Effects of Chromium Picolinate on Some Hemoglobin Properties and (Metabolic) Functions in Healthy Rats

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Abstract: This study examined the effects of chromium picolinate (CrPic) on hemoglobin derivatives, thermodynamic parameters of hemoglobin (Hb), plasma levels of glucose, insulin, glycosylated Hb (HbA$_{1c}$), creatinine, albumin, high density lipoprotein (HDL), cholesterol and body weight of rats. Albino rats were divided into two groups. The control group (group I) received a standard diet; group II received 5 mg of CrPic per kg of diet for six weeks. The results of Hb derivatives, as determined by multicomponent spectrophotometric analysis, revealed significant increases in the percentages of inactive Hb derivatives (sulfohemoglobin, SHb, methemoglobin, MetHb and carboxyhemoglobin, HbCO), $P < 0.01$, concomitant with a significant decrease in the percentage of the functional Hb (in the oxyhemoglobin, HbO$_2$, form) in the CrPic-treated group, compared with controls ($P < 0.01$). The results of thermodynamic parameters revealed significant decreases in enthalpy ($\Delta H°$) and entropy ($\Delta S°$), $P < 0.01$, concomitant with a significant increase in free energy ($\Delta F°$) of Hb extracted from the CrPic-treated group, compared with controls ($P < 0.01$), indicating a conformational change of Hb in the treated group, compared with controls. CrPic supplementation lowered the blood levels of glucose by 11% ($P < 0.00005$) and insulin by 65% ($P < 0.00005$), indicating an improvement in insulin sensitivity by enhancing intracellular insulin receptors. While no significant change in HbA$_{1c}$ level was observed. No significant effect of CrPic on plasma creatinine, albumin, HDL and cholesterol levels were observed. No significant effect of CrPic on body weight of rats was also observed. These results suggested that CrPic at the given doses that improved insulin sensitivity, has no toxic effects on liver and kidney functions of healthy rats, while it has toxic effects on Hb function.

Key words: Chromium picolinate · Hemoglobin derivatives · Thermodynamic parameters · Glucose · Insulin · HbA$_{1c}$, creatinine · Albumin · HDL · Cholesterol

INTRODUCTION

Chromium picolinate (CrPic; chromium(III) tris (picolinate) is a popular dietary supplement, especially in the United States whereas the manufacture and sale of this compound and other chromium (III) complexes has become multimillion dollar industry [1]. This may be of commercial benefit for industry, but it also illustrates that a great number of individuals in the population are exposed to these type of compounds. CrPic is available over-the-counter and can be bought at health food stores, supermarkets, pharmacies and mail order companies in a variety of forms including pills, sport drinks, nutrition bars and chewing gums [2,3]. The compound is primarily used by the general public for reduction of body weight and by athletes for improvement of body composition [1]. It has also been suggested that CrPic could improve the glycaemic control in patients with type-2 diabetes [4- 6] and that it has an antidepressant effect for patients with atypical depression [7]. Most of these beneficial health effects of CrPic have been questioned by various authors claiming, for example, that this type of nutritional supplementation has little or no demonstrated effects on body composition of healthy individuals [8 - 10] and that the data from the studies on diabetes patients are inconclusive [11]. There have also been claims that suggest that CrPic has beneficial effects on plasma glucose and insulin concentrations and other blood

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variables in healthy subjects. These claims have not been substantiated, as shown by another recent review [3] and meta-analysis [11].

It is not only the potential benefits of nutritional supplementtion with chromium (III) complexes that are under dispute; the same situation seems to be true when it comes to the detrimental effects of CrPic. In general, the knowledge about the toxicological profile of CrPic is rather limited and the data that do exist are contradictory, especially when it comes to the potential genotoxicity of the compound. Indeed, there are some isolated case-reports of acute adverse drug reactions in humans following from the ingestion of CrPic, including, for example, anemia, thrombocytopenia, hemolysis, chest pain, liver dysfunction, interstitial nephritis and cognitive, perceptual and motor changes [12-15] but the significance of these case-reports is almost impossible to evaluate. Other authors could not find any clinically significant hematological, renal or hepatic changes in subjects that had been ingesting CrPic supplement on a daily basis over a period of at least 3 months [6,16].

In the literature, only few studies re available on the general toxicity of CrPic. The most extensive toxicity study of CrPic so far seems to be another NTP study evaluating the subchronic toxicity of CrPic in rats and mice [17]. In the latter study, groups of 10 male and 10 female F344/N rats and B6C3F1 mice were exposed for 13 weeks to a diet containing 0-50000 ppm CrPic (in the form of the monohydrate) without significantly affecting the body weight gain, survival, organ weights, hematology and clinical chemistry parameters. Similar results were obtained in another, less exhaustive study on Sprague-Dawley rats [4,5]. In the latter study, the rats were fed a diet containing 10-100 ppm Cr(III) in the form of CrPic for 20 weeks without any evidence of toxicity in terms of changes in body weight, organ weights or hematology.

Taken together the data on the potential toxicity of CrPic are indeed contradictory. The major objective of the present study was therefore to evaluate the potential toxic effects of this compound on Hb, liver and kidney functions in healthy rats.

MATERIALS AND METHODS

Chemicals: Commercial chromium picolinate was purchased from Arab company for Pharmaceuticals and Medicinal Plants, Cairo, Egypt and all commercial kits used in this study were purchased from Biodiagnostics company, Cairo, Egypt.

Animals: The animals care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland. Thirty male Swiss albino rats weighing, in average, 102 ± 6.4 gm obtained from the National Research Center were used in the present experiment. Rats were housed 8 per cage at a constant temperature ($24 \pm 2°C$) with alternating 12 hours light and dark cycles and fed standard laboratory food and water ad labium.

Experimental Design: Thirty Albino rats were divided into two groups, 15 rats for each. The control group (group I) received a standard diet; group II received 5 mg of CrPic per kg of diet for six weeks.

Blood Collection: Animals were anesthetized and blood samples were collected by heart puncture at the end of experimental period. Heparin was used as anticoagulant. Part of blood was used for determination of the percentages of Hb derivatives and thermodynamic parameters and the other part for biochemical analyses.

Hemoglobin Extraction: The blood was centrifuged at 3000 rpm for 5 minutes and the plasma was removed. The packed erythrocytes were washed three times with 4-fold phosphate-buffered saline (PBS) to remove the plasma remnant. After each procedure, erythrocyte PBS mixture was centrifuged at 3000 rpm for 5 min. The packed erythrocytes were brought to 2.0-times the original blood volume with ice-cold distilled water to obtain hemolysate. After mixing thoroughly, the hemolysate was centrifuged at 10,000 rpm for 20 minutes to remove erythrocytes ghosts.

Hemoglobin Derivatives Determination: The muticomponent spectrophotometric method for the determination of four Hb derivatives was carried out as described by Atef *et al.* [18]. The method was used for determination of SHb, MetHb, HbCO and the remaining functional Hb in the HbO_2 form. The measurements were carried out at four wave lengths ($\lambda = 500, 569, 577$ and 620 nm) using Shimadzu UV/ VIS double-beam spectrophotometer model-240, Japan.

Thermodynamic Parameters of the Spin Equilibrium Reaction of HbO_2: In this experiment, the absorbance (A_{578}) of the Hb aqueous solution with a concentration of 3.4×10^{-5} M, at the spin state band of HbO_2 ($\lambda = 578$), was measured at various temperatures in the range 25-40°C (5°C intervals). The spin-state equilibrium constant

at each temperature was calculated as described previously [19], using the following equation:

$$K = A_{578} / 1 - A_{578} \qquad (1)$$

The enthalpy ($\Delta H°$), free energy ($\Delta F°$) and entropy ($\Delta S°$) of the equilibrium reaction were calculated by means of Van't Hoff equations [19].

Determination of Glucose in Plasma: The plasma level of glucose was determined spectrophotometrically according to the method of Jatana [20].

Determination of Insulin in Plasma: The plasma level of insulin was determined according to the method of Focardi *et al*. [21].

Determination of Glycosylated Hemoglobin in Plasma: The plasma level of HbA1c was determined spectrophotometrically according to the method of Bunn [22].

Determination of Creatinine in Plasma: The plasma level of creatinine was determined spectrophotometrically according to the method of Davalos-Misslitz *et al*. [23].

Determination of Albumin in Plasma: The plasma level of albumin was determined spectrophotometrically according to the method of Cosgrove *et al*. [24].

Determination of High Density Lipoprotein in Plasma: The plasma level of HDL was determined spectrophotometrically according to the method of Widhaim and Pakosta [25].

Determination of Cholesterol in Plasma: The plasma level of cholesterol was determined spectrophotometrically according to the method of Sundvall *et al*. [26].

Statistical Analysis: Data are presented as means ± S.D. Student's t-test was used for determination of the level of significance of difference between the two groups. The difference is considered significant at P<0.05.

RESULTS

The results of percentages of Hb-derivatives before and after treatment of rats with CrPic are shown in Table (1). Results revealed significant increases in the percentages of inactive Hb derivatives (SHb, MetHb and HbCO), $P < 0.01$, concomitant with significant decrease in the percentage of the functional Hb (in the HbO_2 form) in the CrPic-treated group, compared with controls, $P < 0.01$.

The results of thermodynamic parameters of Hb (Table 2) revealed significant decreases in enthalpy ($\Delta H°$) and entropy ($\Delta S°$), $P < 0.01$, concomitant with a significant increase in free energy ($\Delta F°$) of Hb extracted from the CrPic-treated group, compared with controls ($P < 0.01$).

Th results of plasma levels of glucose, insulin and HbA_{1c} before and after treatment of rats with CrPic are shown in Table (3). The results revealed significant decreases in plasma level of glucose by 11% ($P < 0.00005$) and plasma insulin by 65% ($P < 0.00005$) were obvious in the CrPic-treated group, compared with the control group. While insignificant change in the plasma level of HbA_{1c} was observed.

Table 1: Percentages of Hb derivatives before and after treatment of rats with CrPic

Group	SHb %	MetHb %	HbCO %	HbO₂ %
Control (n=15)	0.71±0.033	1.48±0.64	2.98±0.09	92.1±0.31
CrPic-treated (n=15)	0.96±0.029**	2.22±0.053**	3.34±0.09**	87.88±0.37**

** $P < 0.01$

Table 2: Thermodynamic parameters of the spin equilibrium reaction of HbO₂ before and after treatment of rats with CrPic

Group	$\Delta F°$ (Joul/mole)	$\Delta H°$ (Joul/mole)	$\Delta S°$ (Joul/mole)
Control (n=15)	$4.79 \times 10^2 \pm 0.39$	$7.23 \times 10^3 \pm 0.21$	26.72±0.098
CrPic-treated (n=15)	$7.42 \times 10^2 \pm 0.26$**	$5.56 \times 10^3 \pm 0.19$**	24.55±0.087**

** $P < 0.01$

Table 3: Plasma levels of glucose, insulin and HbA1c before and after treatment of rats with CrPic

Group	Glucose (mg/dl)	Insulin (µU/ml)	HbA₁c %
Control (n=15)	144.6±4.7	59.8±4.9	4.2±0.8
CrPic-treated (n=15)	128.7±3.4***	20.9±1.4***	4.2±0.9

*** $P < 0.00005$

Table 4: Plasma levels of creatinine, albumin, HDL and cholesterol before and after treatment of rats with CrPic

Group	Creatinine (mg/dl)	Albumin (g/dl)	HDL (mg/dl)	Cholesterol (mg/dl)
Control (n=15)	0.44±0.02	2.7±1.2	32±4.4	57.8±6.4
CrPic-treated (n=15)	0.43±0.03	2.6±1.1	31±4.1	56.9±5.7

Table 5: Body weights of rats before and after treatment with chromium picolinate during a period of 6 weeks

Group	1st	2nd	3rd	4th	5th	6th
Control (n=15)	102±6.4	154±7.9	183±8.9	247±10.8	273±11.6	300±15.8
CrPic-treated (n=15)	105±6.3	157±7.8	184±9.8	253±10.7	279±11.7	307±16.3

The results of plasma levels of creatinine, albumin, HDL and cholesterol of rats before and after treatment with CrPic are shown in Table (4). No significant effect of CrPic on plasma creatinine, albumin, HDL and cholesterol levels were observed. Also, No significant effect of CrPic on body weight of rats was also observed (Table 5).

DISCUSSION

Chromium (Cr) potentiates the action of insulin and improves glucose tolerance after long–term supplementation. The present study showed that CrPic supplementation lowered the blood levels of glucose and insulin, indicating an improvement in insulin sensitivity by enhancing intracellular insulin receptors. These results are consistent with those reported previously by Frauchiger *et al.* [27] who studied the effects of chromium supplementation on postprandial metabolism in healthy young men. They found that after addition of 400 and 800 μg CrPic incremental area under the curve (AUC) for capillary glucose was 23% (P = 0.053) and 20% (P = 0.054), respectively, lower than after the white bread meal. These differences reached significance if the subjects were divided into responders (n = 10) and non-responders (n = 3). For responders AUC after 400 and 800 μg Cr was reduced by 36 and 30%, respectively (placebo 175±22, Cr400 111±14 (P < 0.01), Cr800 122±15 mmol. min/L (P < 0.01). They concluded that CrPic may have acute effects and might be beneficial in lowering the glycemic index of a meal.

CrPic was found to have beneficial effects on zucker obese rats, models for the early stages of type 2 diabetes [28,29], while smaller, but significant effects were also noted in healthy rats. In the latter study CrPic was found to decrease plasma insulin and glucose in healthy rats. Cefalu *et al.* [30] observed beneficial effects of CrPic administration (18 μg Cr/kg body mass daily, equivalent to 540 μg for a 60 kg subject) on insulin sensitivity in a rat model for type 2 diabetes mellitus (T2DM). Another study [31] reported metabolic effects of CrPic in a rat model of T2DM. The addition of CrPic lowered glucose at an average of 63%. Effects of chronic CrPic treatment in uninephrectomized rat were reported previously [32]. Plasma insulin concentration was lower in the CrPic-treated group, suggesting improved insulin sensitivity.

Investigations over the last two decades have suggested that there is a naturally-occuring biomolecule that binds trivalent Cr that can explain how Cr is involved in carbohydrate and lipid metabolism. This molecule, chromodulin (originally termed low-molecular-weight Cr-binding substance), is a naturally-occurring oligopeptide composed of glycine, cysteine, aspartate and glutamate with the carboxylates comprising more than half the total amino acid residues [33,34]. Despite its small size (molecular weight approximately 1438 for the bovine liver material), the molecule tightly binds four equivalents of Cr^{3+}. Chromodulin can increase insulin receptor activity as much as eightfold [35]. Based on these results, it has been proposed that chromodulin functions as part of a unique autoamplification system for insulin signaling [36,37]. In this mechanism apochromodulin is stored in insulin-sensitive cells. In response to increases in blood insulin concentration insulin binds to its receptor, bringing about a conformation change that results in autophosphorylation of tyrosine residues on the internal side of the receptor. This process transforms the receptors into an active tyrosine kinase and transmits the signal from insulin into the cell. In response to insulin Cr is moved from the blood into insulin-sensitive cells. Here, the Cr flux results in the loading of apochromodulin with Cr. The holochromodulin then binds to the receptors, presumably assisting in the maintenance of the receptors in its active conformation, amplifying the receptor's kinase activity. When the signaling is to be turned off, a drop in blood insulin levels facilitates relaxation of the conformation of the receptor and the holochromodulin is excreted from the cell into the blood. Ultimately, chromodulin is efficiently excreted in the urine. The Fe-transport protein transferrin has recently been shown to be responsible for maintaining Cr^{3+} levels in the blood plasma and for transporting Cr to tissues in an insulin-responsive manner [35,38].

Only 6 years after the initial report of potentially beneficial effects from CrPic supplementation, Stearns *et al.* [39] reported the first chemical evidence for concerns over the use of CrPic. The complex generated chromosome damage in a Chinese hamster ovary cell model. Subsequently, damage was demonstrated in murine macrophages [40] and another study using the same cell line observed oxidative damage associated with CrPic [41]. Stearns and colleagues [42] and Manygoats *et al.* [43] in continuing work with Chinese hamster ovary model have observed mitochondrial damage and apoptosis generated by the supplement is mutagenic. Previous study [44] reported a significant increase in the urinary excretion of 8-hydroxy-deoxyguanosine in rats, indicating oxidative DNA damage in vivo. The effects have been postulated to arise from the released picolinate ligand [39,42] or from reactive oxygen species catalytically generated by the intact complex [2,45].

Chromium picolinate is a very stable hydrophobic molecule which allows it to be readily absorbed from the digestive tract and readily cross membranes. But it appears that once inside cells, trivalent chromium (Cr^{3+}) picolinate can be reduced to divalent chromium (Cr^{2+}) which can participate in the Fenton reaction in the presence of hydrogen peroxide to generate hydroxyl radicals which cause DNA damage [2,46]. Chromium (III) induced oxidative stress in goldfish liver and kidney [47] and brain [48]. This high oxidative stress may explain the increase in the rate of HbO_2 autoxidation [49] and therefore the increase in the level of inactive oxidized Hb (MetHb) after treatment of rats with CrPic observed in the present study. Methemoglobin is a form of the oxygen-carrying protein hemoglobin, in which the iron in the heme group is in the Fe^{3+} state, not the Fe^{2+} of normal hemoglobin. Hemoglobin can be considered to exist in active and inactive states. When the iron atom is in the ferrous form, the protein is active and can bind oxygen reversibly. The oxidation to the ferric form (MetHb) leads to an inactive protein. Methemoglobin is unable to carry oxygen.

When red cells reach the end of their life due to aging or defects, they are broken down, the hemoglobin molecule is broken up and the iron gets recycled. When the porphyrin ring is broken up, the fragments are normally secreted in the bile by the liver. This process also produces one molecule of carbon monoxide (CO) for every molecule of heme degraded [50]; this is one of the few natural sources of carbon monoxide production in the human body and is responsible for the normal blood levels of carbon monoxide and carboxyhemoglobin (HbCO) even in people breathing pure air. This may explain the higher HbCO levels observed in the present study that accompany the higher rate of hemolysis induced by CrPic treatment [14] and Hb and heme degradation.

The binding of oxygen is affected by molecules such as carbon monoxide (CO). CO competes with oxygen at the heme binding site. Hemoglobin binding affinity for CO is 200 times greater than its affinity for oxygen, meaning that small amounts of CO dramatically reduces hemoglobin's ability to transport oxygen. Hemoglobin bonds to carbon monoxide preferentially (200: 1 more so) compared to bonding to oxygen, so effectively HbCO will not release the carbon monoxide and therefore hemoglobin will not be available to transport oxygen from the lungs to the rest of the body.

Since the inactive components of Hb (SHb, MetHb and HbCO) are unable to transport oxygen, the net concentration of active or functional Hb (in the HbO_2 form) is an indicator of the actual degree of anemia. The increase in the percentages of MetHb and HbCO components may explain the decrease in the concentration of the functional Hb in (the HbO_2 form) induced by CrPic treatment of healthy rats observed in the present study.

No significant changes in the plasma concentration of creatinine, albumin, HDL and cholesterol after treatment with CrPic, when compared to controls, were observed. These results indicated that the renal and liver functions are not affected by CrPic. These results are consistent with many previous studies. Mahmood *et al.* [51] studied the effects of CrPic on the kidney of the obese Zucker rat. This study reported that indices of renal function or histopathology were not affected by CrPic treatment. Anderson *et al.* [5] studied the effects of CrPic on cholesterol, protein and creatinine of healthy rats. In this study Harlan Sprague Dawley rats were fed a stock diet to which was added 0, 5, 25, 50 or 100 mg of Cr per kg of diet as picolinate. This study demonstrated no toxic effects of CrPic on these blood indices. Also histological evaluation of the liver and kidney of control and animals fed 100 mg/kg CrPic also did not show any detectable differences. Another study [32] suggested that chronic CrPic did not adversely affect renal function of uninephrectomized rat.

The results of the present study are contradictory with previous studies. In the first study [31] the addition of CrPic to fat-fed streptozotocin (STZ)-treated rat lowered total cholesterol by 9.7% ($P < 0.001$), triglycerides by 6.6% ($P < 0.001$) and creatinine level by 25% ($P < 0.01$).

Histopathologic findings suggested that CrPic-treated group had normal renal tubular appearance compared with STZ-treated group. Normal appearance of hepatocytes was observed in the CrPic-treated group. A second study [52] reported that CrPic treatment decrease elevated serum creatinine as well as elevated serum levels of hepatic enzymes of STZ-diabetic rats.

The present study showed no effects of CrPic on body weight of healthy rats. These results are consistent with previous studies [52,53].

The results of the present study suggested no toxic effects of CrPic on liver and kidney functions of healthy rats, while it has toxic effects on Hb function.

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