin resistance and diabetes are states that alter the metabolism of minerals, mainly due to imbalances in carbohydrates metabolism and oxidative stress. In this study, insulin resistance was induced by feeding rats a high-fructose diet. This state was assessed by comparing the HOMA – Insulin Resistance index in the control group and high-fructose diet fed group (data not shown). The effect of high-fructose diet on tissue mineral content is presented in Tables 1, 2 and 3. It has been shown that a high-fructose diet given for 8 weeks affected mineral accumulation in the rat organs. In particular, rats fed for 8 weeks a high-fructose diet had a decreased hepatic Cu level (by 13%), while splenic Cu level increased (by 27%). Additionally, a fructose diet slightly reduced the kidney Fe content. The tissular Ca, Mg, Zn and Cr concentrations remained constant in rats fed high-fructose diets with a standard Cr level.

Table 1

Content of minerals in liver of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	156 ± 34	146 ± 28	153 ± 37	148 ± 34
Ca	707 ± 58^{a}	739 ± 51^{ab}	837 ± 85^{b}	833 ± 87^{b}
Fe	316 ± 37	351 ± 50	296 ± 57	348 ± 38
Zn	100 ± 9	103 ± 10	94.8 ± 5	106 ± 10.7
Cu	22.9 ± 2.5^{b}	19.9 ± 2.7^{a}	22.5 ± 2.9^{b}	23.7 ± 2.1^{b}
Cr	0.71 ± 0.18	0.48 ± 0.06	0.66 ± 0.18	0.68 ± 0.19

Abbreviations: C – control group, H-FRU – high-fructose diet fed group with 0.1 mg Cr kg^{-1} b.m. day^{-1}, H-FRU + 1 Cr – high-fructose diet fed group with 1 mg Cr kg^{-1} b.m. day^{-1}, H-FRU + 5 Cr – high-fructose diet fed group with 5 mg Cr kg^{-1} b.m. day^{-1}; means in a row with different letters differ significantly ($p<0.05$)

Chromium(III) is an element involved in carbohydrate metabolism. An increasing body of evidence supports the hypothesis that Cr is necessary to the proper functioning of the insulin receptor; however, the mechanism by which it improves blood glucose or lipid levels is still being investigated. Despite several advantages of Cr supplementation in diabetics, there are some concerns about its safety. There are some examples of research on humans, in which the authors suggested that ingestion of a high Cr supplemental dosage (above 1200 mg Cr per day) might cause dermatitis (Fowler 2000) or renal impairment (Cerulli et al. 1999). Other adverse effects have

Table 2

Content of minerals in kidney of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	230 ± 37	193 ± 52	156 ± 31	171 ± 32
Ca	764 ± 89	826 ± 58	934 ± 62	887 ± 53
Fe	368 ± 58	337 ± 59	339 ± 37	359 ± 35
Zn	81.2 ± 7.3	82.6 ± 8.8	79.9± 4.9	80.4 ± 8.2
Cu	39.4 ± 6.8	35.5 ± 6.2	30.8 ± 2.8	33.3 ± 3.1
Cr	0.57 ± 0.23^{a}	0.66 ± 0.36^{ab}	0.86 ± 0.18^{b}	1.35 ± 0.33^{c}

Abbreviations: cf. Table 1

Table 3

Content of minerals in spleen of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	145 ± 42	134 ± 38	138 ± 32	136 ± 35
Ca	612 ± 73	564 ± 63	639 ± 73	662 ± 71
Fe	2571 ± 638	2398 ± 310	2744 ± 638	2624 ± 441
Zn	41.1 ± 4.2	42.3 ± 7.2	48.1 ± 6.3	43.3 ± 6.0
Cu	8.71 ± 1.81^{ab}	11.1 ± 2.09^{b}	5.63 ± 0.81^{a}	6.62 ± 0.80^{a}
Cr	5.30 ± 1.22	4.90 ± 1.41	5.11 ± 1.37	4.92 ± 1.39

Abbreviations: cf. Table 1

not been observed. On the other hand, another aspect that should be taken into account during Cr(III) supplementation is its possible interaction with other minerals.

This study investigated the effect of 4-week CrProp supplementation on tissue content of minerals in rats fed high-fructose diets (Tables 1, 2 and 3). Supplementary CrProp (1 and 5 mg kg^{-1} b.w. day^{-1}) did not affect tissue Ca, Fe and Zn levels, but significantly increased hepatic Mg level (by 13%) and hepatic Cu content (by 13 and 19%, respectively), while decreasing splenic Cu (by 40 and 50%, respectively) in rats fed high-fructose diets. Moreover, CrProp given in a higher dosage (5.0 mg kg^{-1} b.w. day^{-1}) doubled the Cr content in the kidneys.

The Cr(III) – Fe interaction seems most likely, since these elements have the same transport protein, i.e. transferrin. Some studies have confirmed a relationship between an increased dietary Cr(III) intake and iron

stores, especially if high Cr(III) dosages were used (LAMPSON, 2002, CAMPBELL et al. 1997). For example, in our previous study, we confirmed that 8-week CrProp supplementation (in dosages of 1 and 5 mg kg^{-1} b.m.) decreased the Fe kidney concentration (KRÓL, KREJPCIO 2010). Similarly, in a study by CLODFELDER et al. (2005) a reduced kidney Fe level was observed in obese type 2 diabetic rats after supplementation of 1000 µg Cr kg^{-1} b.w. day^{-1}. Similarly, the same dosages used in another experiment normalized an increased liver Fe content in rats fed high-fat diets and injected with streptozotocin (KRÓL, KREJPCIO 2011). However, DOGUCAN et al. (2009) did not report any changes in tissue Fe concentration in a similar animal model of type 2 diabetes after 10 weeks of chromium(III) histidinate supplementation. In that study, Fe levels in analyzed tissues did not change, probably due to short supplementation period. To the authors' best knowledge such interaction has not been noticed in human studies, when Cr(III) compounds were given in dosages of 200 to 1000 µg Cr day^{-1} for at least 2 months (CAMPBELL et al. 1997, 2002, VOLPE et al. 2001, KRÓL et al. 2011).

The mechanism of Cr(III) interaction with other elements is unknown although such a relationship has been reported in animal studies. RHEE et al. (2004) proved that in diabetic prone BHE/cdb rats Cr deficiency increased Zn content in the liver, while decreasing Cu tissue content and enhancing Mg and Fe accumulation in the liver.

In the study by DOGUCAN et al. (2009) mentioned above, Cr(III) supplementation decreased Cu and increased Zn content in the liver and kidneys of fat-fed and streptozotocin-treated type II diabetic rats. These data correspond well with results of ŚCIBOR, ZAPOROWSKA (2007), who found that an aqueous solution containing 0.42 mg Cr kg^{-1} b.w. day^{-1} given for 12 weeks increased Zn content in the kidneys of healthy Wistar rats. Additionally, there was no influence on Cr, Fe or Cu levels in either the liver or kidneys of these animals. In another study (SAHIN et al. 1999), pregnant rabbits supplemented with chromium(III) picolinate had a decreased Cu concentration in their liver and kidneys, while Zn levels in those organs increased.

In human subjects, there were no changes in Zn or Cu indices after Cr(III) supplementation for a period of up to 12 weeks in patients with type 2 diabetes as well as moderately obese subjects (ANDERSON et al. 2001, VOLPE et al. 2001, KRÓL et al. 2011).

CONCLUSION

On the basis of the results, it can concluded that CrProp supplementation disturbs mineral homeostasis in the rats' organs. In particular, CrProp given for 4 weeks in dosages of 1 and 5 mg kg b.w. day^{-1} affects Mg, Cu and Cr levels, although it does not influence tissue Ca, Fe and Zn content

in rats fed high-fructose diets. Based on the results, it should be said that the most important issue in Cr(III) supplementation and its interaction with other elements in the body is the dosage used and the duration of supplementation.

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