Published in final edited form as:

Biol Trace Elem Res. 2013 March ; 151(3): 406–414. doi:10.1007/s12011-012-9580-9.

Long-term exposure to $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$ in Wistar rats fed normal or high-fat diets does not alter glucose metabolism

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Abstract

The essentiality of chromium(III) has been the subject of much debate, particularly in healthy subjects. Chromium(III)-containing supplements are widely used for body mass loss, building of lean muscle mass, and improving glucose and lipid metabolism. $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$, Cr3, is one of the most-studied chromium nutritional supplements. The current study evaluates the effects of long-term (15 months) supplementation with Cr3 on body mass and glucose metabolism in Wistar rats on traditional and cafeteria-style (high fat, high carbohydrate) diets. Male Wistar rats were randomly assigned to one of four treatment groups: 1) control diet (milled Harlan Teklad LM-485 rodent diet), 2) control diet + 1 mg Cr3/kg body mass/day, 3) a cafeteria-style (CAF) diet (high fat, high carbohydrate), or 4) CAF diet + 1 mg Cr3/kg/day. Cr3 supplementation had no effect on fasting blood glucose levels or blood glucose levels in response to glucose and insulin challenges. Rats consuming the CAF + Cr3 diet tended to have a significantly higher body mass than rats consuming the CAF diet, but necropsy results showed no difference in visceral fat or body wall thickness between groups. These data suggest that long-term Cr3 supplementation does not significantly affect body mass in rats consuming a normal diet or glucose levels or metabolism in rats consuming either diet.

Keywords

$[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$; rat; cafeteria diet; glucose

INTRODUCTION

The idea that chromium(III) is an essential element for proper glucose and fat metabolism in mammals has been debated for more than 50 years. Initially, Schwarz and Mertz [1, 2] proposed that a “glucose tolerance factor” (GTF) was naturally present in the body and that this compound contained chromium [3, 4]. This belief was in part a result of studies in which rats on chromium-deficient diets developed impaired glucose tolerance. When chromium was given to deficient rats by stomach tube, the impairment was reversed [1]. However, the use of metal cages and the failure to measure the actual chromium content of

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Conflict of Interest

JBV is the inventor or co-inventor of patents on the use of Cr3 as a nutritional supplement or therapeutic agent.

the diet has raised concerns about the actual status of the diet; the discontinuance of the use of term “glucose tolerance factor” has been recommended [4].

The National Academy of Sciences set the adequate dietary chromium intake, AI, at 25 µg/day for women and 35 µg/day for men [5]. By this standard, almost all (>98%) Americans have a chromium-sufficient diet [4, 6]. Case studies of humans on total parenteral nutrition (TPN) who developed glucose intolerance and other problems such as neuropathy and encephalopathy have also been cited as evidence of the essentiality of chromium(III) because the addition of chromium improved their symptoms [4, 7]. However, the validity of such an assumption has been questioned. In a review of the case studies, Stearns [3] notes that among the five cases, the time on TPN varied from 5 months to 13 years and that when factors such as TPN chromium levels (present as contamination) and complete absorption were taken into account, no real chromium deficiency was present. Additionally, not all patients shared the same combination of symptoms “chromium-deficient” patients were treated with varying amounts of chromium once symptoms developed, and glucose intolerance did not improve with addition of chromium in one of the cases [3]. The doses of chromium used in the TPN fluid far exceed the AI, especially when it is considered that the chromium in the TPN fluid is administered intravenously, rather than orally. Any effects from chromium should, thus, be considered pharmacological rather than nutritional.

Animal studies since the initial studies of Schwarz and Mertz have attempted to determine whether chromium is essential. For example, rats have been given purified, low Cr diets with high levels of carbohydrates (55% sucrose, 33 µg Cr/kg diet) [8, 9]. Dietary carbohydrate stress is known to increase urinary chromium loss, so that high-carbohydrate diets might be able theoretically to generate chromium “deficiency” in animals for the purpose of studying the effects of deficiency and subsequent supplementation [4]. This diet compared to a diet supplemented with chromium resulted in no effects on body mass, fasting glucose levels, or glucose levels in intravenous glucose tolerance tests. Supplemental chromium, in a pharmacologically-relevant dose, was shown to result in increased insulin sensitivity [8, 9]. Similar results have been obtained using a high fat diet [10]. However, a study using a purified (chromium-free) diet (16 µg Cr/kg diet) and metal-free housing found no impairment in “chromium-deficient” rats [6], lending support to the idea that chromium(III) is not an essential element; however, the study did also find that pharmacological doses of chromium could enhance insulin sensitivity [6].

Just as the essentiality of chromium(III) has been heavily debated, the effects of nutritional chromium(III) supplementation have been debated, particularly in healthy subjects [11, 12]. Chromium(III) is widely used as a nutritional supplement for body mass loss, building of lean muscle mass, and improving glucose and lipid metabolism, despite a lack of conclusive support of these claims in human subjects [13, 14, 15, 16, 17]. Dietary chromium is absorbed with 0.4–2% efficiency [4], so coupling chromium with a suitable ligand is desirable to increase bioavailability. Commercially available chromium supplements (particularly chromium picolinate) are very popular as “natural” complements to traditional pharmaceutical treatments for type 2 diabetics and metabolic syndrome, but the results are inconclusive as to whether or not supplementation is useful [12, 18, 19, 20].

The effects of the nutritional supplement $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$, or Cr3 (also called Kemtrace Cr [21]), on healthy rats and rat models of insulin resistance, colorectal cancer, and type 1 and 2 diabetes have been examined; it has been found to have beneficial effects on insulin sensitivity, the incidence of colorectal cancer, and, in most cases, on lipid parameters [22]. Similar results on insulin sensitivity have been observed in studies with cattle [23]. Cr3 is more water-soluble than chromium nicotinate and chromium picolinate (the two most popular chromium supplements) and does not break down in the GI tract like

chromium chloride and chromium nicotinate [24]. Cr3 has been shown to decrease fasting plasma triglycerides and cholesterol in healthy and diabetic rats, as well as decreasing plasma insulin concentrations and increasing insulin sensitivity in diabetic rats [24]. The compound has been found to be nontoxic in *in vitro* and *in vivo* assays and to not damage DNA *in vitro* although the compound can be oxidized by biological oxidants in the absolute absence of reducing agents [22].

To date, most of the available literature exploring the effects of chromium supplementation in rats have been short-term studies; few have looked at the effect of long-term chromium supplementation. No reports to date have appeared on the effects of long-term supplementation with Cr3, particularly at pharmacological levels. Thus, the current study evaluates the effects of long-term supplementation on body mass and glucose metabolism in Wistar rats on traditional and cafeteria-style (high fat, high carbohydrate) diets.

MATERIALS AND METHODS

Animals and Exposure

Forty-eight male Wistar rats, obtained from Charles River Breeding Laboratories (Portage, MI), were housed in a USDA-approved animal facility in rooms maintained at $25 \pm 2^\circ\text{C}$ and 50 % to 70 % humidity with a 12 hour photoperiod. After a two week acclimation period, each rat was uniquely identified by ear punch and randomly assigned to one of four treatment groups: 1) control diet (milled Harlan Teklad LM-485 rodent diet) (n = 11), 2) control diet + 1 mg Cr3/kg body mass/day (n = 12), 3) a cafeteria-style (CAF) diet (33 % powdered Harlan Teklad LM 485 rodent chow, 33% sweetened condensed milk, 27 % water, and 7 % granulated sucrose) [25] (n = 12), or 4) CAF diet + 1 mg Cr3/kg/day (n = 12). One rat originally purchased was found to have a severe dental misalignment and was not included in the study. Rats were individually housed in shoe-box type polycarbonate cages with recycled bedding and were allowed to consume the appropriate diet and water *ad libitum*. At the onset of the study, the rats were approximately 8 weeks of age and had an average body mass of 278g. There was no significant difference in initial body mass of the rats among groups.

Cr3 was synthesized according to the methods of Earnshaw et al. [26] and generously provided by the Vincent laboratory at The University of Alabama. The authenticity of the Cr3 was established by high resolution electron impact mass spectrometry [27]. Milled rodent diet was purchased from Harlan Teklad (Madison, WI). Cr3 was added to the appropriate diets in sufficient quantities to achieve the appropriate concentration of the test compound. All calculations were based on data from a previous study (unpublished data) that indicated male Wistar rats consume an average of 33 g diet/day. Extensive stability studies indicate that chromium test compounds are extremely stable and that no degradation in the diet would be expected [28].

Because the purpose of this study was to determine the effects of pharmacological levels of chromium, no special measures were taken to prevent exposure of the rats to small amounts of chromium that may be introduced into the diet through methods of feed preparation or from the cage hardware. The diet purchased, Teklad LM-485 (7012), contained added chromium in the form of chromium potassium sulfate (0.48 mg/kg of diet).

Data Collection

This study had a duration of 15 months. During this time, body mass and food consumption were measured twice weekly. Blood samples were collected monthly via saphenous venipuncture. Blood glucose was measured at the time of collection using a One-Touch Ultra™ glucose meter. Data collection continued for 15 months, during which time 3

glucose challenges and 2 insulin challenges were also administered. Glucose was administered intraperitoneally at a dosage of 2 g/kg body mass, and blood glucose levels were measured prior to injection and 30, 60, 90, and 120 minutes after injection. Bovine zinc insulin (5 units/kg body mass) was administered subcutaneously, and blood glucose levels were measured as in the glucose challenges.

After 15 months, rats were euthanized and necropsied. Final body mass, body wall thickness, organ masses (liver, kidneys, lungs, and heart), and visceral fat mass were measured. Data from animals that died prior to 15 months were not included in the analyses, but those animals were necropsied and measured.

Statistical Methods

Data means were analyzed by one-way ANOVA using SPSS (SPSS, Inc., Chicago, IL) followed by a Fisher's LSD post-hoc multiple comparisons test to determine specific significant differences ($p < 0.05$). All data are presented as the mean $\pm$ the standard error of the mean (SEM).

RESULTS AND DISCUSSION

No significant differences ($p > 0.05$) were present among fasting blood glucose levels (Figure 1). Results of a glucose challenge, administered at months 4 and 14, and their respective area under the curve (AUC) analysis are shown (Figures 2–5). Differences were present in the rats' responses to glucose challenges, but AUC analyses (Figures 4 and 5) show that the differences were not consistent or biologically meaningful. Thus, rats on either diet with or without Cr3 supplementation could handle an increase in glucose load without an appreciable increase in blood glucose levels. Cr3 exposure did not affect the rats' responses to the insulin tolerance tests administered at months 10 and 14 of the study, as shown in the blood glucose levels (Figure 6 and 7) and the respective AUC (Figures 8 and 9). Thus, all of the rats were able to manage their blood glucose levels after the administration of exogenous insulin equally well.

Rats consuming the CAF + Cr3 diet tended to have a significantly ($p < 0.05$) higher body mass than rats consuming the CAF diet (Figure 10). As chromium supplements are purported to reduce fat mass and increase muscle mass, one would expect the increase in body mass to be attributed to changes in these parameters. However, an examination of the visceral measurements (Table 1) reveals that no statistically significant increases in body wall thickness (a measure of muscle tissue) or reduction in visceral fat occurred. In addition, if Cr3 supplementation had increased lean muscle mass and decreased visceral fat mass, this effect should have been paralleled in the rats consuming the control diet. However, these parameters are nearly identical between these groups. Rats consuming the CAF diet, with or without Cr3 supplementation, had significantly higher body mass than rats consuming either of the control diets (Figure 10). This difference appears to stem primarily from an increase in visceral fat and not from an increase in lean muscle tissue (Table 1).

The results of this study indicate that long-term supplementation with Cr3 is not beneficial to healthy or obese rats with regard to blood glucose levels or body composition. Studies of the effects of chromium supplementation of rats have been shown to vary depending on whether the rats were healthy or what type of disease model was being examined [22]. Regardless of strain, chromium has no effect on the body composition or food intake of healthy rats [22]. Other than studies with Cr3, chromium administration generally has no effect on blood variables in healthy rats. In obesity, type 2 diabetes, and cardiovascular disease models that have mutations of the leptin receptor (Zucker obese, Zucker diabetic fatty, and JCR:LA-cp rats), chromium administration generally appears to have no effect on

fasting glucose levels but does lower glycated hemoglobin levels [22]. Chromium also appears to have beneficial effects on total cholesterol levels, while effects on other lipid variables are variable, and on insulin sensitivity. For rats with streptozotocin-induced diabetes (a type 1 diabetes model), the results are too variable for any conclusion to be drawn. When streptozotocin treatment is combined with a high-fat diet, the rats tend to have lower fasting blood glucose levels with no effects on insulin or lipid levels [22]. Rats on high carbohydrate or high fat diets tend to have increased insulin sensitivity and beneficial effects on lipid levels without accompanying effects on blood glucose levels [22].

A few studies have appeared previously on the use of Cr3 in various rat models, although they were considerably shorter in duration. Healthy Sprague Dawley rats treated daily with 20 µg Cr/kg body mass as Cr3 intravenously for 12 weeks had lower blood plasma insulin, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, but not glucose, levels [29]. When given intravenously at 20 µg Cr/kg body mass, Cr3 lowered blood plasma insulin, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, but not glucose, levels in healthy Sprague Dawley rats after 4, 8, 12, 16, 20 and 24 weeks; also 2-h plasma glucose and insulin levels after a glucose challenge were lowered [30]. In rats with streptozotocin-induced diabetes, this treatment had no consistent statistically significant effects although plasma insulin, total cholesterol, and triglycerides tended to be lower [30]; the streptozotocin treatment appeared to have increased the spread of the value of the measured variables resulting in a loss of sufficient power to resolve any effects. Finally, similar intravenous treatment of Zucker obese rats resulted in lower blood plasma insulin, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, but not glucose, levels; also 2-h plasma insulin, but not glucose, levels after a glucose challenge were lowered [30]. In contrast, Cr3 only lowered plasma insulin levels in Zucker lean rats, suggesting a difference between effects in healthy strains; however, total cholesterol and triglycerides tended to be lower in these animals [30]. Two-h plasma insulin, but not glucose, levels after a glucose challenge were lowered for both types of Zucker rat [30].

The effects of oral (gavage) administration of the complex have been examined [31]. At levels of 250, 500, or 1000 µg Cr/kg body mass, the treatment at all concentrations lowered fasting plasma insulin, triglycerides, total cholesterol and LDL cholesterol levels of healthy Sprague Dawley rats while having no effect on plasma glucose or HDL cholesterol. These levels were lower after 4 weeks of treatment and remained lower for the next 20 weeks of treatment. The maintenance of glucose levels with less insulin indicates increased insulin sensitivity. Both plasma glucose and insulin levels were lowered in 2-h glucose tolerance tests. In Zucker obese rats, the early stage type 2 diabetes model, receiving 1000 µg Cr/kg body mass, the results were similar to those from intravenous administration. In this study, the effects of the biomimetic cation on Zucker Diabetic Fatty (ZDF) rats were also examined using 1000 µg Cr/kg body mass. Again, fasting plasma insulin, triglycerides, total cholesterol and LDL cholesterol levels were all lower while glucose concentrations were consistently but not statistically lower. HDL levels were lowered from their very high levels; 2-h plasma insulin levels were also lowered. Plasma glycated hemoglobin levels, a measure of longer term blood glucose status, were examined in the healthy, Zucker obese and ZDF rats after 4, 12 and 24 weeks of treatment. No effect was seen for the healthy rats; however, significant effects were noted for the diabetic models. For the ZDF rats, glycated hemoglobin was lower after 12 and 24 weeks of treatment, reaching almost a 22% drop compared with ZDF controls by week 24; for the Zucker obese rats, glycated hemoglobin was 27% lower at week 24 [31]. The Zucker obese rats receiving Cr3 in that study gained more body mass than rats in any other group, which is also similar to what was observed in the current study – rats consuming the CAF diet supplemented with Cr3 had the highest body mass and were significantly heavier than rats on the unsupplemented CAF diet.

The effects of the cation on healthy and model diabetic rats have also been examined by Krejpcio and co-workers. Male Wistar rats were provided a control diet or a diet containing 5 mg Cr/kg diet as the cation for 10 weeks [32,33]. Blood plasma insulin levels were lowered 15.6% by the Cr-containing diet, while glucose transport by red blood cells was increased 9.6%. In another study, this group utilized male Wistar rats with streptozotocin-induced diabetes. Using similar diets for 5 weeks, the rats that had the Cr diet had lower blood serum glucose levels (26%) and increased HDL levels (14%) [34]. Cr3 supplementation of the diet (AIN-93M or high-fructose diet) of male Wistar rats for 8 weeks (0, 1, and 5 mg Cr/kg body mass daily) has been shown to result in increased insulin sensitivity without affecting blood plasma glucose or lipid levels; no effects were observed on body mass as an effect of supplemental chromium [18]. Thus, the results of the short term study with male Wistar rats are very similar to those of the current study. In another study from the same laboratory, male Wistar rats were fed a control (AIN-93M) diet or high fat diet with or without chromium supplementation as Cr3 (0, 1, or 5 mg Cr/kg body mass) for 5 weeks; rats were subsequently injected with streptozotocin before being fed the same diets for another week [35]. Cr3 increased insulin sensitivity and lowered serum total and LDL cholesterol and triglycerides levels but had no effect on blood glucose levels. No effects on body mass were observed except the group on the high-fat diet receiving the highest dose of Cr3 had lower body mass weeks 3 through 5 than rats on the high-fat diet not receiving any supplemental chromium; the effect disappeared after streptozotocin treatment. The effect of Cr3 on blood glucose was again similar to that of the current study.

The data obtained in this study strongly suggest that long-term Cr3 supplementation does not significantly affect body mass or metabolic responses in blood glucose concentration to glucose or insulin in male Wistar rats consuming a normal diet or a high-fat, high-carbohydrate cafeteria-style diet. Thus, the results of this long-term study on Cr3 supplementation are consistent with short-term studies on the effects of Cr3 on healthy Wistar rats or Wistar rats receiving a high-carbohydrate or high-fat diet. These results, along with the results of many shorter-duration studies [4, 6, 36, 37] clearly support the idea that chromium is not an essential element for proper glucose metabolism. The effects of long-term supplementation on insulin sensitivity and on lipid parameters such as total cholesterol, low-density lipoprotein, and high-density lipoprotein levels in normal rats, particularly those on the high-fat, high-carbohydrate diet were not explored in this study but are areas that will require further research.

Acknowledgments

This project was made possible by NIH grant number P20 RR016475 from the INBRE Program of the National Center for Research Resources to Emporia State University and by a faculty research creativity award from Emporia State University to Melissa M. Bailey.

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Fig. 1.
Effect of Cr3 on fasting blood glucose in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

Fig. 2. Glucose challenge, month 4, in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

Fig. 3. Glucose challenge, month 14, in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

Fig. 4.
AUC, glucose challenge, month 4.

Fig. 5.
AUC, glucose challenge, month 14.

Fig. 6. Insulin challenge, month 10, in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

Fig. 7. Insulin challenge, month 14, in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

Fig. 8.
AUC, insulin challenge, month 10.

Fig. 9.
AUC, insulin challenge, month 14.

Fig. 10. Effect of Cr3 on body mass in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation. ^aDiffers significantly from all other groups ($p < 0.05$) ^bDiffers significantly from both control groups ($p < 0.05$)

Table 1

Effect of Cr3 on visceral measurements in Wistar rats fed normal and cafeteria-style diets with and without Cr3 supplementation.

	Control	Control + Cr3	CAF	CAF + Cr3
Liver (g)	25.75 ± 1.37	26.29 ± 1.08	28.05 ± 2.28	28.44 ± 1.15
L. lung (g)	2.2 ± 0.17	2.36 ± 0.12	2.27 ± 0.16	2.56 ± 0.20
R. lung (g)	2.65 ± 0.19	2.25 ± 0.15	2.00 ± 0.16	2.64 ± 0.25
Heart (g)	2.75 ± 0.18	2.60 ± 0.13	2.77 ± 0.21	2.86 ± 0.17
L. kidney (g)	2.58 ± 0.10	2.57 ± 0.12	2.78 ± 0.39	2.44 ± 0.13
R. kidney (g)	2.61 ± 0.10	2.64 ± 0.15	3.08 ± 0.41	2.44 ± 0.13
Visceral fat (g)	31.45 ± 3.26	32.33 ± 3.01	47.92 ^a ± 2.83	57.18 ^a ± 5.18
Body wall thickness (mm)	5.83 ± 0.30	5.85 ± 0.28	6.77 ± 0.25	6.89 ± 0.46

Data are mean ± SEM

^aDiffers significantly from Control and Control + Cr3 (p < 0.01)