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Evaluation of anti-diabetic potential of chromium(III) propionate complex in high-fat diet fed and STZ injected rats

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ABSTRACT

The aim of this study was to examine the anti-diabetic potential of the chromium(III) propionate complex (CrProp) in a diabetic rat model. Male Wistar rats (n = 28, 8-week old) were divided into 4 groups (with 7 rats each) and fed at libitum: the control diet (AIN-93M), and high-fat diets with or without supplementary CrProp (10 and 50 mg Cr kg^{-1} diet; 1 and 5 mg kg^{-1} body mass per day) for 5 weeks, and subsequently injected with STZ to induce diabetes. Rats were further fed the same diets for another week until the end of the experiment. Blood indices and the contents of minerals (Fe, Zn, Cu and Cr) in rat tissues were determined by atomic absorption spectrometry. Supplementary CrProp did not affect blood glucose level, but significantly improved insulin sensitivity (HOMA-IR index) and reduced serum levels of triacylglycerols, total and LDL cholesterols. Both supplementary dosages of CrProp (10 and 50 mg Cr kg^{-1} diet) normalized the increased liver Fe content, reduced hepatic and renal Cu levels and elevated renal Cr contents in diabetic rats. In conclusion, CrProp has a significant anti-diabetic (insulin-sensitizing and hypolipidemic) potential; thus it might be a candidate for a therapeutic agent in diabetes.

1. Introduction

Diabetes, regardless of its etiology and type, is a chronic metabolic disorder of glucose and lipid metabolism and when inadequately treated brings about serious metabolic consequences, leading to various complications such as renal failure, cardiovascular disease, blindness or non-fatty liver disease (Arrese, 2010; Cheung et al., 2010; Goldfine and Fonseca, 2010; Kotseva et al., 2010). The prevalence of diabetes in all age groups, countries and regions of the world has been increasing dramatically over the last decades. For example, diabetes is the sixth leading cause of death listed on US death certificates, contributing to ~225,000 deaths annually (Gambert and Pinkstaff, 2006). Therefore, effective prevention and treatment measures, including dietary modifications, supplementation, medication, life-style factors, are being extensively sought to attenuate the epidemic shift and improve well-being of diabetic patients.

One of the numerous bioactive compounds applied in pharmacotherapy to attenuate metabolic disturbances in diabetes, is a trace element – trivalent chromium Cr(III), used in various chemical forms (e.g. Cr picolinate, nicotinate, and various complexes with amino acids) in dietary supplements and therapeutic drugs, available over the counter in many countries. However, the role of Cr in carbohydrate and lipid metabolism, despite over 50 years of intense studies in animals and over forty years on humans, has not been fully elucidated and has been a matter of considerable debate. Due to the contradictory results of clinical and experimental studies, supplementation with Cr(III) is not routinely recommended by diabetic associations. An extended discussion on the role of Cr in glucose and lipid metabolism have been the subject of recent publications (Di Bona et al. 2011; Rhodes et al. 2010; Staniek and Krejpcio, 2009; Król and Krejpcio 2010) and will not be repeated in this article.

Irrespective of controversial scientific opinions concerning Cr, the market for Cr supplements and pharmaceuticals has been growing fast during the last decades, generating revenue of millions of dollars income yearly. The results of recent studies published by Di Bona et al. (2011) revealed that a diet with as little chromium as reasonably possible (16 μg kg^{-1} diet) had no effect on body composition, glucose metabolism, or insulin sensitivity compared with a chromium-"sufficient" diet. Based on the results of these and other studies, the authors concluded that chromium can no longer be considered an essential element.

If Cr(III) is not an essential element for mammals, but at certain dosages improves impaired glucose and lipid homeostasis, its action could be called "pharmacological" at best.

The anti-diabetic potential of Cr(III) compounds depends, among other things, on the chemical form, solubility, dosage, rate of absorption, stability in the physiologic milieu and duration of treatment.

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The pharmacological effects of supplementary Cr(III), administered mostly in the form of Cr(III) trispicolinate (CrPic) and Cr-yeast, have been studied extensively both using various experimental animals (mostly mice and rats, both healthy and diabetic) and in clinical studies on diabetic human subjects (Albarracin et al., 2008; Cefalu et al., 2010; Pei et al., 2006; Racek et al., 2006; Król et al., 2011). Given the popularity of CrPic as a dietary supplement and food supplement worldwide, investigation of any potential health risk caused by this compound is necessary. Conflicting results have appeared on CrPic toxicity. Recently the European Food Safety Authority (EFSA, 2010) issued its Scientific Opinion that CrPic is a safe source of Cr added for nutritional purposes to foodstuffs for particular uses and to foods intended for the general population.

Among the various chemical forms of Cr(III), the Cr(III) propionate complex $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]+$, known as CrProp or Cr_3, is of particular interest. Clodfelder et al. (2005) reported that CrProp is absorbed with very high efficiency of 40–60%, while popular Cr supplements such as $CrCl_3$, Cr(III) nicotinate or CrPic are absorbed at only 0.5–1.3% of the gavaged dose. The difference in the degree of absorption is readily explained by the stability and solubility of the cation in the physiological milieu.

The physicochemical characteristics of CrProp, its toxicity and biochemical properties have been studied in recent years. In regard to the CrProp anti-diabetic potential, most of research has been conducted on laboratory animals, mainly at The University of Alabama (the Vincent laboratory) (Harton et al., 1997; Sun et al., 2002; Clodfelder et al., 2004, 2005; Clodfelder and Vincent, 2005).

In previous studies by Krejpcio (Staniek et al., 2010a,b), CrProp was demonstrated to possess low acute toxicity (LD50 >2000 mg kg^{-1} body mass) and low genotoxic potential in rats (Staniek and Krejpcio, 2010). Furthermore, CrProp was shown to improve insulin sensitivity in high-fructose diet fed rats (the insulin-resistant model) (Król and Krejpcio, 2010). Supplemental CrProp administered orally at dosages of 10 and 50 mg Cr kg^{-1} diet (equivalent to 1 and 5 mg Cr kg^{-1} body mass per day for 8 weeks) to Wistar rats fed a high-fructose diet was able to ameliorate insulin resistance symptoms, without generating toxic effects. However, a prolonged treatment with this compound affected Fe status (lowered renal Fe level by 10%).

The question whether the "pretreatment" with CrProp (before and shortly after the onset of diabetes) has a therapeutic potential in rats, to the authors' knowledge, has not been clearly addressed to date. The objective of this study was to evaluate the anti-diabetic potential of "pretreatment" with CrProp, given orally at dosages of 10 and 50 mg kg^{-1} diet, equivalent to 1 and 5 mg Cr kg^{-1} body mass per day (5- and 25-fold of the basal level) for 6 weeks, to high-fat fed/streptozotocin (STZ) injected rats.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (n = 28, 8 weeks old) were purchased from the Licensed Laboratory of the Animal Breeding Center (Poznan, Poland). After arrival at the animal care facility, rats were kept under controlled temperature (21 ± 2 °C) and humidity (55–60%) with a 12 h/12 h day/night cycle throughout the experiment. After a 5 day adaptation period, animals were divided into 4 groups (of 7 rats each, initial mean body weight = 216 g), and kept in metal-free individual cages. Rats were fed semi-purified diets: a control (C) or high-fat diets (HF) composed according to the AIN-93M recommendations (Reeves et al., 1993), of casein (20%), soybean oil (7%), wheat starch (53.2%), sucrose (10%), potato starch (5%), L-cysteine (0.3%), vitamin mix AIN-93M (1%) and mineral mix AIN-93M (3.5%), enriched where necessary with additional Cr as CrProp. The high-fat diets (40% calories from fat) were obtained from the basal AIN-93 diet, by replacement of wheat starch with fat (lard 15% and soybean oil 10%). The four experimental groups were the control (C), high-fat (HF), high-fat supplemented with 10 mg Cr kg^{-1} diet (HF + Cr10), and high-fat supplemented with 50 mg Cr kg^{-1} diet (HF + Cr50). The dosages of Cr provided by these diets were 0.2, 0.2, 1 and 5 mg Cr kg^{-1} body mass, respectively, thus supplemental diets provided approx. 5-fold and 25-fold higher dosages of Cr in comparison to the basal diet. The chemical composition of experimental diets is presented in Table 1.

Table 1
Chemical composition of diets used in experiment (mean ± SD).

Ingredient	Diets			
	C	HF	HF + Cr10	HF + Cr50
Energy (MJ 100 g^{-1})	1.83 ± 0.01	2.27 ± 0.02	2.28 ± 0.03	2.29 ± 0.02
Protein (%)	18.7 ± 1.23	17.5 ± 0.14	17.7 ± 036	17.9 ± 0.13
Fat (%)	7.14 ± 0.31	25.4 ± 1.07	26.4 ± 0.21	26.4 ± 0.08
Carbohydrates (%)	61.7	47.9	46.9	46.2
Dry mass (%)	91.8 ± 0.19	93.7 ± 0.31	94.0 ± 0.32	93.5 ± 0.18
Ash (%)	4.06 ± 0.71	2.89 ± 0.23	2.99 ± 0.11	2.90 ± 0.12
Cr (μg g^{-1})	2.01 ± 0.51	2.10 ± 0.28	10.1 ± 0.79	50.4 ± 4.71

Abbreviations: C – control (AIN-93M), HF – high-fat, HF + Cr10 – high-fat with Cr(10 mg kg^{-1}), HF + Cr50 – high-fat with Cr(50 mg kg^{-1}).

Animals had free access to food and distilled water. Food intake was monitored daily and body mass gain was recorded weekly.

After 5 weeks of feeding with these diets, rats were intraperitoneally injected with STZ (35 mg kg^{-1} body mass in citric buffer, pH = 4.4) to induce diabetes, while rats of the control group were injected only with the carrier (citric buffer). On the second day after injection blood drops taken from the tail vein were placed on test strips to measure blood glucose concentration (Optium Medisense glucometer, Abbott Co.) to check hyperglycemia. After STZ injection all animals, irrespective of the type of diet, exhibited elevated fasting blood glucose levels (>16 mmol/l) and were classified as "diabetic" (HF/STZ). Subsequently, all the experimental groups of rats were fed the same diets as before for an additional week until the end of the experiment.

2.2. Test chemicals

The chromium(III) propionate complex (CrProp) in the form of nitrate salt (chemical formula $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]+(NO_3)$ was synthesized at the laboratory of the Department of Product Ecology, Poznan University of Economics, according to the method described previously by Earnshaw et al. (1966). The contents of elemental Cr were determined by the AAS method (an AAS-3 spectrometer with BC correction, Zeiss, Germany). The authenticity was established using the physicochemical characteristics of CrProp as described previously (Wieloch et al., 2007).

2.3. Data collection

At the end of the experiment, after 16-h fasting, rats were anaesthetized with an intraperitoneal thiopental injection and dissected to collect blood from the aorta and remove inner organs (the liver, kidneys, heart, spleen, pancreas, testes) for appropriate biochemical tests.

Organs were washed in saline, weighed and stored at −20 °C until analyzed. All the procedures used in this study were accepted by the Animal Bioethics Committee of Poznan, Poland (Approval # 59/2005).

2.4. Laboratory analyses

2.4.1. Blood morphology

Blood hemoglobin (Hb) level was determined by the Drabkin cyanohemoglobin method (Flaherty, 1991). Red Blood Cell Count (RBC) and other blood morphology parameters (hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), platelets (PLT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large-cell ratio (p-LCR), red cell distribution width based on standard deviation (RDW-SD) were obtained using the CELLDYN-1700 analytical hematology system (Abbot Laboratories, 1995).

2.4.2. Blood Biochemistry

Blood serum indices were determined by the following methods: glucose concentration by the hexokinase method (Sacks et al., 2002), while total, LDL and HDL cholesterol levels and triacylglycerols (TAG) concentration by the colorimetric methods (Miki, 1999; Riesen,1998; Shephard and Whiting, 1990) using Olympus AU 560 equipment. Plasma insulin concentration was measured by the RIA method using kits specific for rats (Linco Research, St. Charles, MO, USA).

Enzyme ALT and AST activity was measured by the kinetic methods (Schumann and Klauke, 2003), while urea concentration was determined by the kinetic method using urease and glutamine dehydrogenase (Newmann and Price, 1999), total