Chapter 6
Chromium: Is It Essential, Pharmacologically Relevant, or Toxic?

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Contents

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Abstract Over fifty years ago, the element chromium (as the trivalent ion) was proposed to be an essential element for mammals with a role in maintaining proper carbohydrate and lipid metabolism. Evidence for an essential role came from dietary studies with rodents, studies on the effects of chromium on subjects on total parenteral nutrition, and studies of the absorption and transport of chromium. Over the next several decades, chromium-containing nutritional supplements became so popular for weight loss and muscle development that sales were second only to calcium among mineral supplements. However, the failure to identify the responsible biomolecules(s) that bind chromium(III) and their mode of action, particularly a postulated species named glucose tolerance factor or GTF, resulted in the status of chromium being questioned in recent years, such that the question of its being essential needs to be formally readdressed. At the same time as chromium(III)'s popularity as a nutritional supplement was growing, concerns over its safety appeared. While chromium has been conclusively shown not to have beneficial effects on body mass or composition and should be removed from the list of essential trace elements, chromium(III) compounds are generally nontoxic and have beneficial pharmacological effects in rodents models of insulin insensitivity, although human studies have not conclusively shown any beneficial effects. Mechanisms have been proposed for these pharmacological effects, but all suffer from a lack of consistent supporting evidence.

Keywords chromium • insulin sensitivity • insulin signaling • rats • type 2 diabetes

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1 Introduction

Recently, a paradigm shift has occurred in terms of the status of chromium. While first proposed to be an essential element in the late 1950s and accepted as a trace element in the 1980s, scientific studies have continued to fail to produce convincing evidence of this status. In the 1990s, statements to justify the status of chromium despite the results of studies such as "Chromium is a nutrient and not a drug, and it will therefore benefit only those who are deficient or marginally deficient in Cr" [1] were common in review articles [1–3]. Recent studies have led to a reinterpretation of the status of chromium. The status of chromium as an essential element is no longer supported by experimental data. In fact, chromium is now best understood as a therapeutic agent. However, the potential benefits of the use of chromium as a therapeutic agent are uncertain, and its mechanism of action in increasing insulin sensitivity and possibly influencing lipid metabolism at a molecular level is poorly understood.

This review will examine the data on which chromium was proposed to be an essential element and describe the problems with this interpretation, discuss the evidence for a therapeutic role for chromium in animal models of diabetes and insulin resistance, and evaluate the potential toxicity as chromium(III) complexes when used at pharmacologically relevant doses.

2 Is Chromium Essential?

2.1 Current Opinions

Chromium reduces body fat, causes weight loss, causes weight loss without exercise, causes long-term or permanent weight loss, increases lean body mass or builds muscle, increases human metabolism, and controls appetite or craving for sugar, while 90% of US adults do not consume diets with sufficient chromium to support normal insulin function, resulting in increased risk of obesity, heart disease, elevated blood fat, high blood pressure, diabetes, or some other adverse effect on health. Any or all of the above representations may come to mind when thinking about chromium and its relationship to human nutrition. Most people think of chromium in terms of weight loss and lean muscle mass development as a result of nutraceutical product marketing. However, the Federal Trade Commission (FTC) of the United States ordered entities associated with the nutritional supplement chromium picolinate to stop making each of the above representations in 1997 because of the lack of "competent and reliable scientific evidence" [4].

Overwhelming scientific evidence currently indicates that chromium does not affect body mass and body composition of healthy individuals and that chromium nutritional deficiency is rare (if it exists at all) [5]. Yet, although the ruling by the FTC is over 15 years old, such representations can still be found in the popular media. In the United States, the National Research Council of the National Academies of Science recognized chromium as an essential trace element in 1980 and reviewed this position in 1989 and 2002 [6–8]. However, in 1980 and 1988, chromium was determined to have an estimated safe and adequate daily dietary intake (ESADDI) of 50–200 μg, while in 2002 this was changed to an adequate intake (AI) of 30 μg. The Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) [9] in 2009 determined that chromium deficiency in farm animals had never conclusively been observed such that 'no evidence of the essentiality of Cr(III) as a trace element in animal nutrition' exists. As discussed in Section 2.2, the status of chromium is at best uncertain currently, and the element should probably be removed from the list of essential trace elements.

2.2 Evidence

2.2.1 "Low Chromium" Rodent Diets

Over fifty years ago, Cr was suggested to be an essential trace element in the mammalian diet. In this work reported by Mertz and Schwarz [10], previously considered the pioneering work in the field, rats were fed a torula yeast-based diet, which compromised the health of the rats. The rats developed necrotic liver degeneration and apparently impaired glucose tolerance in response to an intravenous

glucose load [10]. Selenium was discovered to reverse the liver disorder but not the glucose intolerance; as a result, the authors proposed a new dietary requirement, coined glucose tolerance factor (GTF) was absent from the torula yeast-based diet and responsible for the glucose intolerance [11]. In an effort to identify the missing dietary component, a variety of chemicals and some foods were added to the diet. Most notably, inorganic compounds containing over 40 different elements (200–500 mg element/kg body mass) could not restore glucose tolerance, while several inorganic Cr(III) complexes (200 mg Cr/kg body mass) restored glucose tolerance [12]. Brewer's yeast and acid-hydrolyzed porcine kidney powder were identified as natural sources of the missing dietary component and were found to contain appreciable quantities of Cr [12]. When given by stomach tube (500–1000 mg/kg body mass), brewer's yeast, porcine kidney powder, and concentrates made from them restored proper glucose metabolism in rats on the torula yeast-based diet [12]. Consequently, Mertz and Schwarz proposed the active ingredient of GTF was Cr^{3+}, making Cr an essential trace element for the mammalian diet [12].

As this became the primary evidence for an essential role for Cr, one must ask what exactly this work established? The Cr content of the regular laboratory rat diet and of the torula yeast-based diet have not been determined; thus, the rats were not shown to actually receive a diet lacking or deficient in Cr; the studies only indicated that adding Cr to the diet could lead to potential effects on apparent glucose intolerance. Subsequently, the Cr content of torula yeast has been determined, but the Cr content has been found to range significantly in value [13,14]; the content probably varies based on the growth conditions. As a result, the content of the original diet simply cannot be established. In addition, the rats were housed in wire mesh cages, possibly with stainless steel components (as the metal composition of the wire was not reported), allowing the rats to obtain Cr by chewing on these components. Thus, the actual Cr intake of the rats in these studies is impossible to gauge. As subsequent studies have shown that rat in metal free cages on purified diets fail to develop Cr deficiency, these studies fail to establish that the animals developed a Cr deficiency.

The results do raise another possible explanation, one that was not originally considered – the Cr added to the torula yeast-based diet was having a pharmacological or therapeutic effect and not correcting a nutritional deficiency. The magnitude of the doses of Cr utilized in these studies need to be put into perspective. An American consuming a nutritionist-designed diet [15] or self-selected diet [16] consumes about 30 μg of Cr daily. This value, 30 μg Cr/day, is the value set as the adequate intake (AI) by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences (USA) [8]; as defined, the AI indicates that >98% of the population receiving this quantity of an item display no health problems from deficiency. Given the average body mass of a human, 65 kg, gives an adequate Cr intake of less than 0.5 μg Cr/kg per day. Rats on the torula yeast-based diet that was supplemented with Cr compounds received at least 400 times this quantity, a supra-nutritional dose. These comparisons, of course, make the assumption that the biochemistry of Cr is similar in rodents and primates.

Attempts have been made to establish the nutritional status of Cr using nutritionally compromised diets supplemented with Cr, most notably in the 1990s [17–19]; the rationale behind these diets was that stresses that increase urinary Cr loss could potentially lead over time to chromium deficiency. However, these studies suffer from some of the same flaws in assumptions as the initial studies. Rats were provided a high-sugar or high-fat diet (supposedly a "low-Cr" diet with ca. 30 μg Cr/kg diet) with additional mineral stresses for 24 weeks, resulting in compromised lipid and carbohydrate metabolism in the rats. The addition of 5 ppm Cr to the drinking water of rats on the stressed diets led to plasma insulin levels tending to be higher in intravenous glucose tolerance tests after 24 weeks on the diet [17]. Unfortunately, the Cr intake compared to the Cr loss in the rats was not determined so that whether the rats were maintaining a Cr balance cannot be established. However, as described in Section 2.2.2, the amount of urinary Cr loss is directly dependent on the amount of Cr intake so that the rats should not have developed a Cr deficiency. Consequently, the results should be interpreted in terms of supplemental Cr having a beneficial effect on diet-induced insulin resistance, a pharmacological rather than nutritional effect.

An analysis of the actual Cr content of the diet is in order. A male Wistar rat (as used in Refs [17–19]) on average in a subchromic study consumes 20 g of food a day and has an average body mass of 217 g [20]. Twenty grams of food containing 33 μg Cr/kg food provides 0.66 μg Cr. Thus, 0.66 μg Cr/d for a 217 g rat is 3.0 μg Cr/kg body mass per day, six times what a human intakes. Thus, the "low-Cr" diet was not deficient, unless rats require more than six times the Cr dose that humans do. In contrast, a male Wistar rat on average drinks 147 mL of water [20]. This volume of water supplemented with 5 ppm Cr provides 735 μg Cr daily or 3.39 mg/kg body mass. This is approximately 100 times the adequate intake of an American male (35 μg Cr/day) [8]. Again, indicating the lowering of plasma-insulin levels by addition of Cr can only be considered a pharmacological effect.

Finally, a most recent study appears to have unambiguously demonstrated that Cr has a pharmacological rather than a nutritional effect in mammals [21]. Whether Cr is an essential element was examined for the first time in carefully controlled metal-free conditions using a series of purified diets containing various Cr contents. Male lean Zucker rats were housed in specially designed metal-free cages for six months and fed the *AIN-93G* diet with no added Cr in the mineral mix component of the diet (containing 16 μg Cr/kg diet), the standard *AIN-93G* diet (containing added 1,000 μg Cr/kg), the standard *AIN-93G* diet supplemented with 200 μg Cr/kg, or the standard *AIN-93G* diet supplemented with 1,000 μg Cr/kg. The Cr content of the diet had no effect on the body mass or food intake. Similarly, the Cr content of the diet had no effect on the glucose levels in glucose tolerance or insulin tolerance tests. However, a distinct and statistically significant trend toward lower insulin levels under the curve after a glucose challenge was observed with increasing Cr content in the diet; rats on the supplemented *AIN-93G* diets had significantly lower areas ($P < 0.05$) than rats on the low-Cr diet. The study revealed that a diet with as little Cr as reasonably possible had no effect on body composition, glucose metabolism, or insulin sensitivity compared with a "Cr-sufficient" diet; however, pharmacological

quantities of Cr had a concentration-dependent effect on lowering insulin levels in glucose tolerance tests, indicating that Cr may have a pharmacological effect increasing insulin sensitivity in healthy rats [21].

In summary, a complete paradigm shift has occurred in the field of Cr nutrition, where for four decades, Cr had been considered to have only a nutritional, not a pharmacological effect. Now Cr is realized to have a pharmacological effect rather than a nutritional one. Nutritional studies cannot be used to determine whether Cr is an essential element. Studies using as little Cr as possible in the diet have failed to establish any signs of Cr deficiency. Without conclusive positive evidence, Cr cannot be considered an essential trace element. A demonstration that Cr could potentially be an essential element will probably require the isolation of a biomolecule that is essential to some critical biological process and requires Cr to perform its essential function. As described below (Section 3.3), this has not occurred.

2.2.2 Absorption and Transport

Cr is absorbed by passive diffusion when intaken orally. This has been convincingly demonstrated by a double perfusion technique using segments of the small intestine of rats; these studies revealed that over a 100-fold range of $[Cr_3O(propionate)_6(H_2O)_3]^+$ (Cr3) concentrations (10–1000 ppb), chromium absorption was a nonsaturable process [22]. Additionally, studies following the fate of orally administered ^{51}Cr have not observed a change in % Cr absorption over a range of intakes [23,24]. Most recently, rats gavaged with a dose of $CrCl_3$ absorbed approximately 0.2% of the Cr over a 2000-fold range of doses (0.01–20 mg Cr) [24]. Another interesting conclusion that can be drawn from the intestinal perfusate studies is that Cr appears to be actively transported out of the intestinal cells, as approximately 94% of the Cr entering the cells was cleared from the cells (leaving only approximately 6% behind to be stored). However, no transporter is known for Cr^{3+}. This suggests the possibility that Cr^{3+} may be bound to some chelating ligand and actively transported in this form; this is an area requiring further research. Changes in diet could affect the amount of Cr absorption and potentially affect the mechanism, although changes in mechanism have not been demonstrated. For example, the presence of added amino acids, phytate (high levels), ascorbic acid, and oxalate, but not low levels of phytate, in the diet reportedly altered the extent of Cr uptake (reviewed in [25]), although the changes (while statistically significant in some cases) were relatively small in a small percentage of absorption.

Once in the bloodstream, Cr^{3+} binds almost exclusively to the Fe-transport protein transferrin. The association of transferrin and Cr has been reviewed previously [26]. Cr-loaded transferrin has been demonstrated to transport Cr *in vivo* [27,28]. Injection of ^{51}Cr-transferrin into rats resulted in incorporation of ^{51}Cr into tissues. The transport of Fe into tissue by endocytosis of transferrin has been found to be insulin sensitive, as the transport of Cr; injection of labeled transferrin and insulin resulted in a several fold increase in urinary Cr [28]. Thus, transferrin, in an insulin-dependent fashion, can transfer Cr to tissues from which it is excreted

in the urine. The binding of Cr to transferrin is quite tight, although the apparent binding constants for the two metal binding sites differ by approximately 10^5 [29]; the *in vitro* binding of Cr^{3+} from inorganic salts has been shown to be quite slow [29], although these studies were performed in the presence of ambient bicarbonate concentrations. This also suggests that Cr may be carried to transferrin as a chelate complex. However, recent studies in the author's laboratory reveal that at the bicarbonate concentration of human blood (~20 mM) the binding of Cr^{3+} is quite rapid (B. Liu, G. Deng, K. Wu, and J. B. Vincent, unpublished results). Once Cr is brought into the cell by endocytosis, it must leave the endosome to enter the cell cytosol. As Cr^{3+} is not readily reduced by any biological reducing agents, so that it can be transported by divalent metal ion transporters (in a fashion similar to Fe), it must be transported by another mechanism; this is another area requiring further research [30].

A human study of chromium absorption as a function of Cr intake has often been cited as evidence of an essential role for Cr; however, this single study requires reproduction. Anderson and Koslovsky have reported an inverse relationship between dietary chromium intake and degree of absorption observed in human studies [31]. The data suggest that absorption of Cr varies approximately from 0.5 to 2.0% for Cr intakes of ~15–50 μg per day. This difficult to perform study is far from definitive; for example, a distinct difference is found if the data are separated into male and female subjects. For males, no statistical variation occurs for chromium absorption as a function of intake, while an apparent inverse trend is observed for the female subjects. However, these data are in striking contrast to this same lab's studies reported two years earlier [32]. Chromium absorption was determined to be ~0.4% for free-living individuals; when Cr intake was increased by over fourfold, urinary chromium excretion increased over fourfold while maintaining ~0.4% absorption of chromium for both males and females. The difference between the two studies lies in the range of Cr intakes of ~15–50 μg per day for the former and ~60–260 μg per day for the latter, suggesting that an inverse relationship between Cr intake and absorption, if it exists, exists only at the lowest portion of the range of intakes. The former study requires a careful examination in terms of statistical analysis and propagation of error, in addition to reproduction, before this study can be used as evidence for an essential role for Cr in humans (or female humans).

Cr concentrations in the human urine and blood serum are proportional to Cr intake [32,33], while human urine Cr concentrations do not correlate with serum glucose, insulin, or lipid parameters or with age or body mass [32]. Additionally, in rats, Cr concentrations in the liver and kidney correlate with Cr intake [34]. Urinary Cr loss is increased in type 2 diabetic subjects [35,36], raising the question of whether the increased Cr loss could result in a conditional Cr deficiency; however, studies with model diabetic rats (alloxan-treated rats [37] and Zucker diabetic fatty rats [38]) have shown that the increases in urinary Cr excretion are the result of increases in Cr absorption (perhaps simply as a result of increased water consumption). Thus, urinary Cr loss is controlled by absorption of Cr, and Cr apparently is not a conditionally essential element.

An increase in urinary Cr excretion has been reported for human subjects on self-selected diets in response to a glucose challenge, while no effect was observed for individuals taking a Cr supplement (200 μg Cr as $CrCl_3$ for 3 months) [33]. Urinary Cr loss after a glucose challenge was found not to be predictable and suggested to not reflect Cr status [33]. Yet, the extent of movement of chromium to the urine in response to a glucose challenge did change, from an increase at normal Cr intake to no increase when supplemented with Cr (the inverse of the expected observation). Also in this study, the Cr intake of the individuals in the study was not established. The results from humans on self-selected diets are consistent with studies of urinary Cr loss in subjects on diets supplemented with a variety of varying carbohydrates [39]. The greater the increase in the amount of insulin in the blood in response to the various carbohydrates, the more Cr was lost in the urine [39]. Thus, Cr appears to be mobilized in response to insulin, rather than directly to glucose or other carbohydrates. A range of responses to the carbohydrates was noted. Some of the subjects who in response to the diets had the highest circulating blood insulin levels had decreased abilities to mobilize Cr for excretion in the urine (within 90 min); thus, a group of subjects with decreased carbohydrate tolerance appeared to have decreased urinary Cr loss [39]. The Cr content of the self-selected diets of individuals in the study was not determined, and the subjects do not appear to have been questioned about whether they were consuming any Cr-containing supplements [39,40].

Urinary Cr excretion after a glucose tolerance test does not differ between control men or hyperinsulinemic men or differ between men on diets with differing high amylase cornstarch contents [41]. Eight of 10 healthy individuals have been found to have increased urinary Cr loss (ng Cr/min) for 4 hours after an oral glucose tolerance test compared to the 4 hours before the test such that the mean Cr loss was significantly greater after the test than before, while no mean effect was observed for 13 diabetic subjects [42]. Finally, Morris and coworkers conducting hyperinsulinemic euglycemic clamp studies have shown that increases in blood insulin levels, not specifically blood glucose levels, are responsible for a decrease in plasma Cr and an accompanying increase in urinary Cr loss [43], consistent with their earlier studies demonstrating increased urinary Cr loss after an oral glucose challenge [44]. Thus, humans appear to increase urinary Cr loss in response to an increase in blood insulin concentrations (whether from a carbohydrate or insulin challenge) although the magnitude of the change appears to be quite variable, including some individuals who may not respond potentially as a result of decreased glucose tolerance. This increase apparently results from the increased movement of Cr bound to transferrin as noted above.

Rats have been conclusively shown to increase Cr excretion in response to an insulin or glucose challenge [27,28,45]. If Cr were essential and had a role under physiological conditions in insulin sensitivity, this increase in urinary Cr loss in response to insulin could potentially serve as a biomarker for Cr. However, studies on rats on the purified diets containing as low as possible to very high Cr contents (described in ref. [21]) show that the increase in rate of urinary Cr loss does not correlate with Cr intake, even at the lowest Cr content [46]. At the highest Cr intake and thus highest background rate urinary Cr loss, insulin did not stimulate an

increase in rate of Cr loss [46]. These results are very similar to those described above in humans [33]; thus, insulin-stimulated Cr loss is not a biomarker for Cr status, and the movement of Cr in response to insulin does not provide evidence for its being essential.

One cannot help but notice that Cr appears to be set up in terms of transport to play a role in glucose metabolism. In the bloodstream, Cr binds tightly to one site of transferrin. While transferrin is kept only 30% saturated with iron and has similar binding constants for both Fe^{3+} binding sites, Cr^{3+} binds more rapidly and more tightly to the site that Fe^{3+} binds to more slowly; thus, transferrins appear primed to carry Cr in addition to Fe. As transferrin movement is insulin-sensitive, Cr bound to transferrin is delivered to tissues in an insulin-sensitive fashion; this transport of Cr is primarily to the skeletal muscle [27,28], where most glucose is metabolized in response to insulin. Cr is then rapidly removed from these tissues.

2.2.3 Total Parenteral Nutrition

Starting in the late 1970s, studies of patients on total parenteral nutrition (TPN) have been used to support the proposal that chromium is an essential element [47–50]. This stems from patients on TPN who developed impaired glucose utilization or glucose intolerance and neuropathy or encephalopathy [47,48,51–55]. The symptoms were reversed by chromium infusion and not by other treatments, including insulin administration alone. While limited to less than ten individual cases, these studies have been interpreted as providing evidence of clinical symptoms associated with chromium deficiency that can be reversed by supplementation. Another patient on TPN who developed symptoms of adult-onset diabetes and hyperlipidemia but died had low tissue chromium levels [56]. Additionally, the effects of chromium supplementation on five patients on TPN requiring a substantial amount of exogenous insulin have been examined. Three subjects displayed no beneficial response while two showed a possible beneficial response to chromium supplementation [57]. Subjects received TPN containing 10 μg Cr/day followed by supplementation with an additional 40 μg Cr/day for 3 days and then restoration of the normal TPN.

Curiously the development of symptoms that were reversible by chromium supplementation does not correlate with serum chromium levels [49], indicating that either serum chromium levels are not an indicator of chromium deficiency or that another factor is in operation. Additionally, these incidences of diagnosed potential chromium deficiency have been questioned recently as they lack consistent relationships between the chromium in the TPN, time on TPN before symptoms appear, serum chromium levels and symptoms [58].

The most notable features of these studies are the quantities of Cr administered. In the cases where apparent deficiencies were reported, the TPN solutions provided 2–240 μg Cr/day. For comparison, all the Cr in the TPN is introduced into the bloodstream, while only 0.5% of Cr in the regular diet is absorbed into the bloodstream. Thus, 30 μg of Cr in a typical daily diet presents only ~0.15 μg Cr to the bloodstream.

The TPN solutions are consequently providing 13–67 times the required amount of chromium; thus, based on these data, *the TPN solutions cannot be considered Cr-deficient*. Subjects were, in turn, treated with 40–250 μg Cr/day added to the TPN solution to alleviate their conditions, clearly pharmacological doses, as the largest dose provided 1.7×10^3 times more chromium than a standard diet. Consequently, the results with the insulin-resistant TPN patients can only be considered as providing evidence for a pharmacological role of chromium. The data are not relevant for examining whether chromium is an essential element.

Not surprisingly, as TPN provides ten or more micrograms of chromium per day, TPN patients are accumulating chromium in their tissues [59,60]. Calls are appearing for the re-examination of the chromium levels in TPN solutions in terms of a need to reduce recommended levels [61].

In summary, evidence to designate chromium an essential element does not exist. While the possibility always exists that evidence could surface in the future to support a biological role for chromium, such assumptions cannot be taken into current considerations. The next review of the status of chromium by the Committee on the Scientific Evaluation of Dietary Reference Intakes of the National Academies of Science (USA) must seriously consider revising its status.

3 Is Chromium Pharmacologically Relevant?

3.1 Rodent Disease Model Studies

Several rat models of type 2 diabetes have been utilized to examine the effects of Cr(III) administration [5]. Three models have symptoms arising from mutations of the leptin receptor: the JCR:LA-cp, Zucker obese and Zucker diabetic fatty rats. Leptin is a hormone produced by adipocytes that signals the brain that the appetite should be suppressed. Consequently, as the leptin signaling system is blocked at the receptor, the JCR:LA-cp and Zucker obese rats become markedly obese and insulin-resistant and possess somewhat elevated blood glucose levels and elevated levels of blood insulin, triglycerides, and cholesterols. The Zucker diabetic fatty (ZDF) rats have an additional, uncharacterized mutation that results in these rats developing symptoms very comparable to type 2 diabetes in humans, including elevated blood glucose levels, in addition to the high triglycerides and cholesterol levels. In contrast to the obese models, the ZDF rats have smaller body masses than healthy Zucker rats. Some general statements for studies of Cr(III) complex administration using these three models can be made. When Cr is administered at a young age, it has no effect on body mass and food intake [62–69]. Cr administration generally appears to have no effect on fasting blood glucose levels but to lower glycated hemoglobin levels. (This might be explained by the data of Vincent and coworkers [66], which show that while glucose levels tend to be lower in Cr-treated animals at several instances during the administration period, that this effect is not significant; however, glycated hemoglobin levels, which serve as a window to the average

exposure of red blood cells to glucose over 60–90 days, reflect a beneficial effect on blood glucose over this time.) Cr appears generally to be beneficial to lipid metabolism, lowering total cholesterol levels; however, effects on other lipid variables are inconsistent. Thus, in these rat models of diabetes and obesity-related insulin resistance, Cr appears to have beneficial effects on insulin resistance, marginally beneficial effects on blood glucose, and beneficial effects on the grossly elevated plasma lipid levels. Unfortunately, only a tiny percentage of human type 2 diabetes cases are the result of mutations in leptin or its receptor.

The Goto–Kakizaki rat is a non-obese model of type 2 diabetes; the origins of the diabetes at a molecular level are not known. Two studies have examined the effects of $[Cr(pic)_3]$ (1–100 mg/kg daily) for either 4 or 32 weeks [70,71]. Unfortunately the reports do not indicate whether the dose is of Cr as the compound or of the compound (in which case ~12.5% of the dose would be Cr). No effects were observed on body mass, fasting blood glucose or insulin levels, or glucose or insulin areas under the curve in a glucose tolerance test. For this model, Cr(III) appears to have no appreciable effect.

The chemical streptozotocin when administered intravenously or intraperitoneally relatively selectively kills the beta cells of the pancreas, destroying nearly all the body's ability to produce insulin. Thus, rats treated with the chemical serve as an excellent model of type 1 diabetes (not type 2 diabetes). To generate a better model for type 2 diabetes studies, the addition of a high-fat diet has been utilized in addition to the chemical treatments or streptozotocin has been given to newborn rats, rather than adults. Four studies have examined the effects of Cr supplementation on these type 2 models where they found lower fasting glucose, total cholesterol, and triglycerides concentrations [72–75]. Studies using just streptozotocin have given inconsistent results but are also very different in design from one another making interpretation difficult [5].

In summary, the results of the studies with rats undergoing modified streptozotocin treatments (lower fasting glucose but not insulin and effects on lipids) are different from those of the Goto–Kazizaki rats (no effects) that are in turn different from the results from the leptin-receptor mutation models (lower fasting insulin but not glucose and effects on lipids). No great dependence appears on dose (when the doses are supranutritional), length of time of Cr administration or form of Cr. The origin of the diabetes appears to make a significant difference on the potential benefits of Cr administration.

Mouse models of diabetes with mutations to the genes for leptin, the ob/ob mouse, and leptin receptor, the db/db mouse, have also been studied in terms of effects of Cr(III) administration. Both these models display obvious obesity. Unfortunately, not all the studies have used well-defined forms of Cr. The results of these studies have been conflicting in terms of fasting blood glucose and cholesterol concentrations, although glucose and insulin levels in glucose tolerance tests consistently tend to be lower [76–84] (reviewed in [5]).

Thus, with one exception, Cr(III) treatment of rat and mouse models of type 2 diabetes have had beneficial effects, although the effects differ from one model to the other. These differences in the models may be significant to the results observed in human clinical trials.

3.2 Clinical Studies

While human studies of the effects of chromium supplementation have failed to observe effects in healthy subjects, clinical trials of the effects of chromium supplements on type 2 diabetic subjects have failed to generate consistent results. A recent review that included only studies that were placebo-controlled and used a chemically well-defined form of Cr identified 19 studies that met the criteria [5]. (Not including well-defined studies eliminates Cr sources such as "Cr-enhanced yeast"). Nine of the 19 reports reported no effects from supplementation; another may or may not have seen significant changes depending on how the statistical analysis is performed. Studies using 150–1000 μg Cr daily for 6 weeks to 16 months have reported no effects from Cr, while studies using 200–1000 μg for 10 days to 6 months reported beneficial effects. Studies using over 100 subjects, that should have more power to distinguish potential differences, reported no effects in one case and beneficial effects from supplementation in the others. Several studies are quite small, lacking the statistical power to potentially observe effects.

Similarly, no pattern was identified in terms of beneficial effects on particular symptoms from Cr supplementation. Fourteen studies examined fasting blood glucose levels. Five reported that levels dropped with supplementation while nine observed no effect. Four studies observed no effect on fasting insulin levels while levels were lower in three studies. Triglyceride levels were unaffected in four studies and lower in two studies. Glycated hemoglobin levels were reported to be lower in four studies, but no change was reported in five studies. Effects on cholesterol levels were slightly more consistent. Seven studies reported no lowering of total cholesterol while three noted decreases. For HDL, six studies reported no effect, while a single study reported an increase in levels; for LDL, five studies reported no effects, while only a single study reported a decrease. In response to some type of a glucose challenge, four studies observed no effects on glucose levels while three saw positive effects; in terms of insulin response, one study had mixed results depending on the time interval that Cr was administered, while another reported positive effects. Behavior of the blood variables across the studies was simply found to be too inconsistent to draw any firm conclusions. This inconsistent behavior existed whether these studies are broken down by the compound used, the amount of Cr, the number of subjects, or the length of the study [5].

Two thorough meta-analyses of the effects of Cr supplements on type 2 diabetic subjects have been reported. Althius et al. [85] in 2002 performed a meta-analysis on studies under a contract from the Office of Dietary Supplements of the National Institutes of Health (USA). Using their criterion for inclusion (trials containing a Cr treatment group and a control), the authors identified only four studies of subjects with type 2 diabetes for analysis. The combined data from the studies, except those from a study by Anderson and coworkers [86], showed no effect from chromium on glucose or insulin concentrations. Thus, they concluded that the data on diabetics were inconclusive. The authors also examined the effects of Cr supplements on healthy subjects or subjects with impaired glucose tolerance (but not type 2 diabetes)

in 14 trials including 425 subjects; no associations between Cr administration and glucose or insulin concentrations were found.

Another meta-analysis was reported by Balk et al. in 2007 [87], the most thorough meta-analysis on Cr supplementation in terms of blood variables reported to that date. Forty-one randomized controlled trials were identified that examined the effects of chromium supplementation on glucose metabolism and lipids concentrations in ≥10 non-pregnant adults (i.e., healthy and diabetic subjects) for ≥3 weeks. However, almost half were determined to be of poor quality. Nine studies were funded by the food or supplement industry, 18 were by non-industry sources, and 14 did not indicate the funding source. Ten studies used Brewer's yeast, 15 studies used $CrCl_3$, 5 studies used Cr nicotinate and 15 studies used $[Cr(pic)_3]$; some studies compared multiple sources of Cr. No benefit from Cr supplementation was identified for healthy individuals [87]. Eighteen studies were identified that examined type 2 diabetic subjects. Cr supplementation was found to statistically improve glycemic control in type 2 diabetics. The effects were fairly small but significant overall. When broken down by Cr source, the effects were small but significant for subjects on yeast and $[Cr(pic)_3]$ but not $CrCl_3$. Most significantly, the authors determined the results were not definitive because of the poor quality and heterogeneity of the studies. Overall Cr did not affect lipid levels, while $[Cr(pic)_3]$ lowered glycated hemoglobin levels. However, lower glycated hemoglobin levels were only observed in 3 interventions out of 14, two of which came from a single, large study (that of Anderson and coworkers [86]). Amongst fasting glucose studies, a trend was observed that industry-sponsored studies were more likely to observe beneficial effects. The authors also expressed concerns that the Brewer's yeast results suggested that another component in the yeast may be having an effect because effects were observed at lower doses of Cr. As a bottom line the authors concluded that Cr supplementation 'may have a modest effect' on glucose metabolism in type 2 diabetics but that 'the large heterogeneity and the overall poor quality limit the strength of our conclusions' and that more randomized trials are required [87]. The study was supported by a contract from the Agency for Healthcare Research and Quality (US Department of Health and Human Services).

Three studies meeting the appropriate criteria have appeared since the Balk et al. meta-analysis. These are a small study by Lai [88] with Cr yeast with a 10 subject treatment and 10 subject control that observed small effects on plasma glucose, insulin, and glycated hemoglobin; a study with Cr yeast utilizing 57 subjects by Kleefstra et al. [89] that observed no effects; and a study by Cefalu et al. [90] with 93 subjects that observed no effects with 1000 μg Cr daily as $[Cr(pic)_3]$. These studies, because of the participant size of the last two, would have significantly affected the results of the meta-analysis if they could have been included, making any effect of Cr on fasting glucose in type 2 diabetics even more questionable. One must also note that any meta-analysis is likely to be biased toward the positive as studies with negative results tend to be published less frequently than positive reports. Basically, the results come down to the following: (i) clinical trials on Cr(III) complex supplementation for healthy subjects observe no effects from treatment, (ii) clinical studies on Cr(III) complex supplementation are equivocal for type 2 diabetic

subjects, and (iii) the results of the trials with diabetic subjects are basically only considered equivocal, rather than without observable effect, because of the results of the single large, well-designed study by Anderson and coworkers [86]. This study is unique in being the only study using subjects from China and needs to be independently repeated.

In a review in 1998, Anderson [91] split studies on Cr supplementation of type 2 diabetics into two groups: subjects receiving ≤200 μg Cr daily and subjects receiving >200 μg Cr daily. Using all the studies identified with diabetic subjects to that date, Anderson suggested that >200 μg Cr were required for diabetic subjects to generate an observable effect. The effect appeared to be largest for $[Cr(pic)_3]$ where this apparent effect was the result of only the single study by Anderson and coworkers [86]). Subsequently, this requirement has commonly been cited. However, studies since 1998 have failed to follow the trend identified by Anderson.

Cefalu and coworkers [90,92] in a preliminary and then in a subsequent report potentially may have found a relationship that might explain the different results between populations in the various studies. In a double-blind, placebo-controlled study, 93 subjects with a fasting plasma glucose level of at least 6.94 mmol L^{-1} received 1000 μg Cr daily as $[Cr(pic)_3]$ or placebo for 24 weeks [90]. Comparison of the treatment and control groups found no effects on body mass, percentage body fat, free fat mass, or abdominal fat deposits, fasting glucose, glycated hemoglobin, or insulin sensitivity. Yet, effects were observed when the Cr-receiving subjects at the end of the study were divided into responders (≥10% increase in insulin sensitivity from baseline) and non-responders. At baseline, responders had lower insulin sensitivity and higher fasting glucose and glycated hemoglobin levels than non-responders. Thus, Cefalu and coworkers might potentially have identified predictors for type 2 diabetic subjects that might preferentially respond to Cr treatment. These results will need to be carefully tested in additional studies where the 'responder' group is identified before the Cr administration to establish whether a subsequent difference is actually manifested.

According to the American Diabetes Association in its 2010 Clinical Practices Recommendations, 'Benefit from chromium supplementation in people with diabetes or obesity has not been conclusively demonstrated and therefore cannot be recommended' [93]. The American Diabetes Association dropped any mention of chromium in its 2011, 2012, and 2013 recommendations.

In December 2003, Nutrition 21, the major supplier of chromium picolinate, petitioned the United States Food and Drug Administration (FDA) for eight qualified health claims:

1. Chromium picolinate may reduce the risk of insulin resistance.
2. Chromium picolinate may reduce the risk of cardiovascular disease when caused by insulin resistance.
3. Chromium picolinate may reduce abnormally elevated blood sugar levels.
4. Chromium picolinate may reduce the risk of cardiovascular disease when caused by abnormally elevated blood sugar levels.
5. Chromium picolinate may reduce the risk of type 2 diabetes.

6. Chromium picolinate may reduce the risk of cardiovascular disease when caused by type 2 diabetes.
7. Chromium picolinate may reduce the risk of retinopathy when caused by abnormally high blood sugar levels.
8. Chromium picolinate may reduce the risk of kidney disease when caused by abnormally high blood sugar levels [94].

After extensive review, the FDA issued a letter of enforcement discretion allowing only one (No. 5) qualified health claim for the labeling of dietary supplements [94,95]: 'One small study suggests that chromium picolinate may reduce the risk of type 2 diabetes. FDA concludes that the existence of such a relationship between chromium picolinate and either insulin resistance or type 2 diabetes is highly uncertain.' The small study was performed by Cefalu et al. [96]. This study was a placebo-controlled, double-blind trial examining 1000 μg/day of Cr as $[Cr(pic)_3]$ on 29 obese subjects with a family history of type 2 diabetes; while no effects of the supplement were found on body mass or body fat composition or distribution, a significant increase in insulin sensitivity was observed after four and eight months of supplementation.

This raises the question of why the discrepancy between human and rodent studies exists. Rodent studies observing beneficial effects generally provided rats between 80 and 1000 μg Cr/kg body mass daily. Based on mass, this would correspond to 5.2 to 65 mg Cr daily for an average 65 kg human. Even when corrected for the increased metabolic rate of rats compared to humans, this range corresponds to ~1 to 13 mg of Cr daily. Thus, human clinical trials may have only started to approach the dose necessary to see a beneficial effect in humans. The amount of Cr used in clinical trials needs to be increased before ruling out that Cr has no effect on type 2 diabetic subjects. However, one cannot rule out that something is unique about rodents that allows Cr to have beneficial effects. Unfortunately, studies of Cr supplementation on farm animals are also equivocal and often use doses in proportion to body mass even smaller than those used in human clinical trials [5,97].

Recently, Vincent [5] has proposed that in order to definitely determine whether Cr supplementation has an effect on diabetics, human clinical trials should:

(1) be performed with sufficient power to be able to realistically observe effects, on subjects whose baseline characteristics are well established, and for periods of time of at least 4–6 months. Knowing baseline characteristics is particularly important, given the possibility at the current dosages that only subjects with the highest degrees of insulin resistance may be responsive to Cr.
(2) be performed with larger doses of Cr(III). Studies using JCR:LA-cp or ZDF rats utilized 80–1000 μg Cr/kg daily corresponding to approximately 5.2–65 mg daily for a human (based on body mass). If corrected for the increased metabolic rate of rats, this still correspond to ~1–13 mg daily. Studies are needed using 5–7 mg Cr(III) daily for 4–6 months or longer.
(3) be carefully monitored for any deleterious effects, especially when using the higher doses of Cr(III).

3.3 Proposed Mechanisms of Action

3.3.1 Insulin Signaling

When many bioinorganic chemists or nutritionists think of a biological form of chromium, glucose tolerance factor (or GTF) may be their first thought. As has been reviewed many times recently [5,25,98], the studies postulating the existence of GTF are flawed, and the material isolated from Brewer's yeast and also called GTF is an artifact of its isolation. The term GTF should be removed from the lexicon of the chemistry and nutrition communities. What then can be said about the action of chromium at a molecular level?

Given that Cr(III) appears to have pharmacological effects in increasing insulin sensitivity and altering lipid metabolism in rodent diabetes models, Cr must interact directly with some biomolecules(s) to generate these effects. To begin to elucidate how Cr can affect insulin sensitivity at a molecular level, the effects of Cr on cultured mammalian cells have been probed. However, research results are contradictory such that the state of the field is not immediately clear (reviewed in [98]). Using the lesson learned from toxicology studies of $[Cr(pic)_3]$ (see Section 4.2), some of the discrepancies might be explained based on the stability of the Cr(III) complexes and what form of Cr(III) is actually being presented to the cells (and whether this form is biologically relevant); yet, this does not aid in elucidating the site of action of Cr. Most of these studies have used adipocytes (or preadipocytes) or skeletal muscles, cells known to incorporate Fe via endocytosis of transferrin. These cells should, thus, intake Cr via transferrin endocytosis. Given that Cr-loaded transferrin can be readily prepared, the physiologically relevant form of Cr, i.e., Cr transferrin, should be used in cell culture studies to deliver Cr to the cells.

One result is nearly uniform across cell culture studies utilizing skeletal muscle, adipocytes, or adipocyte-like cells – Cr enhances glucose uptake and metabolism in a fashion dependent on insulin (see, for example, [99]). Numerous pathways by which a Cr biomolecule could manifest itself in these effects have been proposed. However, research results in *in vitro* and *in vivo* systems are contradictory, such that the state of the field is not immediately clear (Table 1). Attention has been focused on two sites of action in the insulin signaling cascade as the potential sites of Cr action, insulin receptor (IR) and Akt.

The most thorough studies observing increased IR signaling from Cr(III) treatment were reported by Brautigan and coworkers [100]. Preincubation of Chinese hamster ovary (CHO) cells overexpressing IR with $[Cr(pic)_3]$, Cr histidine (actually a complex mixture of numerous Cr-histidine complexes), or $[Cr_3O(propionate)_6(H_2O)_3]^+$ (Cr3) activated IR tyrosine kinase activity in the cells at low doses of insulin. While the concentration dependence was only examined for Cr histidine, the effect was concentration-dependent. Neither insulin binding to the cells nor IR number was affected. Additionally, the addition of Cr did not inhibit dephosphorylation of the IR by endogenous phosphatases or added PTP1B (phosphotyrosine phosphatase 1B). Also, Cr apparently did not alter redox regulation of PTP1B (i.e., by trapping

Table 1 Selected studies of effects of chromium administration on insulin signaling pathway.[a]

Cell or organism	Chromium compound	Effect	Refs.
Skeletal muscle	$CrCl_3$, $[Cr(pic)_3]$, Cr peptide complexes	Up-regulation of insulin receptor mRNA levels	[152]
Insulin-resistant 3T3-L1 adipocytes	$[Cr(pic)_3]$	No effect on insulin receptor and Akt mRNA levels	[120]
Chinese hamster ovary cells	$[Cr(pic)_3]$, Cr3, Cr histidine	Activated IR kinase activity	[100]
JCR:LA rat	$[Cr(pic)_3]$	Increased insulin receptor, IRS-1, and Akt phosphorylation and increased PI3K activity	[63]
3T3-L1 adipocytes	$Cr(D\text{-}phe)_3$	Increased phosphorylation of Akt but not insulin receptor	[153]
3T3-L1 adipocytes	$[Cr(pic)_3]$	No effect on phosphorylation of insulin receptor, IRS-1, or Akt	[113,115]
KK/HIJ mice skeletal muscle	Milk powder enriched with trivalent Cr	Increased IRS-1 tyrosine phosphorylation, increased Akt activity, and decreased IRS-1 serine-307 phosphorylation	[154]
3T3-L1 adipocytes	Cr histidine	Increased insulin-stimulated glucose uptake and insulin-stimulated tyrosine phosphorylation of IR	[101]
C2C12 skeletal muscle cells	Cr oligo-mannuronate	Enhanced phosphorylation of IR, PI3K, and Akt and AMPK	[155]

[a] Table adapted from [5].

the oxidized inactive form or by preventing its reduction and reactivation). $CrCl_3$ and Cr histidine were found not to activate the kinase activity of a recombinant fragment of IR. The authors concluded that Cr inside the cell modified the receptor in some manner, activating its kinase activity [100]. Subsequently, Brautigan, et al. [101] demonstrated that Cr histidine stimulated tyrosine phosphorylation of IR in 3T3-L1 adipocytes in the presence of insulin but not of MAPK (mitogen-activated protein kinase) or 4E-BP1, markers for activation of transcription and translation, respectively, in the presence of insulin; glucose uptake in the presence of insulin was also stimulated by Cr histidine. The effects of Cr histidine were also examined in competition with those of vanadate [101]; the results were interpreted in terms of Cr having an action involving IR activation and potentially in another action beyond IR activation that increases GLUT4 transport.

Sreejayan and coworkers [81] using $Cr(D\text{-phenylalaninate})_3$ ($Cr(D\text{-}phe)_3$) have generated evidence for an association between Cr and Akt. $Cr(D\text{-}phe)_3$ (5 or 25 μM for 10 days) was found to increase insulin-stimulated glucose uptake by cultured mouse 3T3-adipocytes. Treatment of the cells with 5 μM Cr for 0.5 to 4 hours or 0.1 to 100 μM Cr for 2 hours did not increase insulin-stimulated phosphorylation of IR (Tyr1146) significantly, while under similar conditions insulin-stimulated Akt phosphorylation (Thr308) was increased significantly.

To reconcile the heterogeneous results in the studies with cultured cells (Table 1), the complexes need to be studied under uniform conditions – the same cells treated in the same manner for the same period of time with the same Cr complex at the same concentrations. Additionally the Cr compounds need to be examined over a range of concentrations over varying periods of time with each of the cell types. The stability of the Cr complexes in the culture media needs to be established. Only in this manner will the actual Cr species in contact with the cells be established. Similarly, the distribution, concentration, and form of the Cr in the cells needs to be determined. Control experiments using just the ligands need to be performed to determine if any effects arise from just the ligands. Without this type of comprehensive treatment, progress in interpreting the body of cell culture experiments is going to be difficult if not impossible as has already been found in toxicology studies (see Section 4.2). Studies would probably be best performed if Cr-transferrin, the form of Cr by which the metal is delivered to cells, were utilized.

One specific biomolecule has been proposed as the biologically active chromium-binding molecule. This is the only biomolecule other than transferrin known to bind Cr *in vivo*, low-molecular-weight Cr-binding substance (LMWCr or chromodulin). This molecule occurs in the tissue, the bloodstream, and the urine and appears to bind Cr in the tissues for its elimination from the body via the urine. The history of studies of this molecule has been exhaustively reviewed [5,102] and is beyond the coverage of this review. The inability of the organic portion of this Cr-peptide complex to be characterized generated significant controversy, as the situation bore similarity to the previous inability to characterize the organic component of GTF [103]. Another important concern is that a Cr-loading procedure is necessary in the purification of LMWCr, so that the peptide could be followed (by its Cr content) through the isolation procedure; thus, the animal providing the tissue or body fluid is usually administered a Cr(III) or Cr(VI) source or such a source is added to the tissue homogenate or fluid [5,102]. Rupture of CrO_4^{2-}-treated mammalian cultured cells resulted in Cr being bound to a low-molecular-weight species with spectroscopic properties similar to LMWCr [104]. This was interpreted in terms of LMWCr being an artifact generated during isolation; however, the unnatural method of presenting CrO_4^{2-} in high concentration to cultured cells also suffers from the types of problems discussed above when using cultured cells. Thus, this study only shows that apoLMWCr can potentially bind Cr in a cell extract and potentially bind Cr tight enough to remove it from other biomolecules, consistent with the results of the isolation procedures of LMWCr described above. The Cr environment of LMWCr has been characterized by a variety of techniques including paramagnetic NMR, EPR, X-ray absorbance, and variable temperature magnetic susceptibility [105,106]. The peptide component has recently been sequenced by mass spectrometry [107]; the sequence begins with four glutamate residues whose cyclizing blocked attempts at Edman degradation sequencing. The peptide binds four chromic ions with identical binding constants and cooperativity as apoLMWCr (within experimental error) [107]. LMWCr has been found to stimulate insulin-dependent glucose incorporation and metabolism in isolated rat adipocytes [99,104] and *in vitro* to stimulate

(or perhaps retard the deactivation of) the kinase activity of the insulin-activated insulin receptor [108,109].

A mechanism for LMWCr in amplifying insulin signaling has been proposed [110,111]. This proposal was put forward when Cr was thought to be essential; the mechanism needs to be altered, so that it would be in vogue under conditions of Cr supplementation, so that abnormally high concentrations of holoLMWCr are generated. In this mechanism, apoLMWCr is stored in insulin-sensitive cells. Responses to increases in blood insulin concentrations result in activation of the insulin-signaling cascade: insulin binds to its receptor bringing about a conformational change that results in the autophosphorylation of tyrosine residues on the internal side of the receptor, transforming the receptor into an active tyrosine kinase and transmitting the signal from insulin into the cell. In response to this signaling, transferrin moves from the bloodstream into cells, carrying in part Cr3þ into the cells. The Cr flux results in loading of LMWCr with Cr. The holoLMWCr then binds to the insulin receptor, presumably assisting to maintain the receptor in its active conformation and amplifying insulin signaling. This mechanism requires demonstration that it can (or cannot be) active *in vivo* to verify (or refute); clear demonstration that the IR is directly involved in increasing insulin sensitivity by Cr would support this mechanism. As Cr is probably not an essential element, LMWCr could be part of a Cr detoxification system as suggested by Yamamoto, Wada, and Ono [112]; Cr supplementation, which leads to increased Cr concentrations in the body, could lead to increased concentrations of holoLMWCr, capable in turn of affecting insulin signaling. Studies need to determine the origin of LMWCr, i.e., what protein is it made from and what enzymes are involved? Is the holoLMWCr biologically active at physiological levels (suggesting a potential biological role for Cr) or is it significantly active only when Cr concentrations are high? Does LMWCr interact with the IR *in vivo*, or does it manifest its effects elsewhere?

3.3.2 Cholesterol and Fatty Acid Metabolism

Elmendorf and coworkers have examined the effects of $CrCl_3$ and $[Cr(pic)_3]$ on 3T3-L1 adipocytes [113–117] (however see [118,119]). In their first report [113], $CrCl_3$ and $[Cr(pic)_3]$ were shown to increase GLUT4 transport to the plasma membrane in the presence of insulin. Cr treatment did not affect IR, insulin receptor substrate-1 (IRS-1), PI3K, or Akt regulation but decreased plasma membrane cholesterol. Subsequently, the effects of $[Cr(pic)_3]$ were shown to be dependent on the glucose concentration of the media with the effects being observed at 25 mM, but not 5.5 mM [114]. $[Cr(pic)_3]$ activated AMPK (AMP-activated protein kinase) and improved defects in cholesterol transporter ABCA1 trafficking and cholesterol accrual in the high glucose treated cells [117].These researchers have postulated that Cr manifested its effects via affecting the cholesterol homeostasis and the membrane fluidity [113–117]. Yao and coworkers [120,121] determined that $[Cr(pic)_3]$ increased glucose uptake and metabolism and GLUT4 transport in 3T3-L1 adipocytes; the effects

were independent of insulin. Cr (60 nM) had no effect on IR or Akt phosphorylation but was found to activate MAPK independent of its effect on GLUT4 translocation. They also looked at the effects of Cr at both 25 and 5.5 mM glucose in their studies described above; similar results were observed at both glucose concentrations in contrast to Elmendorf and coworkers.

The use of exclusively [$Cr(pic)_3$] in some of the studies examining membrane properties generates some questions that may be related to differing results between cell studies. While not particularly lipophilic, despite being neutral in charge [122], the compound still appears to be able to partition to a significant degree to cell membranes. This membrane incorporation of [$Cr(pic)_3$] results, for example, in increased membrane permeability [123]. Thus, some of the observations related to cholesterol homeostasis may be specifically related to the use of [$Cr(pic)_3$], its lipophilicity, and its stability in cell culture media. Notable in this regard is a recent report showing that [$Cr(pic)_3$] associates with the lipid interface in reverse micelle model membranes and that a similar association could explain the increased association of the insulin receptor, phosphorylated IRS-1, and phosphorylated Akt in detergent-resistant membrane microdomains [124].

3.3.3 Inflammation and Oxidative Stress

Jain and Kannan have shown that monocytes exposed to high glucose concentrations have lower levels of the cytokine TNF-α (tumor necrosis factor-α) in the presence of 100 μM $CrCl_3$ for 24 hours at 37°C [125]. Treatment with $CrCl_3$ also inhibited stimulation of TNF-α secretion in these cells by 50 μM H_2O_2. Lipid peroxidation and protein oxidation in the presence of H_2O_2 was also inhibited by $CrCl_3$. As increased TNF-α secretion may be associated with insulin resistance, Jain has proposed in an interview that increased insulin sensitivity arising from Cr administration may be mediated by lowering of TNF-α levels [126]. In a follow-up study, $CrCl_3$ in combination with estrogen lowered lipid peroxidation in high glucose-treated monocytes [127]. The combination was also found to decrease interleukin-6 (IL-6) secretion. Cr was proposed to potentiate the effects of estrogen [127]. Curiously, another group has shown that Cr(III) treatment (350–500 ppm) results in increased TNF-α production by macrophages (in the absence of high glucose concentrations) [128]. This activation by chromium ($CrCl_3$) may be regulated by tyrosine kinases [129]. The results in the presence of high glucose could also point to an association between reactive oxygen species and chromium, but these studies must be considered extremely preliminary. Additionally, the fate of Cr in these cell culture studies needs to be examined. Subsequent studies in Zucker diabetic fatty [65] and streptozotocin-induced diabetic rats [130] have found that Cr(III) administration can lower blood levels of TNF-α, IL-6, and C-reactive protein, although differences appeared to be observed depending on choice of Cr(III) complex administered. Cr(III) administration has also been reported to lower blood levels of TNF-α in a clinical trial of type 2 diabetic subjects, although again differences appeared to be observed depending on choice of Cr(III) complex administered [131].

4 Is Chromium Toxic?

4.1 Chromate

Lay and coworkers [132,133] have proposed that chromate generated enzymatically (i.e., from hydrogen peroxide or other species generated by enzymes) from Cr(III) in the body could act as a phosphotyrosine phosphatase (PTP) inhibitor, in a similar manner to vanadate, and that the site of action of Cr is at the PTPs. The proposal that chromate could be involved in chromium action *in vivo* is based on the ability of hydrogen peroxide to oxidize Cr(III) compounds to chromate, suggesting the apparent beneficial effects of Cr actually stem from side effects of its toxicity [133]. To demonstrate this, Lay and coworkers exposed chromium picolinate, $CrCl_3$ and the basic chromium carboxylate cation Cr3 to 0.10–1 mM hydrogen peroxide for 1–6 h in 0.10 M HEPES buffer at pH 7.4. This resulted in the formation of chromate in efficiencies of from 1% ($[Cr(pic)_3]$ for 6 h with 1 mM H_2O_2) to 33% (the cation for 6 h with 1 mM H_2O_2). The cation could also be oxidized with hypochloride or glucose oxidase or xanthine oxidase (enzymes that produce H_2O_2). However, when one considers the amount of Cr humans consume from their diet and from nutritional supplements and the low % absorption and that cell concentrations of peroxide are 10^{-7} to 10^{-8} M while numerous reductants (such as ~5 mM ascorbate) are present, the probability that cell concentrations of chromate could even approach the K_i of chromate for phosphatases is negligibly small [5]. Similarly, toxicity from chromate at these concentrations is unlikely. Given the enormous doses of Cr(III) complexes shown to have no detrimental effects (see Section 4.2), this proposed mechanism of toxicity from chromate generated from Cr(III) sources can be ignored.

4.2 Chromium Picolinate and Other Cr(III) Complexes

The potential toxicity of Cr picolinate, $[Cr(pic)_3]$, the most popular form of Cr supplement over the last two decades, has been an area of intense debate, but consensus has probably recently been reached (for recent reviews see [5,134,135]). In mammalian cell culture studies and mammalian studies in which the complex is given intravenously [5,134], $[Cr(pic)_3]$ is clearly toxic and mutagenic, unlike other commercial forms of Cr(III) supplements. The first study to raise concerns about potential toxic effects, by Stearns and coworkers [136], demonstrated, using CHO cells, that $[Cr(pic)_3]$ as a solid suspension in acetone or the mother liquor from the synthesis of $[Cr(pic)_3]$ (before the compound precipitates from solution) caused chromosomal aberrations. Subsequent studies have shown that the complex gives rise to a variety of types of oxidative damage and is clastogenic [137–143]. This led, for example, in fruit flies (*Drosophila*) to dominant female sterility, appreciable delays in development of larvae and adults, and lower success rates in pupation and eclosion; the Cr dosage in these studies was approximately equivalent to a

human consuming one 200 μg Cr-containing supplement a day [144]. The ability of $[Cr(pic)_3]$ to generate chromosomal aberrations in polytene chromosomes of the salivary glands of *Drosophila* larvae was also examined; in the $[Cr(pic)_3]$-treated group, 53% of the identified chromosomal arms were positively identified as containing one or more aberrations, while no aberrations were observed for the identified chromosomal arms of the control group [145]. No effects on *Drosophila* were observed for other Cr(III) compounds examined [144,145]. However, when given orally to mammals, $[Cr(pic)_3]$ does not appear to be toxic nor appear to be a mutagen or carcinogen.

An NIH-commissioned study of the effects of up to 5% of the diet (by mass) of male and female rats and mice for up to 2 years found no harmful effects on female rats or mice or male mice and at most ambiguous data for one type of carcinogenicity in male rats (along with no changes in body mass in either sex of rats or mice) [146]. Despite numerous claims that $[Cr(pic)_3]$ is absorbed better than inorganic forms of Cr used to model dietary Cr, $CrCl_3$, Cr nicotinate (the second most popular form of Cr sold as a nutritional supplement), and $[Cr(pic)_3]$ are absorbed to a similar degree in rats [24,147,148]. Only 1% of absorbed Cr from the supplement is found in the bloodstream as $[Cr(pic)_3]$, suggesting that little of the intact molecule is absorbed [149]. When ingested, the complex probably hydrolyzes near the stomach lining, releasing the Cr, which is subsequently absorbed. The picolinate ligands also alter the redox properties of the Cr center such that it is more susceptible to undergoing redox chemistry in the body than hexaaqua Cr(III) [150,151]. The hydrolysis of the complex is probably fortuitous, releasing the Cr before the intact $[Cr(pic)_3]$ complex can be absorbed to an appreciable level and potentially enter into redox chemistry, in contrast to the cell studies where the very stable, neutral complex could be absorbed intact. The message of these conflicting results is that applying solutions of Cr(III) compounds to cultured cells in general does not present Cr(III) to the cells in a comparable fashion to that in which Cr(III) is presented to cells in the body; the difference may be crucial to the results and interpretation of the study.

In summary, Cr(III) supplementation appears to be safe at levels currently used in nutritional supplements and in pharmacology studies, in line with assessments by the Food and Drug Administration (USA) and European Food Safety Authority. However, as no benefit has been demonstrated for Cr supplementation of healthy individuals, any potential risk from supplementation would appear to outweigh potential benefits at the current time.

5 Concluding Remarks and Future Direction

At present Cr cannot be considered as an essential element as (i) nutritional data demonstrating Cr deficiency and improvement in symptoms from Cr supplementation are lacking and (ii) no biomolecules have convincingly been demonstrated to bind Cr and have an essential function in the body. No beneficial effects have

convincingly been demonstrated from Cr supplementation by healthy humans. Cr(III) supplementation appears to be safe at levels currently used in nutritional supplements and in pharmacology studies. While studies with rodent models reproducibly demonstrate beneficial effects from Cr supplementation at pharmacological doses, the scientific literature for clinical trials in diabetic humans lacks consistent and reproducible outcomes.

Future clinical studies need to be more carefully designed including the utilization of an appropriate number of subjects and appropriate amount of administered Cr, the use of well characterized Cr(III) compounds, and the examination of whether particular subgroups of type 2 diabetic subjects are likely to benefit from chromium supplementation. Further studies are required to investigate the mechanism and mode of action of Cr(III) at the molecular level in enhancing insulin sensitivity and potentially improving cholesterol metabolism.

Abbreviations and Definitions

AI	adequate intake
AMPK	AMP-activated protein kinase
CHO	Chinese hamster ovary
Cr3	$[Cr_3O(propionate)_6(H_2O)_3]^+$
$Cr(D\text{-}phe)_3$	$Cr(D\text{-}phenylalaninate)_3$
$[Cr(pic)_3]$	chromium picolinate
4E-BP1	4E-binding protein-1
ESADDI	estimated safe and adequate daily dietary intake
FDA	Food and Drug Administration
FEEDAP	Panel on Additives and Products or Substances Used in Animal Feed
FTC	Federal Trade Commission
GLUT4	glucose transporter type 4
GTF	glucose tolerance factor
HDL	high density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IL-6	interleukin-6
IR	insulin receptor
IRS-1	insulin receptor substrate-1
LDL	low density lipoprotein
LMWCr	low-molecular-weight chromium-binding substance
MAPK	mitogen-activated protein kinase
PI3K	phosphatidylinositol 3-kinase
PTP	phosphotyrosine phosphatase
PTP1B	phosphotyrosine phosphatase 1B
TNF-α	tumor necrosis factor-α
TPN	total parenteral nutrition
ZDF	Zucker diabetic fatty (rats)

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