The Safety and Efficacy of High-Dose Chromium

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Abstract

The data on the standards for chromium requirements and the safety of various chromium compounds and doses are reviewed. The 350-fold difference between the acceptable daily intake and the calculated reference dose for humans of 70 mg per day seems without precedent with respect to other nutritional minerals. Previous claims of mutagenic effects of chromium are of questionable relevance. While studies have found DNA fragmentation (clastogenic effects) by chromium picolinate, anecdotal reports of high-dose chromium picolinate toxicity are few and ambiguous. The beneficial effects of chromium on serum glucose and lipids and insulin resistance occur even in the healthy. Serum glucose can be improved by chromium supplementation in both types 1 and 2 diabetes, and the effect appears dose dependent. Relative absorption of various chromium compounds is summarized and the mechanism of low molecular weight chromium binding substance (LMWCr) in up-regulating the insulin effect eight-fold is discussed. There is evidence of hormonal effects of supplemental chromium besides the effect on insulin. Chromium supplementation does result in tissue retention, especially in the kidney, although no pathogenic effect has been demonstrated despite considerable study.

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Introduction

Chromium, an essential nutrient for human life, has been used at dosages higher than the minimum nutritional level to offset problems of malabsorption or to pharmacologically influence the chemistry of blood sugar control in diabetics. Questions have arisen as to whether chromium at higher dosages can cause DNA fragmentation (clastogenic effect), which is separate from any issue of biochemical toxicity. While evidence for this concern is sparse, the question deserves to be examined along with potential benefits of higher chromium doses.

Chromium compounds (along with other higher-than-divalent minerals) are not readily absorbed. Along with the question of chromium dosage level, there is also the question of potential variation in absorption or utilization with different chromium compounds. The data on chromium surveyed below includes historical background of biological effects, as well as results of chromium supplementation on hypoglycemia, diabetes, serum cholesterol, serum dehydroepiandrosterone (DHEA), and calcium excretion. The safety of various oral chromium doses is discussed. The phenomenon of glucose tolerance factor (GTF) and the mechanism of low molecular weight chromium-binding substance (LMWCr) on the insulin receptor are reviewed. This presentation is intended to demonstrate important concepts with respect to high-dose chromium, not be a comprehensive survey of chromium biochemistry.

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Historical Background

In 1929 Glaser et al[1] discovered brewer's yeast exhibited a potentiating effect on the hypoglycemic action of insulin. In 1958 the potentiating effect was rediscovered when rats fed a Torula yeast-based diet began to show signs of glucose intolerance, which was reversed by a diet of brewers yeast.[2] This discovery led to the isolation of a "glucose tolerance factor" or GTF.[2,3] Trivalent chromium (CrIII) was found to be the active component of GTF. The biological activity of chromium was found to be contingent on the valence, Cr III being the only biologically active form.

Chromium is now recognized as one of 15 trace elements critical for proper physiological functioning of lipid and carbohydrate metabolism. Deficiency of chromium has been linked to a number of disorders, including symptoms of type 2 diabetes, such as decreased glucose tolerance,[4-7] increased serum insulin levels,[8] and decreased number of insulin receptors.[9] Chromium deficiency can also mimic many signs of cardiovascular disease, such as elevated serum cholesterol and triglycerides, as well as decreased high-density lipoprotein cholesterol (HDL).[10]

The most dramatic examples of clinical syndromes due to chromium deficiency have been seen in long-term total parenteral nutrition (TPN). Although chromium had been previously found in animal studies to be essential in glucose control, it was not until 1977 that the first definitive human case of glucose intolerance with neuropathy and weight loss was reported in a patient receiving long-term TPN.[11] The administration of 200 mcg/day of chromium chloride corrected all symptoms, and in three weeks the patient was able to discontinue all insulin medication. This patient was the first documented case that showed humans could have similar clinical presentations of glucose intolerance as test animals, and that the clinical syndrome could be reversed by the administration of Cr III. Similar dramatic reversals of high blood glucose have since been reported with the supplementation of chromium in TPN patients, although these cases did not include neurological deficits.[12-14] Another case, involving not only hyperglycemia and insulin resistance, but also peripheral neuropathy, ataxia, postural tremor, and muscle weakness, was treated successfully by the administration of 250 mcg/day of Cr III, and resulted in normalized blood glucose, as well as improved insulin resistance and nerve conduction.[15]

Chromium in Glucose Homeostasis

One aspect of the diabetic model often focuses on a deficiency of Cr III leading to poor blood sugar control. Although this is true, the picture is more complicated. Chromium is part of a glucose/insulin system that maintains homeostatic control of blood glucose in the organism. From this systemic point of view there is a range of imbalance along a glucose/insulin axis, which can lead to hypoglycemia on one end of the spectrum and diabetes on the other with "normal persons" in the middle. Chromium has been shown in a number of studies to have profound effects on glucose homeostasis in all these categories.

Chromium deficiency has been associated with hyperglycemia in test animals as well as humans. The condition is reversed by supplementation.[12-15] Chromium has also been shown to have a positive influence on individuals with no diabetic symptoms. Morris et al[16] studied plasma chromium levels in healthy volunteers and compared fluctuations to diabetic patients. Serum chromium levels in healthy individuals were found to be inversely related to insulin peaks in response to a glucose challenge. The observed rapid decrease in chromium in response to glucose (as high as 50 percent in 45 minutes) was not due to urinary excretion. In diabetic volunteers chromium levels were not found to fluctuate with respect to insulin.

The effects of chromium supplementation (200 mcg/day of trivalent chromium as chromium picolinate) were studied over a period of 10 weeks on six healthy individuals in order to measure changes in insulin sensitivity to endogenous and exogenously administered insulin. After seven weeks of supplementation, fasting blood glucose decreased by eight percent and fasting insulin decreased by a statistically significant 28 percent.

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There was a significant improvement in endogenous insulin by week four. Half of the volunteers had a significant increase (30%) in sensitivity to exogenous insulin.[17] Similar results were produced in earlier work of Anderson et al[18] who found that a three-month course of chromium III chloride supplementation (200 mcg/day) given to hyperglycemic subjects resulted in significantly lower glucose and insulin (the concentration at 90 minutes post chromium administration) leading to improved glucose tolerance.

The effects of supplemental Cr III were studied in 29 individuals who were not found to be deficient in chromium nor diabetic, but were obese and had a family history of type 2 diabetes.[19] This trial showed a significant increase in insulin sensitivity at 1000 mcg/day of chromium III picolinate over a period of eight months. "Insulin sensitivity" was defined as the ability of insulin to enhance glucose disappearance and inhibit hepatic glucose production. The combination of these three studies support the idea of a continuum of functioning within the glucose/insulin axis in which seemingly healthy people can have improved blood glucose and insulin sensitivity by supplementation of chromium.

Hypoglycemia

Hypoglycemia is on the opposite end of the glucose/insulin axis. If Cr III is a key factor in overall glucose/insulin homeostasis, it should be effective in controlling clinical symptoms of low blood sugar. Studies have shown supplemental Cr III can have a beneficial effect on both subjective and objective parameters of hypoglycemia. In a placebo-controlled crossover trial it was found that three months of supplementation with chromium III chloride (200 mcg/day) was effective in alleviating the symptoms of hypoglycemia while significantly raising the minimum glucose levels 2-4 hours after a glucose challenge.[9] Similar results were obtained with the administration of 125 mcg/day of yeast-based chromium for three months.[20] Evidence for the successful clinical use of Cr III for hypoglycemia is apparently based on these two reports.

Diabetes

Chromium deficiency is associated with the blood sugar irregularities of diabetes. Recent studies have demonstrated that chromium is effective in treating various types of diabetes, including types 1 and 2, gestational, and steroid-induced diabetes.[21-24]

Treatment of type 2 diabetes with chromium has led to improvement in blood glucose, insulin, and hemoglobin A1C (HbA1c) levels. The use of organic chromium complexes has been found to give superior results when compared to inorganic salts.[25] Recent studies have shown a dose-dependent response to chromium. Although 200 mcg of an absorbable organic form of chromium such as picolinate can improve blood indices for type 2 diabetics, it was not sufficient to reverse all glucose abnormalities.[25] A double-blind, placebo-controlled study (employing 180 Chinese type 2 diabetics) described random supplementation of placebo, 200 mcg or 1000 mcg of chromium as picolinate daily for a period of four months. The higher dose of 1000 mcg was found to have a more pronounced effect during the four-month trial. Fasting and two-hour glucose levels (after glucose challenge) were significantly lower in the 1000 mcg group, both at two months and four months, while the 200 mcg group had no significant drop. The 1000 mcg dosage of chromium also led to significant decrease in HbA1c after the second month of treatment, while the 200 mcg group did not reach significantly reduced HbA1c until the fourth month. (Note the HbA1c of the 200 mcg group reached approximately 7.4 micromol/d, while the 1000 mcg group reached approximately 6.6 micromol/d in the same four-month period, suggesting that the higher chromium dose did not merely speed the arrival of the same endpoint.) The higher chromium dose also resulted in a decrease in cholesterol levels, which was not seen in the group receiving the lower dose of chromium (200 mcg).[22] A follow-up survey of the original Chinese study (833 type 2 diabetic patients) demonstrated the effect of supplementation was long lasting. Fasting glucose as well as postprandial glucose still showed significant improvement after 10 months of treatment with 500 mcg/day of chromium as picolinate.[21]

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Although the majority of research on diabetes has focused on type 2 diabetes, a few small studies have tested the efficacy of Cr III on type 1 diabetes and found it effective.[23] One study supplemented 162 patients (48 had type 1 diabetes, the others type 2) with 200 mcg/day of chromium picolinate. Seventy-one percent of the type 1 patients responded positively, allowing a 30-percent decrease of insulin dose. Blood sugar fluctuations also responded positively, decreasing as soon as 10 days after treatment. Supplementation of chromium as picolinate (600 mcg/day) in a 28-year-old woman with an 18-year history of type 1 diabetes reduced HbA1C from 11.3 percent to 7.9 percent in three months.[24]

Approximately 2-5 percent of pregnant women are diagnosed with gestational diabetes mellitus (GDM), which is thought to be the most common medical complication of pregnancy. Almost 180,000 women this year will be diagnosed in the United States. Gestational diabetes often develops in the second trimester when the mother's pancreas is unable to produce enough insulin to overcome the insulin resistance brought on by the added demands of pregnancy.[26,27] It has been hypothesized that supplemental Cr III should be able to reduce insulin sensitivity and benefit women with GDM. A trial of 20 women (25-43 years old with GDM and between 20-24 weeks gestation) tested the effect of Cr III supplementation on GDM. After eight weeks of supplementation the women were found to have significant improvement in fasting insulin levels as well as one-hour glucose and insulin levels during glucose tolerance testing with either 4 mcg/kg or 8 mcg/kg of Cr III. The 8 mcg/kg/day group had significantly lower postprandial glucose levels (approximately 40 mg/dL less than placebo) than the 4 mcg/kg group. The data show a dose-dependent decrease in blood glucose between the two groups.[28] This small trial demonstrates the potential usefulness of chromium supplementation for gestational diabetes.

Dysregulation of blood sugar and insulin resistance are significant sequelae of the chronic use of corticosteroids. Chromium has been found to be effective in reversing diabetes caused by the therapeutic use of glucocorticoids. Chromium picolinate (600 mcg/day) was effective in lowering blood glucose by almost half, decreasing from 13.9 mM/L to 8.3 mM/L in 47 of 50 such patients. Patients were also able to reduce insulin and/or hypoglycemic medications by half within one week of beginning chromium supplementation.[29]

Chromium and Cholesterol

Recent studies have demonstrated the cholesterol-lowering effect of trivalent chromium in both human and animal studies. Trivalent chromium at 5 mcg per gram of food given to 20 Wistar rats for 10 weeks resulted in a significant decrease in cholesterol levels.[30] Human studies have also demonstrated significant reduction in cholesterol levels with daily supplementation. Chromium as picolinate (200 mcg/day) given in a placebo-controlled trial to 28 healthy volunteers with slightly elevated total cholesterol for 42 days demonstrated a statistically significant decrease in total cholesterol (7%), low-density lipoprotein (LDL) cholesterol (12%), and apolipoprotein B, while showing no significant change in triglycerides (TG).[31]

A blinded crossover study found that a nicotinic acid-complexed form of trivalent chromium at 200 mcg/day slightly lowered fasting total and LDL cholesterol, triglycerides, glucose concentrations, and 90-minute postprandial glucose levels in individuals with type 2 diabetes. However, the authors did not regard the results as statistically significant.[32] A study of 26 non-obese young adults using chromium as picolinate (220 mcg/day Cr III) showed no reduction in total cholesterol after 90 days.[33] In a contrasting study of 23 male athletes, it was found that supplementation with either 200 or 800 mcg/day of Cr III as chromium nicotinate produced sizeable decreases in total cholesterol and LDL cholesterol, with some reduction in HDL cholesterol. There was demonstration of a dose-response relationship.[34] In another study using an inorganic form of chromium III (chromium chloride) for 76 patients with established atherosclerosis, doses of 250 mcg/day were not shown to decrease total cholesterol levels while triglycerides decreased, HDL cholesterol increased, and VLDL continued to decrease over the 7-16 month period.[35] In this

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study triglycerides did not fall significantly within the first three months of supplementation; therefore, longer periods of time and/or higher doses may be required to see a triglyceride-lowering effect.

Perhaps the most significant data on the reduction of cholesterol attributable to chromium supplementation is demonstrated in the 1997 study by Anderson et al[22] of 180 individuals with type 2 diabetes using either 200 mcg/day or 1000 mcg/day of Cr III as chromium picolinate. There were no significant effects of chromium supplementation on HDL cholesterol or triglycerides. Examination of the graphs in the paper indicates little difference in total cholesterol over four months in the 200 mcg/day supplementation group. However, total cholesterol appeared to drop steadily during the four-month period in the 1000 mcg/day group. In summary, there appears to be evidence that chromium supplementation can lower serum cholesterol, but it may require a longer time or higher doses of chromium with diabetic patients.

Chromium, DHEA, and Osteoporosis

A placebo-controlled trial of 27 postmenopausal women given 200 mcg/day of chromium as picolinate for 60 days found a decrease in insulin levels (38%), plasma glucose (26%), and urinary calcium (19%), while dehydroepiandrosterone (DHEA) levels increased by 24 percent. The authors suggest that a 47-percent decrease in urinary hydroxyproline/creatinine ratio indicates that chromium might be effective in the prevention of osteoporosis.[36]

Chromium Dosage Criteria

The U.S. Environmental Protection Agency (EPA) has replaced acceptable daily intake (ADI) with calculated reference dose (RfD). The RfD is calculated from the No Observed Adverse Effect Level (NOEL) or the Lowest Observed Effect Level (LOAEL) from animal or human experiments. From this data, safety factors are applied consisting of "uncertainty factors" and a "modifying factor."[37] The RfD has been calculated for Cr III at 70 mg per day, reflecting the weight of a 70 kg man at a rounded per kg dosage of 1 mg from the actual RfD of 1.47 mg/kg. The RfD reflects a staggering 350 times the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of 50-200 mcg.[37] The ratio of RfD to ESADDI is less than 2 for zinc, about 2 for manganese, and about 6 for selenium as compared to 350 for chromium.[38] The ESADDI value for chromium has been criticized for the arbitrary "safe and adequate" dosage being 350 times less than that of the RfD, given the lack of toxicity or adverse effects of Cr III.[37,38] This suggests dietary supplementation may be greatly underdosed.

Safety/Toxicity of Chromium

Chromium occurs in both trivalent and hexavalent (Cr VI) forms. Chromium toxicity has been almost exclusively linked to the hexavalent form. Ingestion of hexavalent chromium is 10-100 times more toxic than trivalent compounds.[39] Cr VI is chiefly known as a man-made product of industry. Cr III is found naturally in foods and is associated with nutritional supplements in various complexed forms. The most popular complexed form is chromium picolinate, although chromium nicotinate and chromium citrate are also used as nutritional supplements.

Five Anecdotal Reports of Toxicity

Although no *in vivo* study has demonstrated toxicity of chromium in either the trivalent inorganic salt form or the trivalent organic-complexed form, there have been only five anecdotal case reports of suspected toxicity associated with use of chromium picolinate supplements.

After ingesting 1200 mcg of chromium picolinate over a period of two days, a 24-year-old female body builder began to develop muscle cramps, which were later diagnosed as rhabdomyolysis. After the removal of her program of more than 30 supplements, hospital treatment for three days resolved symptoms, but left some residual renal dysfunction.[40]

Another report of an adverse reaction involved ingestion of 600 mcg of chromium picolinate daily by a 49-year-old female nurse who presented with renal insufficiency. She was

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diagnosed with severe chronic active interstitial nephritis that was ascribed to chromium intake over a six-week period that ceased five months prior to diagnosis.[41] The concomitant use of terazosin, hydrochlorothiazide, and verapamil used to treat her hypertensive condition were not considered as significant contributing factors to the nephritis. Thiazide diuretics have been associated with allergic interstitial nephritis (but onset is usually rapid) and verapamil is associated with increased deterioration of pre-existing renal failure.

A 33-year-old schizophrenic and depressed woman (height 5'2" and weight 90.2 lbs) was admitted to the emergency room with a 1-2 week history of severe fatigue, malaise, fever, chills, weight loss to cachexia, jaundice, urinary tract infection, anemia, thrombocytopenia, hemolysis, liver dysfunction (aminotransferase enzymes 15-20 times normal, total bilirubin three times normal), and renal failure (serum creatinine 5.3 mg/dL; blood urea nitrogen 152 mg/dL). The renal failure was ascribed to her ingestion of 1200-2400 mcg/day of chromium picolinate for a period of 4-5 months for enhancement of weight loss. She was taking the supplement on admission to the hospital. Her history included concurrent monthly injection of paroxetine (Paxil) and fluphenazine (Prolixin). Hospital treatment consisted of dialysis and transfusions.[42] No consideration was given to the potential associated side effects of liver and kidney damage from fluphenazine injection as cited in the manufacturer's package insert.

A 35-year-old male presented with dermatitis ascribed to chromium picolinate ingestion because of a positive skin patch test to potassium dichromate (Cr VI).[43] No chromium dosage was furnished in this case nor details of other supplements ingested. Numerous errors in units were present in the paper with no mention of patient follow-up. Potassium dichromate is a strong oxidizing agent and will show skin reaction in a majority of normal individuals.

A male patient who took chromium picolinate on three separate occasions (200, 400, and 400 mcg) experienced three progressively worse episodes of cognitive, perceptual, and motor changes. The symptoms ("feeling funny," disrupted thinking, and difficulty in driving) subsided within two hours.[44]

One or more of the anecdotal reports cited above may be true individual reactions to chromium supplementation. The majority of cases appear explainable by other factors, especially when compared to clinical trials that have monitored patients for adverse side effects over long periods of time. Although these anecdotal reports of toxicity due to chromium picolinate leave considerable doubt as to the true origin of the toxicity, the commonality among three of the five reports is the occurrence of renal dysfunction. This concern is explored further in the *in vivo* studies below and the section on chromium retention in tissue.

In vivo *Studies*

In vivo laboratory studies have not supported claims of toxicity of chromium picolinate in test animals. A study by Anderson et al[45] examined the possible toxicity of trivalent chromium using both chromium chloride and picolinate at levels of 5, 25, 50, or 100 mg/kg of diet in rats for a 20-week period. The results showed no toxicity at levels as high as 1500 mcg/day (15 mg/kg body weight) as evidenced by analysis of blood chemistry (BUN, creatinine, LDH, ALT, and AST) as well as tissue histology. In another study, Sprague-Dawley rats were given a single dose of 0, 33, 250, or 2000 mg/kg of chromium picolinate and sacrificed 18-24 hours later. Analysis of the tissue revealed no chromosomal aberrations.[46] These results support previous *in vivo* research of the safety of trivalent chromium.

The use of 1000 mcg/day of chromium as picolinate in 180 type 2 diabetic patients for a period of four months showed no toxic reactions in any of the patients.[22] No toxicity was found in a follow-up survey of more than 830 patients who had taken 500 mcg/day of chromium as picolinate for 10 months. The only symptoms reported were decreases in thirst, fatigue, and urinary frequency in a majority of patients. No negative side effects were found after one year of supplementation.[21] In a study 29 obese patients at risk for diabetes were given 1000 mcg/day of chromium as

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picolinate and all patients were found to have normal renal and liver function tests, both before and after the eight-month study.[19]

Oral administration of trivalent chromium has found little, if any, toxicity associated with its use in both test animals and humans. Studies conducted in 1934 on cats found ingestion of trivalent chromium complexes produced no illness or tissue damage. Ten cats were fed 50-1000 mg/day of either chromium carbonate or phosphate for a period of 1-3 months. Histological examination failed to demonstrate any tissue damage or irregularities.[47] A similar study of rats also demonstrated no long-term toxicity of trivalent chromium. In this study, 60 male and 60 female rats were fed chromium oxide at 72-75 mg/kg or 160-180 mg/kg daily for a period of 90-730 days. There were no significant changes in blood indices, teratogenicity, litter size, or carcinogenesis in adults or pups as compared to controls.[48] In another study of toxicity in rats, 20 rats were given 25-ppm chromium chloride in drinking water. No adverse effects were noted after one year of treatment as determined by gross or microscopic pathological changes in tissue or blood.[49] Later studies showed that rats fed up to 100 mg/kg of either chromium chloride or chromium picolinate for a period of 24 weeks were found to have no toxic effects.[45]

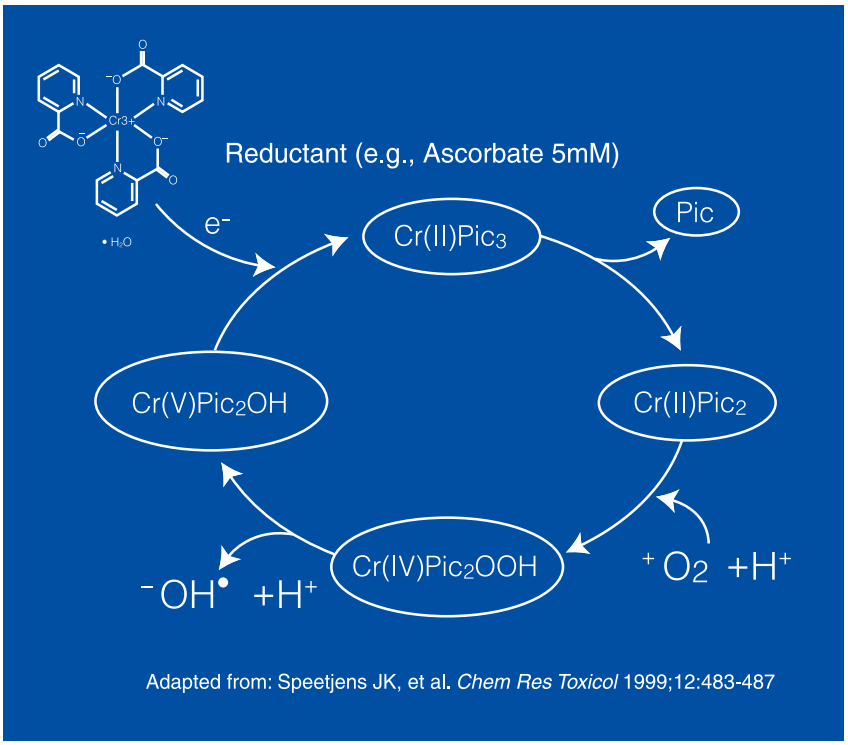
Presently the EPA Federal-State Toxicology and Risk Analysis Committee has set state and federal drinking water standards for all chromium regardless of valence at 100 mcg/L, while Minnesota has refined its standards to include chromium III at 20,000 mcg/L.[50]

The higher range of safety set by Minnesota stems from a number of studies that have examined the effects of chronic ingestion of chromium III in water. Schroeder et al[8] found that 461 rats given drinking water containing 5-ppm Cr III for a period of three years were found to have no signs of sub-clinical toxicity or microscopic abnormalities in tissue, while the rats demonstrated an increase in growth rate and mature weight as well as increased longevity as compared to controls.

Subcutaneous, Intravenous and Intraperitoneal Injection of Cr III

The lethal dose of chromium III chloride, subcutaneously injected, was reported to be 0.8 gm/kg for dogs and 0.5 gm/kg for rabbits. Rats and mice intravenously injected with Cr III showed an LD50 of 10-30 mg/kg.[4] Another study demonstrated the chromium III chloride LD50 to be 1.75

Figure 1. *Proposed Hydroxyl Radical Generation*



Adapted from: Speetjens JK, et al. *Chem Res Toxicol* 1999;12:483-487

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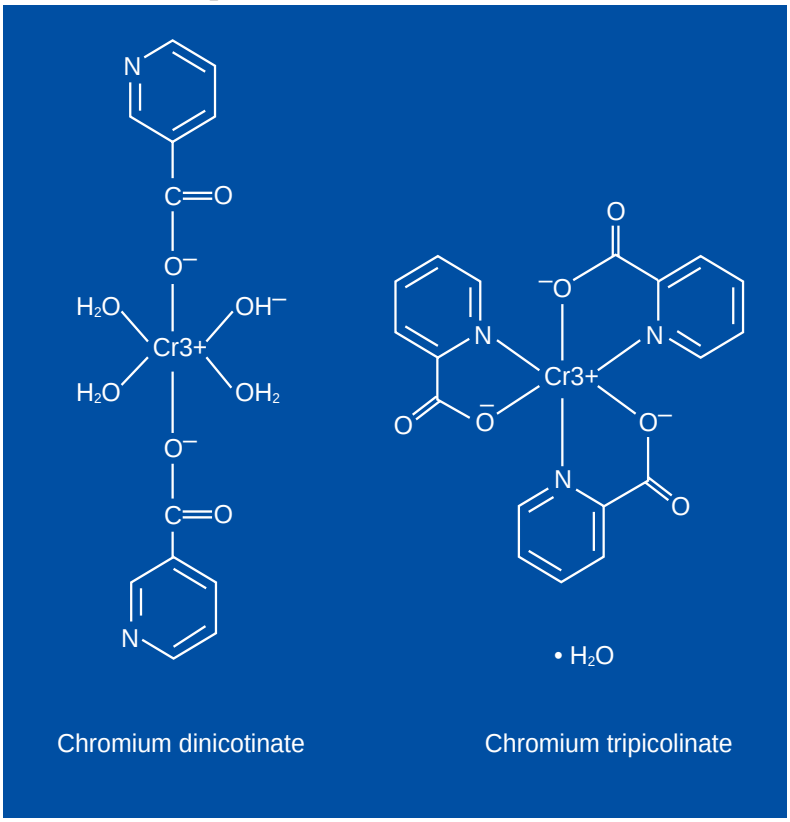
mg/100 g and the chromium III hexaurea chloride complex LD50 to be 1.0 mg/100 g.[4] In an attempt to study chronic toxicity, mice were given intraperitoneal injections of Cr III and Cr VI compounds. Along with the obvious acute toxicity of Cr VI, the median lethal dose in distal time, regardless of oxidation state (more than 10 days after treatment), averaged (17.9 +/- 1.8) mcg chromium/g body weight.[51]

Mutagenic Potential: In vitro *Studies*

The results of many studies support the conclusion that Cr III is relatively nontoxic. Most Cr III compounds have failed to induce genetic defects in bacteria, yeast, or mammalian cell lines.[52] Recently, the safety of chromium picolinate has been questioned with the research of both Stearns and Speetjens. Stearns et al[53,54] cites that organic forms of Cr III such as chromium picolinate and nicotinate have much higher absorption (2-5%) as compared to 0.5-1 percent for chromium III chloride and carbonate or phosphate salts.[4] Stearns contends this increased absorption of organic-complexed chromium could cause toxicity due to the concentration of this heavy metal in various tissues. She also argues that concentration of Cr III in various tissues over time could lead to eventual damage through chromosomal aberrations from clastogenic action of the metal, as has been observed *in vitro*.[53-55] (A clastogen is a specific mutagen that causes breaks in chromosomes.) Chromium picolinate is thought to become clastogenic due to the redox potential of the compound, which can enter the cell intact and possibly generate hydroxyl radicals (Figure 1).

Hydrophobic complexes such as those with the pyridine nitrogen ligands of chromium picolinate are thought to readily enter the lipid bilayer of the cellular membrane. Chromium tightly binds to picolinic acid forming a complex with three picolinate molecules (Figure 2).

Figure 2. *Structure of Chromium Dinicotinate and Chromium Tripicolinate*



The gastrointestinal (GI) tract readily absorbs these hydrophobic compounds. The tight bonding within the complex also makes it difficult to break down in the GI tract as well as in the cell. It has been shown to be relatively stable in gastric juice for more than three hours, requiring 0.1 M concentration of mineral acids to break chromium bonding.[55,56] Chromium picolinate has not been found to transfer chromium to transferrin, albumin, or LMWCr (see GTF section).[55,57] For this reason it was hypothesized that chromium picolinate can, due to the nature of its aromatic bidentate ligand, form hydroxyl radicals. This idea is supported by the clastogenic activity of picolinate alone in cell culture at supraphysiologic levels of 2.0 mM.[53] Stearn's study found that chro-

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mium III picolinate at supraphysiologic dosages of 0.5 to 1.0 mM caused chromosomal aberrations *in vitro* on Chinese hamster ovary cells.[53] Chromosomal damage could not be produced with chromium III chloride or chromium III nicotinate at levels of 10 microM to 2.0 mM.[53] Using a slightly different technique, Stearn's work has been repeated by Speetjens et al.[55] Chromium III picolinate (120 microM total concentration) and ascorbate (5 mM) were mixed directly with suspensions of super-coiled plasmid DNA. The combination of chromium picolinate and ascorbic acid was found to nick the DNA within 20 minutes. This result supports the earlier work of Tsou et al,[58] who found that Cr III in combination with hydrogen peroxide could cause DNA damage through a Fenton-like reaction. It was hypothesized that Haber-Weiss or Fenton cycles could be responsible for the production of hydroxyl radicals through the reduction of chromium III picolinate. Hydroxyl radicals and singlet oxygen have been generated with Cr III and hydrogen peroxide at physiologic pH.[58,59]

Complexes of chromium and amino acids such as arginine, aspartic acid, glycine, hydroxyproline, and lysine were found to be nonmutagenic to various strains of *Salmonella typhimurium* at concentrations of 50 microM.[60] Chromium III glycinate was found to be nonmutagenic to human skin fibroblasts and to have no effect on the DNA repair mechanisms at levels above 0.001 M.[61] Chromium III glycinate complexes were not found to cause DNA damage in cultures of Hamster V79 cells at concentrations of 0.5-1.0 M.[62]

Other researchers have questioned the relevancy of these studies to animals or humans. It is not surprising that supraphysiologic dosages of chromium cause clastogenic reactions in cell culture. It is difficult to extrapolate or compare results from *in vitro* studies to possible human toxicity. McCarty cites that the concentrations required for mutagenesis are four orders of magnitude higher than the physiologic level of serum chromium, which is 2-6 nM in average individuals.[63] Supplementation of 200 mcg daily of chromium picolinate for two months has been shown to increase serum concentrations to 16 nM.[64] This is still far below the level needed for clastogenic activity (50 microM or 3000-fold higher) and the level for DNA nicking (7-fold higher).

Mutagenic Potential: In vivo *Studies*

A number of *in vivo* studies have failed to find any mutagenic potential of Cr III such as was found in the *in vitro* studies. Rats injected with significant amounts of chromium III chloride were found to have detectable chromium in both kidney and liver chromatin, but 40 hours later were found to have no DNA damage as measured by cross-links or single strand breaks.[65] A recent study looked at the levels of DNA damage from 400 mcg/day of chromium as picolinate given to 10 women for eight weeks. Titers of an antibody against an oxidized DNA base (anti-HMdU) showed the supplement dose did not increase oxidative damage to DNA, with a confidence interval of 0.96-1.00.[66] The ability of chromium III nitrate to induce a clastogenic reaction at levels of 500 mg/kg by intraperitoneal injection in mice was measured by the micronucleus test. While hexavalent chromium was found to be positive for chromosomal breakage, there was no mutagenic activity of chromium III nitrate.[67]

Glucose Tolerance Factor and Low Molecular Weight Chromium Binding Substance

Although glucose tolerance factor was recognized almost 50 years ago, attempts to isolate the specific structure have eluded scientists to this day. In 1959, Mertz identified trivalent chromium from yeast as the active constituent of GTF, which, when given to brewer's yeast-fed, chromium-deficient rats, corrected imbalances in carbohydrate metabolism.[2] Determination of the biologically active form of chromium focused on the isolation of GTF from brewer's yeast. Acid hydrolysis with 5N HCL for a period of 18 hours was part of the isolation protocol,[68] which would have destroyed most protein structures associated with the bioactive molecule. Yet this protocol led to the discovery of a low molecular weight molecule that was determined to consist of nicotinic

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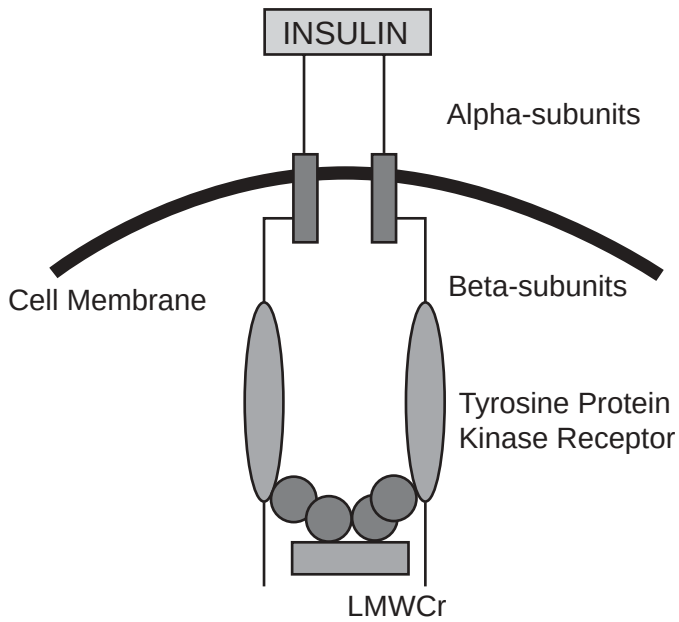
acid, glycine, glutamate, and a sulfur-containing amino acid.[69] Although some early studies used porcine kidney as a raw material in the acid hydrolysis isolation procedure, most used readily available yeast as the raw material for GTF studies.[3] Today, the term GTF is reserved for the organic chromium degradation product from yeast.[69] The question as to whether GTF is a biologically active substance or artifact centers on the ability to stimulate production of CO_2 from glucose in rat adipocytes as a function of insulin concentration. GTF appears to function as a carrier of chromium to the chromium-deficient proteins in the cell.[70]

Analysis of mammalian tissue has resulted in the isolation of a low-molecular-weight chromium binding substance (LMWCr) that in many ways is similar to yeast GTF.

Yamamoto et al[71] isolated two chromium-binding substances: a low molecular weight substance and a high molecular weight substance. The high-molecular-weight chromium binding substance (HMWCr) was isolated from both rabbit liver and mouse organ homogenate and has a molecular weight of 2600 and an ultraviolet absorbance of 260 nm. LMWCr has also been isolated in a number of mammalian organs, including rat lung,[72] rabbit,[73] mouse and canine livers,[71] and from bovine colostrum.[74] LMWCr has a molecular mass of 1500 and has an ultraviolet absorption maximum at 260 nm, which corresponds to the absorption maximum of GTF, also at 260 nm.[69,73] The LMWCr oligopeptide is composed of cysteine, glutamate, aspartate, and glycine. LMWCr differs from GTF in the combination of amino acids and does not contain nicotinic acid.[75] Yet acid hydrolysis of porcine LMWCr, similar to early protocols, yields GTF-like isolates.[57] LMWCr has been found to bind four chromium III ions in a multinuclear assembly much like that of calmodulin. Studies of Vincet et al[57] have discovered that LMWCr is stored in the cytosol of insulin-sensitive cells in an apo (unbound form) that is activated by binding four chromium ions. This activation is the result of a series of steps stimulated by insulin signaling. LMWCr potentiates the action of insulin once insulin has bound to its receptor.[76] (Figure 3)

Figure 3. *Insulin Receptor*



This insulin potentiating or autoamplification action stems from the ability of LMWCr to maintain stimulation of tyrosine kinase activity.[25,57] Once insulin is bound to its receptor, LMWCr binds to the activated receptor on the inner side of the cell membrane and increases the insulin-activated protein kinase activity by eightfold.[75] There is also evidence the autoamplification effect of LMWCr may be enhanced by the inhibition of phosphotyrosine phosphatase, which inactivates tyrosine kinase.[25] Further study is required to understand the inconsistent results of

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Davis et al[77] who found that LMWCr actually activates membrane-associated phosphotyrosine phosphatase in insulin sensitive cells. As insulin levels drop and receptor activity diminishes, LMWCr is transported from the cell to the blood and excreted in the urine.[78] When chromium is absorbed from the gut, it is carried by transferrin, which transfers chromium to the apo-LMWCr. Excess chromium is carried by albumin. It has been estimated that each millimeter of serum contains 2-3 mg of transferrin, of which only 30 percent is saturated with iron, leaving the remaining unsaturated sites able to bind trivalent chromium.[71]

Chromium Absorption

In the last three decades evidence has been collected demonstrating that both exogenous and endogenous factors significantly alter absorption and ultimately bioavailability of chromium. A considerable variance with respect to absorption is reported in the literature. One study of men over age 60 found absorption of trivalent chromium from dietary consumption was approximately 1.8 percent.[79] Other sources cite absorption between 0.5 and 2.0 percent.[80] Variations in absorption of trivalent chromium can be traced to differences in the type of chromium ingested, competing minerals, and the effect of vitamins, proteins, drugs, and other nutritional factors used in combination (Table 1).

Dietary factors such as starch, ascorbic acid, minerals, oxalate, and amino acid intake can have a significant influence on chromium absorption. Carbohydrate intake has been shown to influence chromium urinary excretion and tissue concentration. Mice fed ^{51}Cr-labeled chromium III chloride concomitantly with starch were found to have significantly higher concentrations of chromium in blood and tissue compared to those fed with chromium III chloride mixed with

Table 1. *Absorption and Retention of Various Chromium Compounds*

Type of Chromium	% Blood Absorption	Test Subject
Chloride	0.9 ± 0.2 (4 hrs.)[81] 0.69 (mean range 0.3-1.3)[82] 0.5[83]	Rat Human Rat
Nicotinate	1.3 ± 0.3[81]	Rat
Picolinate	1.1 ± 0.3[81] 2.8 ± 1.14 SD[84]	Rat
Dinicotinic acid diglycine cysteine glutamic acid complex	0.6 ± 0.1[81]	Rat
Chromium from food	1.8 (36.8 mcg Cr/day)[79] 2-3[85]	Human Human
Chromium from brewers yeast	5-10[82]	Human

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sucrose, fructose, or glucose.[86] Diets high in simple sugars have also been shown to increase urinary excretion of chromium by 10-300 percent, with no change in absorption rates.[87] Animals fed ascorbic acid with chromium supplementation demonstrated increased absorption.[88] A study of three women found that the ingestion of ascorbic acid (100 mg) in conjunction with chromium III chloride (1 mg) increased the absorption of chromium as measured in plasma levels.[89]

A number of minerals influence absorption. In rat studies, zinc supplementation reduced chromium absorption, while zinc deficiency had the opposite effect, elevating ^{51}Cr levels.[90] A later study by Anderson et al[81] found no alteration in tissue levels of copper and zinc when mice were fed a diet with 5000 ng Cr III/g of feed. In *in vitro* rat studies, iron, manganese, and calcium have all been shown to depress intestinal transport of chromium at levels of only 100-fold that of chromium, while in the case of titanium, concentrations only 10 times that of chromium inhibited absorption.[91] In a study of rats fed 5000 ng/g of feed of a number of organic chromium compounds (chromium picolinate, nicotinate, acetate, glycinate, histidinate, or chloride), results showed that all compounds tested increased the iron content in the liver and spleen while decreasing iron levels in the heart.[81]

The interaction of iron and chromium is thought to be linked to the shared binding sites on transferrin. Sargent et al[92] first proposed the theory that increased iron stores due to hemochromatosis might result in the competitive inhibition of chromium binding, leading to diabetic symptoms. He found that patients with hemochromatosis did, in fact, have significantly less plasma chromium than iron-depleted patients. Chromium has been found to preferentially bind to the B site of transferrin. When saturation of transferrin with iron increases in hemochromatosis to over 50 percent, iron competes with chromium binding, affecting its transport.[92] This theory is further supported by studies of patients with hemochromatosis who were found to have significantly higher excretion of the unbound plasma chromium as well as a smaller blood pool of chromium due to the saturation of transferrin by iron.[93]

It has been found that substances forming chelates with chromium generally stimulate absorption and that EDTA (ethylenediaminetetracetic acid) or DL-penicillamine significantly increase absorption as measured by ^{51}Cr levels.[91] However Chen et al[94] found no significant difference in absorption when EDTA and ^{51}Cr were administered to rats. Naturally occurring chelating agents, such as phytates and oxalates, have also been found to influence chromium absorption in both *in vitro* and *in vivo* rat studies. Rats fed chromium with oxalate were found to have higher ^{51}Cr blood and tissue levels, while rats fed phytates with chromium had lower blood and tissue levels.

A number of amino acids have also been found to increase absorption of chromium from the intestine. It was found that a mixture of 20 amino acids nearly doubled the rate of absorption. Amino acids like histidine and glutamic acid that readily form complexes with chromium were also shown to increase absorption.[91]

Earlier studies found trivalent chromium had consistent absorption and excretion regardless of previous diet history (unlike the absorption of other elements).[4] In 1996 it was discovered that chromium analyses in biological samples prior to 1980 were inaccurate due to the state of early analytical instrumentation.[95] More recent, post-1980 studies, using more accurate instrumentation, now find dietary absorption to be inversely proportional to dietary chromium intake (as with other minerals). Humans consuming a self-selected diet with an intake of 10 mcg/day Cr III had an absorption of two percent, while an intake of 40 mcg/day provided absorption of only 0.5 percent.[96]

Different forms of trivalent chromium have distinct characteristics of absorption, with inorganic complexes of trace minerals known to have lower levels compared to organic complexes. Chromite ores, chromic oxide, and chromium III chloride have historically been shown to have the lowest levels of absorption. Ingestion of inorganic salts such as chromium III chloride have levels of absorption ranging between 0.4-1.3 percent, with a mean of 0.69 percent.[82,83,97]

Many authors cite absorption levels of 2-

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3 percent of dietary chromium as organic complexes.[4,82] Chromium from brewer's yeast was absorbed in the range of 5-10 percent,[82] although others were unable to duplicate these results.[81] Chromium picolinate was found to have absorption in humans estimated at 2.8 percent ± 1.14 SD.[84] Studies on rats found that 3-8 times more chromium nicotinate was absorbed and retained than was chromium picolinate or chromium chloride. After 6-12 hours, tissues retained on the average 2-4 times more chromium nicotinate than chromium picolinate.[98] Similar results in rat studies using a number of different organic complexes of chromium found the relative absorption/retention as follows: Cr nicotinate > Cr picolinate > Cr chloride.[81] Concentrations of chromium picolinate in the liver and kidney were found to be 2-6 times higher than for chromium chloride- or chromium nicotinate-fed rats, with no detectable toxicity.[45]

Drugs and Chromium Absorption

Chromium absorption is influenced by a number of drugs. Rats fed 40 mg of aspirin had an increased absorption of chromium III chloride as measured by blood levels of ^{51}Cr.[99] Intraperitoneal injection of 5 mg indomethacin increased ^{51}Cr levels in the blood and tissue of rats given ^{51}Cr III chloride.[100] In contrast to aspirin and indomethacin, a number of antacids significantly decrease blood and tissue levels of ^{51}Cr III chloride.[99,101] The decrease in absorption of ^{51}Cr III chloride when combined with antacids (Tums® or Maalox®) has been ascribed to a competitive inhibition by the minerals in the antacids.[99]

Chromium Retention in Tissue

The extent of chromium retention in various tissues is of as much interest as the absorption of chromium compounds. Rats fed a high-chromium diet (5000 ng Cr/g food) compared to a low-chromium diet over a three-week period followed by organ dissection revealed that by far the majority of Cr concentrated in the kidneys as compared to liver, spleen, heart, lung, and gastrocnemius muscle. Table 2 demonstrates that not only is there a preferential organ concentration in the kidney but that the specific chromium compound had substantial effect, with chromium picolinate > nicotinate > chloride.[81]

Chromium dosage at quite high levels in animal studies showed no disturbance in liver and kidney function or tissue histology. In human studies, no abnormalities in liver or kidney function at doses as high as 1000 mcg/day of chromium picolinate were found. It remains to be determined if much higher doses will demonstrate a similar lack of toxicity. There

Table 2. *Chromium Concentration in Rat Organs Following Supplementation*

Form of Cr (ng/g, dry wt.)	Kidney	Liver	Heart	Gastrocnemius
Picolinate	374	49	28	25
Nicotinate	170	14	12	11
Chloride	78	10	4.6	14
Control	20	4.7	12	16

Chromium concentrations in rat organs after chromium supplemented diet (5000 ng Cr/g diet) for three weeks vs. low-chromium diet. Adapted from Anderson et al[81] by analysis of published graphs.

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has been no demonstration of any toxicity so far in the clinical observations of the authors with the use of chromium nicotinate at levels as high as 5000 mcg/day.

Conclusion

Deficiency of chromium can result in hypoglycemia or presentation of diabetes. Even in a healthy person, supplemental Cr can increase insulin sensitivity, and decrease fasting serum glucose and endogenous insulin production. Treatment of type 2 diabetes with Cr has led to improvement in serum glucose, insulin, and HbA1C levels. Organic chromium complexes give better results than inorganic chromium, and higher Cr doses give both faster and better improvement in glucose and lipid levels. Glucose control also improves in type 1 diabetes. Supplemental Cr reduces insulin resistance in gestational diabetes in a dose-dependent manner. Chromium is effective in reversing the diabetes caused by therapeutic use of glucocorticoids. The effects of Cr on blood glucose homeostasis are accomplished by increased activation of insulin receptors through binding of chromium with LMWCr.

Chromium can reduce elevated cholesterol and triglycerides in a dose-dependent relationship. Triglyceride reduction may take a longer time or higher dose in diabetics. One publication demonstrated increased serum DHEA levels in postmenopausal women with supplementation of low-dose chromium. Other data suggested that Cr may help in preventing osteoporosis.

Chromium appears to be the only nutritional mineral with a several hundred-fold difference between the acceptable daily intake level and the calculated reference dose. A search for any report of toxicity from supplemented Cr revealed only a few anecdotal reports of chromium picolinate toxicity. Only one of these seemed to have validity and exhibit an individual subjective reaction that resolved with cessation. Animal studies have failed to show toxicity by histological or laboratory examination at doses of Cr as high as 15 mg/kg body weight. The only trials conducted in humans showed no subjective toxicity or abnormal liver or renal function tests at 1000 mcg daily in diabetics over four months or at 500 mcg after one year. The clastogenic potential reported for Cr III picolinate *in vitro* was not produced by other Cr compounds, nor was it able to be reproduced *in vivo* in animals.

Absorption of chromium is low, as it is for other polyvalent minerals, ranging from less than one percent for Cr III chloride to above one percent for Cr III nicotinate to 1-3 percent for Cr III picolinate in rat studies. Human absorption from food is estimated at about 2-3 percent and 5-10 percent from brewer's yeast. Absorption of inorganic Cr is increased by starch or ascorbic acid. Concurrent supplementation with zinc, iron, manganese, or calcium retards Cr absorption. Concurrent mixed amino acids increased Cr absorption. High-dose Cr in rats increased iron content in liver and spleen, while decreasing the level in heart.

In humans, dietary Cr III absorption is inversely related to dietary intake, varying from 0.5-2 percent. Several drugs increase Cr absorption, while several antacids decrease it, supposedly due to competitive inhibition by the minerals in the antacids. In animal studies, the retention of Cr from supplementation occurred chiefly in the kidneys, followed by liver and heart muscle. The order of retention was Cr III picolinate > Cr III nicotinate > Cr III chloride, which varies from the order of absorption. No disturbances in the organ systems showing high concentration of Cr have yet been detected

The overall chromium picture causes the authors to question whether there is enough chromium in the national diet or if we are absorbing it appropriately. The authors believe higher doses of Cr than are generally prescribed by nutritionally oriented physicians may be appropriate for type 1 and 2 diabetes. At this time, there is no demonstration of general chromium toxicity in animals at a dose that would extrapolate to humans as 1050 mg daily, recalling that the RfD for humans is 70 mg daily.

One of the authors has employed high dose chromium as nicotinate for the treatment of type 2 diabetes patients at the level of 3000-4000 mcg twice daily (based on previously unpublished

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research by Jonathan Wright, MD), with outstanding reductions of glucose and lipid levels in many. None of the patients, followed for months to years, have shown increases in BUN or liver enzymes or other laboratory abnormalities. Further studies on safety and efficacy of this high-dose range are being conducted.

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