

Stability and Absorption of Chromium and Absorption of Chromium Histidinate Complexes by Humans

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ABSTRACT

Increased intake of chromium (Cr) often leads to improvements in glucose, insulin, lipids, and related variables in studies involving humans and experimental and farm animals. However, the results are often variable, depending not only on the selection of subjects but also dietary conditions and the form of supplemental Cr used. Our objective was to find a Cr supplement suitable for humans that was absorbed better than any of those available. Chromium absorption by six adult subjects, three males and three females, was determined based on the amount of Cr excreted in the urine in the initial 2 d following intake of 200 μg of Cr of the various forms of chromium tested. The absorption of the newly synthesized complexes was greatest for those containing histidine. Urinary Cr losses for six control subjects consuming 200 μg of Cr as Cr histidinate increased from basal levels of 256 ± 48 to 3670 ± 338 ng/d compared with 2082 ± 201 ng for Cr picolinate, the currently most popular nutrient supplement, in the 48 h following Cr consumption. Chromium histidinate complexes were stable and absorption was similar to the initial values after more than 2 yr. Mixing of some of the complexes with starch, which was postulated to improve Cr absorption, was shown to essentially block Cr absorption within 1 mo. These data demonstrate that urinary Cr losses need to be determined because stability and absorption of the Cr complexes varies widely and could be responsible for the variability in some of the Cr supplementation studies. Chromium

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histidinate complexes are absorbed better than any of the Cr complexes currently available and need to be evaluated as Cr nutritional supplements.

Index Entries: Chromium; trace elements; diabetes; insulin; glucose; histidine.

INTRODUCTION

Dietary intake of chromium (Cr) appears to be suboptimal based on the numerous studies reporting beneficial effects of various forms of supplemental Cr. However, not all studies have observed the effects of supplemental Cr (1–4). Because Cr is a nutrient and not a therapeutic agent, only subjects whose Cr status is marginal or deficient would be expected to respond. Therefore, the Cr status of the subjects might explain the varying responses to supplemental Cr. However, the Cr supplement consumed could also affect the response to Cr. Ligand displacement, substitution, or exchange reactions are slow for Cr, with half-times often of tens of hours (5) compared with microseconds or less for most metals. Because Cr complexes might be slow to dissociate, some might be poorly absorbed or be converted to forms with very low solubility and low absorption.

Some forms of Cr have been reported to be absorbed much better than other forms tested; that is, Cr nicotinate was reported to be absorbed better than Cr picolinate or any other of the forms tested using radiolabeled compounds (6). Anderson et al. (7) fed nine forms of Cr to rats and found that a Cr–nicotinate–glycine–cysteine–glutamate complex displayed the highest absorption based on tissue concentrations.

The diet might also affect Cr absorption. Mice fed diets containing 50% of the diet as glucose, fructose, sucrose, or starch absorbed more radiolabeled Cr on the starch diets (8).

The forms of Cr, animal species and status, dietary components, as well as components of the Cr supplements such as fillers and binders can all alter Cr absorption and dramatically change the interpretation of Cr supplementation studies. Therefore, some measure of Cr absorption should be part of all Cr supplementation studies.

MATERIALS AND METHODS

Urinary Cr losses of six adult subjects, three males and three females, free of all known diseases and not on medication were employed to determine the absorption of selected Cr complexes. Basal 24-h urinary Cr losses were determined prior to consuming the Cr test compounds, the day Cr was consumed, and the following day. Chromium, 200 µg, of each of the forms was given with 1 cup of water between 8:00 and 9:00 AM. Samples were collected Tuesday, Wednesday, and Thursday of each of the study weeks, with at least 2 wk between test dates. The order of consuming the

various Cr complexes was the same for all subjects. The study was approved by the Human Nutrition Study Committee, US Department of Agriculture, and the Internal Review Board, Clinical Section of Georgetown University, Washington, DC.

Urinary Cr concentrations were determined as described (9). An in-house Cr sample was run with each batch of samples and a urine sample whose Cr concentration had been verified using gas chromatograph–mass spectroscopy was run to verify the accuracy of the Cr determinations (10).

Chromium complexes were synthesized as described with modifications (7). Deionized water, 60 mL, was heated to 80°C and 30 mmol of indicated amino acids added (for the complexes containing 3 M equivalents). If nicotinic acid was present in the complex, it was added before the amino acids. Once compounds were dissolved, 10 mmol chromic chloride were added in five separate additions. After the color changed, the pH was adjusted to 4.5 with concentrated ammonium hydroxide. The solution was kept at 80°C for 60 min after adjusting the pH, and then cooled to room temperature before adjusting the volume to 100 mL. The procedure was modified to accommodate the syntheses of the additional complexes. Complexes were concentrated by rotoevaporation and dried by lyophilization. Chromium pidolate was a generous gift from Laboratories Labcatal (Montrouge Cedex, France). Chemicals utilized were reagent grade purchased from Fisher Chemical Co. (Pittsburgh, PA). The gelatin used in the Cr histidinate absorption studies often used as a filler in making capsules was a standard gelatin NF (Natural Formulary) (175 bloom) (Reuger Chemical Co., Irving, NJ).

Chromium complexes were used directly following synthesis without purification. The amount of Cr in each preparation was determined by graphite furnace–atomic absorption and the amount used for absorption studies was based on these measurements. Elemental analyses were not done because compounds were not pure. Confirmation of the formation of Cr complexes was based on spectral changes from 350 to 700 nm.

The absorption data were analyzed as two-factor mixed linear models using PROC MIXED (SAS Institute, Cary, NC), with the Cr compound as the fixed effect and subject number as a random effect for blocking. The assumptions of the general linear model were tested. To correct variance heterogeneity, the variance grouping technique was used. Because the effect of the Cr compounds were statistically significant, mean comparisons were done with Sidak-adjusted *p*-values so that the experimentwise error was < 0.05.

RESULTS

Following the completion of a human study involving the forms of Cr that were postulated to be absorbed the best, namely Cr nicotinate (6) and a Cr amino acid complex containing glycine, cysteine, glutamic acid, and nicotinic acid (7), we observed that Cr from these Cr complexes was

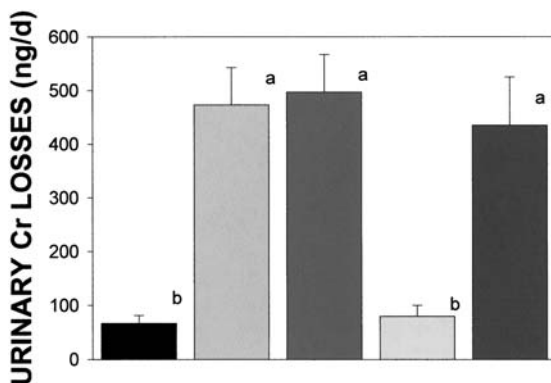


Fig. 1. Starch inhibits chromium absorption in a time-dependent manner. Six control subjects were given 200 μg of Cr and the urinary Cr losses were determined for the next 2 d. Values in the figure are the losses the first 24 h following consumption of the Cr. The first bar (black) denotes the basal losses; the second bar (light gray) denotes Cr chloride alone; the third bar (dark gray) denotes Cr chloride plus corn starch taken the first day after mixing; the fourth bar represents Cr chloride plus starch after 1 mo; the last bar represents Cr chloride as part of a tablet (no starch) that had been prepared more than 20 yr earlier. Chromium, 200 μg of the forms as indicated, was mixed with 400 mg of cornstarch. Bars with different letterscripts are significantly different at $p < 0.05$.

not absorbed under the conditions of our studies (data not shown). Chromium chloride was also tested for comparison and also not absorbed. Chromium complexes were mixed with corn starch to improve absorption (8). Subjects consumed the Cr in capsules containing 200 μg of Cr and 400 mg of starch.

Chromium complexes were subsequently shown to interact with the starch, making the Cr complexes unavailable. Starch, when mixed with the Cr compounds, was shown to lead to decreased solubility and time-dependent spectral changes. Absorption at 600 nm of 200 μg of Cr as Cr chloride mixed with 400 mg of cornstarch decreased almost 50% after 1 mo, with an another approx 50% decrease after 5 mo. The Cr chloride and starch mixture was dissolved in 1 N hydrochloric acid to determine spectral changes at the various times.

The absorption (based on urinary Cr losses) of Cr chloride mixed with cornstarch at zero time and that mixed with starch for 1 mo is shown in Fig. 1. Absorption of the starch–Cr mixture after 1 mo had dropped to baseline levels. Urinary Cr losses following the consumption of Cr tablets (not containing starch) from one of our previous studies (11) were similar to those more than two decades earlier (see Fig. 1, last bar on right).

We then determined the absorption of selected Cr complexes by control subjects to determine a form of Cr that could be used to reproducibly

Table 1
Urinary Cr Losses in Human Subjects Following
Consumption of Designated Chromium Compounds

Form of chromium	Urinary Cr Losses (ng/d)
Basal losses	256 ± 48 ^d
+ Cr chloride	655 ± 74 ^c
+Cr nicotinate	262 ± 69 ^d
+Cr nicotinate (commercial)	160 ± 60 ^d
+Cr-NA-GLY-CYS-GLU*	300 ± 92 ^{cd}
+Cr pidolate	643 ± 131 ^c
+Cr picolinate	2082 ± 201 ^b
+Cr picolinate (commercial)	2048 ± 327 ^b
+Cr methionate	1065 ± 199 ^{bcd}
+Cr glycinate-glutamate-histidinate	2188 ± 169 ^b
+Cr histidinate	3670 ± 338 ^a

Note: Subjects consumed 200 µg of Cr of each of the forms and urine was collected the day of consuming the Cr and the following day (data are for the 2 d combined).

* Chromium nicotinate–glycinate–cysteinate–glutamate complex.

improve Cr nutrition (*see* Table 1). Basal Cr losses were determined on days prior to consuming each of the forms of Cr and were averaged. Chromium chloride alone increased urinary Cr losses more than twofold as observed in our previous studies (11). The Cr nicotinate complexes appeared to be poorly absorbed and the urinary losses were not significantly greater than those for days when additional Cr was not consumed. The absorption of the preparation prepared in our laboratory was similar to that of the complex available commercially (*see* Table 1). The Cr pidolate complex, which improved the antioxidant variables of subjects with type 2 diabetes mellitus (DM) (12), was absorbed similarly to the Cr chloride. The Cr nicotinate–glycinate–cysteinate–glutamate complex (Cr-NA-GLY-CYS-GLU) was poorly absorbed in contrast to that observed in rat studies (7). The Cr picolinate complexes prepared in our laboratory and those available commercially were absorbed greater than the Cr chloride and Cr pidolate complexes. Chromium methionate, a supplement often used in animal studies (13), was not absorbed as efficiently as the Cr picolinate complexes. Complexes containing histidine were absorbed the best of all of the Cr complexes tested. We screened several Cr complexes containing arginine, lysine, serine, valine, glutamine, asparagine, and leucine and

they all appeared to be absorbed similarly to the Cr chloride (data not shown). The addition of histidine to the amino acid complexes tested increased Cr absorption and the complexes that appeared to have the highest Cr absorption were the Cr complexes synthesized with histidine (a molar ratio 3 : 1 for histidine to Cr was used in the preparation) (see Table 1). Chromium absorption was similar after 2.5 yr and when mixed with standard gelatin NF often used for making capsules.

DISCUSSION

We tested the absorption of the Cr complexes based on urinary losses because the majority of the absorbed Cr is excreted in the urine (14). Balance studies for Cr are not meaningful because the amount of Cr absorbed usually ranges from 0.4% to 1.5%. Therefore, 98.5–99.6% of the Cr is not absorbed and remains in the gastrointestinal tract and excreted in the feces. Obviously, fecal collections and Cr analyses would have much larger errors than 1.5% and, therefore, urinary losses are much more suitable. Certainly not all Cr absorbed is lost in the urine, but all Cr found in the urine (except contaminating Cr) has been absorbed. Increased urinary Cr losses do appear to be measures of Cr absorption and not a measure of increased losses from the tissues based on studies involving a stable isotope of Cr (15). The authors demonstrated that using a stable isotope of Cr, the differentiation of the basal Cr losses from the tissues from the absorption of the newly administered Cr in the stable isotope form could be ascertained (15).

We determined the absorption of the complexes in humans because animals did not appear to be suitable models for humans. The complexes that we tested in rats (7) were not absorbed similarly in pigs or in humans (unpublished data). There is also a difference in absorption depending on the amount of Cr given. For example, in rat studies, testing of small amounts of Cr nicotinate complexes as radiolabeled complexes (6) did not yield similar results as when similar complexes were fed at higher amounts in the diet (7). Therefore, we tested the amount of Cr absorbed by humans at levels that would normally be consumed in nutritional supplements designed for humans.

The relative absorption of Cr chloride, Cr polynicotinate, Cr pidolate, and Cr picolinate are similar to those observed in our rat study (7). The complexes that are significantly different are the Cr nicotinate–glycinate–cysteinate–glutamate complexes and the Cr histidinate complexes. The relative ratio of the Cr picolinate with the Cr chloride is similar to that reported by others for human studies and consistent with our previous studies (9,16,17).

Essentially all of the Cr amino acid complexes that we screened in this study had increased Cr absorption when histidine was one of the components. The Cr histidinate added to a gelatin often used as a filler when making supplements was also stable for more than 2.5 yr. These histidinate complexes are not only absorbed better than the other complexes tested

but are comprised of two essential nutrients, namely Cr and histidine, and are, therefore, suitable for nutrient supplements. Complexes comprised of essential nutrients are also suitable as supplements for countries such as France, where all of the components of a supplement must be on the accepted list for human consumption.

In summary, the stability of Cr complexes varies depending not only on the individual complexes but also other dietary components as well as fillers. Studies involving the effects of supplemental Cr should have a measure of urinary Cr losses to ensure that the complexes being studied are absorbed under the conditions of the studies. Results of studies involving other species need to be confirmed with the species used and the dietary conditions. Chromium histidinate is stable, has better absorption than any of the compounds tested in human subjects, and appears to be suitable as a nutrient supplement. Additional studies are needed to show that the absorbed Cr from Cr histidinate complexes is utilized.

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