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ORIGINAL PAPER

Chromium is not an essential trace element for mammals: effects of a "low-chromium" diet

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Abstract Chromium was proposed to be an essential trace element over 50 years ago and has been accepted as an essential element for over 30 years. However, the studies on which chromium's status are based are methodologically flawed. Whether chromium is an essential element has been examined for the first time in carefully controlled metal-free conditions using a series of purified diets containing various chromium contents. Male Zucker lean rats were housed in specially designed metal-free cages for 6 months and fed the AIN-93G diet with no added chromium in the mineral mix component of the diet, the standard AIN-93G diet, 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 chromium content of the diet had no effect on body mass or food intake. Similarly, the chromium content of the diet had no effect on glucose levels in glucose tolerance or insulin tolerance tests. However, a distinct trend toward lower insulin levels under the curve after a glucose challenge was observed with increasing chromium content in the diet; rats on the supplemented AIN-93G diets had significantly lower areas ($P < 0.05$) than rats on the low-chromium diet. The studies reveal that a diet with as little chromium as reasonably possible had no effect on body composition, glucose metabolism, or insulin sensitivity compared with a chromium-"sufficient" diet. Together with the results of other recent studies, these results clearly indicate that chromium can no longer be considered an essential element.

Keywords Chromium · Rats · Glucose tolerance tests · Insulin sensitivity · Essential trace element

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Introduction

Chromium was first proposed to be an essential trace element in 1959 by Schwarz and Mertz [1]. In 1955 Mertz and Schwarz [2] reported feeding rats a *Torula* yeast-based diet that resulted in the rats apparently developing impaired glucose tolerance in response to an intravenous glucose load (in addition to previously identified necrotic liver degeneration). Rats on the diet with *Torula* yeast as the sole protein source had a clearance rate of excess glucose of 2.8% per minute, in contrast to rats on a basal diet, which had a rate of 4.1% (excess glucose was defined as glucose above background concentrations before the glucose challenge was given). The challenge consisted of administering an intravenous injection of 1.25 mg glucose

per kilogram body mass as a 50% aqueous solution after an 18-h fast. The glucose intolerance was at first assumed to be a symptom of the liver disease. Shortly thereafter, a dietary factor (selenium) was discovered which could reverse the liver disorder but not the glucose intolerance; thus, the authors believed they had identified a new dietary requirement absent from the *Torula* yeast-based diet and responsible for the glucose intolerance, which they coined "glucose tolerance factor," or "GTF" [3].

In their 1959 report [1], these researchers identified the active ingredient of GTF as Cr^{3+}. Inorganic compounds containing Li, Be, B, F, Ti, V, Mn, Co, Ni, Cu, Zn, Ge, As, Se, Br, Rb, Sr, Y, Zr, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, I, Cs, Ba, La, Ce, Ta, W, Os, Ir, Au, Hg, Tl, Bi, Th, and U (200–500 μg/kg body mass) could not restore glucose tolerance, whereas several inorganic chromium(III) complexes (200 μg Cr/kg body mass) restored glucose tolerance from a 2.8% per minute or less rate of removal of intravenously injected glucose to the approximately 4% rate of control rats. Brewer's yeast and acid-hydrolyzed porcine kidney powder were identified as natural sources of GTF, and the active (i.e., effective in reversing the inability to handle the glucose load) ingredient could be concentrated from these materials by physical and chemical means [1]. When given by stomach tube (500–1,000 μg/kg body mass), the intact materials and the concentrates could restore proper glucose metabolism in rats on the *Torula* yeast-based diet. From the benefit of 50 years of hindsight, these studies are deeply flawed despite the success of similar studies in identifying other dietary requirements [4, 5]. Based in part on these rat studies, the National Academy of Sciences (USA) established an estimated safe and adequate daily dietary intake (ESADDI) of chromium of 50–200 μg in 1980 [6]. The ESADDI was retained in 1989 [7]. In 2001, the National Academy of Sciences established an adequate intake (AI) of chromium of 35 μg/day for men and 25 μg/day for women [8]. AI is defined as "the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate" and replaces ESADDI. The AI "is expected to cover the needs of more than 97–98% of individuals" [8]. Thus, almost all Americans are believed to be chromium-sufficient, and little if any need exists for chromium supplementation.

The most notable recent efforts with rats to generate a chromium-deficient diet and confirm the status of chromium as an essential element have been reported by Anderson and coworkers. Rats in plastic cages (with no access to metal components) were given a diet consisting of 55% sucrose, 15% lard, 25% casein vitamins, and minerals and providing 33 ± 14 μg Cr/kg diet [9]. The high-sucrose diet was utilized in theory to attempt to induce chromium deficiency; dietary carbohydrate stress leads to increased urinary chromium loss [10]. To compromise pancreas function, low copper concentrations (1 mg/kg) were employed for the first 6 weeks; high dietary iron concentrations were used throughout to potentially aid in obtaining chromium deficiency. A supplemented pool of rats was given water containing 5 ppm $CrCl_3$; unfortunately, the volume of water consumed was not reported, so the chromium intake of the rats cannot be determined. Over 24 weeks, body masses were similar for both groups. At 12 weeks, rats on the diet without supplemental chromium had lower fasting plasma insulin concentrations and similar fasting plasma glucose levels compared with supplemented rats; yet, both concentrations were similar after 24 weeks. In intravenous glucose tolerance tests after the rats had been on the diet for 24 weeks, plasma insulin levels tended to be higher in chromium-deficient rats; rates of excess glucose clearance were statistically equivalent. Glucose area above basal was reported to be higher in chromium-deficient rats; however, at every time point in the glucose tolerance test, the plasma glucose concentrations of each pool of rats were statistically equivalent, suggesting that the difference in area arose from a mathematical error. (The workers reported in a subsequent study utilizing a high-sucrose diet in which the plasma insulin levels were again observed to be elevated; however, the plasma glucose area was not [11].) Thus, a high-sucrose diet combined with other stresses (low copper and high iron) can potentially lead to hyperinsulinemia, possibly reflecting defects in peripheral tissue sensitivity to glucose. This research group also obtained similar results using a high-fat diet that contained 33 μg Cr/kg diet [12]. This diet also contained altered copper content for the first 6 weeks. After 16 weeks on the diet alone, rats had higher fasting plasma insulin levels, but not higher fasting glucose levels, compared with rats also receiving drinking water containing 5 ppm Cr [12]. Similar results were obtained when the fasting insulin and glucose levels of the rats on the diet alone were compared with those of rats on a normal chow diet. Insulin and glucose areas after a glucose challenge were equivalent. Thus, the high-fat diet with the additional stresses appears to induce increased fasting insulin levels, which can be corrected with chromium administration.

Some calculations are needed to put this work into perspective. As noted above, humans lack signs of chromium deficiency with a daily intake of 30 μg Cr; assuming an average body mass of 65 kg, 30 μg/day corresponds to 0.46 μg Cr/kg body mass per day. A 100-g rat eats about 15 g of food a day [13]. Fifteen grams (0.015 kg) of food containing 33 μg Cr/kg food provides approximately 0.50 μg Cr. Thus, 0.50 μg Cr per day for a 0.100-kg rat is 5.0 μg Cr/kg body mass per day, *10 times what humans*

take in per kilogram body mass. Thus, the "low-chromium" diets provided by Anderson and coworkers [9, 11, 12] cannot be said to be deficient or even low in chromium unless rats require more than 10 times the chromium that humans do on a per kilogram body mass basis. Even if the chromium intake for a rat compared with a human is adjusted for metabolic rate (multiplying by approximately 5), the rats were taking in a sufficient diet. Consequently, the effects of the diet cannot be attributed to chromium deficiency; the supranutritional doses of chromium in the chromium-supplemented rats can only be considered as having a pharmacological effect on the rats whose physical condition was impaired by the high-sucrose or high-fat diets and/or the other mineral stresses.

Consequently, to establish whether a diet low in chromium can have deleterious effects that can be prevented by chromium supplementation and provide evidence that chromium is essential for health, rats were maintained in metal-free cages and provided with a purified diet with as low a chromium content as reasonably possible or diets supplemented with a variety of chromium concentrations, and effects of the diet on food intake, body mass, and parameters associated with glucose metabolism and insulin sensitivity were determined.

Materials and methods

Chemicals, assays, and instrumentation

Glucose and insulin (bovine, zinc) were obtained from Sigma–Aldrich. The final concentrations of glucose and insulin were prepared using doubly deionized water. Plasma insulin was measured using an ^{125}I RIA kit from MP Biomedicals. Gamma counting was performed using a Packard Cobra II auto-gamma counter. Blood glucose levels were measured using a One-Touch glucose meter.

Animals

Thirty-two male Zucker lean rats were obtained from Charles River Breeding Laboratories International at 6 weeks of age. Rats were maintained at 22 ± 2 °C and 40–60% humidity with a 12-h photoperiod and were acclimated for 2 weeks prior to treatment. They were housed individually in specially constructed metal-free housing (vide infra) to prevent the introduction of additional chromium into their diets. Rats were fed specific diets and distilled water ad libitum for a 23-week period prior to glucose and insulin challenges. All procedures involving these animals were reviewed and approved by The University of Alabama's Institutional Animal Care and Use Committee.

Treatment

Male Zucker lean rats were separated into four treatment groups, each containing eight rats as follows: (1) rats on a purified AIN-93G chromium-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$, the Cr source designated for the AIN-93G diet], (2) rats on the AIN-93G diet, with chromium not included in the mineral mix, (3) rats on the AIN-93G chromium-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$], and (4) rats on the AIN-93G chromium-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]. Purified AIN-93G rodent diets and modified AIN-93G diets were obtained from Dyets (Bethlehem, PA, USA). Diets were received in powder form.

Housing

Iris Buckle Up boxes were obtained from Target; the boxes were approximately 18 cm high, 45 cm wide, and 28 cm deep. These boxes are made of clear plastic with a removable lid that attaches with latches on both 28-cm sides of the boxes. Holes (4 mm in diameter) were drilled with an electric hand drill in all five sides of the box and in the lid using a square grid pattern with approximately 5 cm between holes. Holes (4 mm in diameter) were also drilled in the corners of the bottom of each box to facilitate urine drainage. Shavings of plastic were removed from the holes, and any rough spots were smoothed using fine sandpaper. An additional hole was drilled in the lid with an appropriate diameter to accommodate the tube of the water bottles, and another hole was drilled in the lip of the box to accommodate a hanging cage card holder. Tube tread no. 116 wet area antifatigue mats were purchased from General Mat Company. The matting is made of vinyl with a tensile strength of 139 kg/cm and is flexible from −10 to 100 °C. The matting was cut with a knife to fit inside the base of the boxes. Both the boxes and the matting could pass through multiple cycles of a cage washing machine without noticeable damage. As the boxes are similar in size to shoebox-type housing, they were kept on a standard rack for animal cages. The cages were placed on absorbent bench paper or newspaper. The rear of the cage was elevated approximately 1 cm using scrap pieces of the matting material placed under the rear of the cage to ensure drainage of urine.

Food and water

Wheaton clear straight-sided, wide-mouth glass jars (about 9 cm in diameter, 9.5 cm in height, 473 mL) and plastic lids (89–400-mm screw cap size) were obtained from Fisher Scientific and were used to hold food. A 5-cm-diameter

circular opening was cut in the polyvinyl-lined plastic lids to allow the animals access to food. To prevent the rats from dumping the powdered food from the jars, a 2-cm-thick Plexiglas disk (about 7 cm in diameter) was placed on the food. The disk had a 14-mm-diameter circle cut out in the center, with six other 14-mm-diameter circles cut in a hexagonal pattern around the center circle; the disks were prepared by The University of Alabama College of Arts and Sciences machine shop.

To provide water, the stainless steel tubes were removed from the water bottles and replaced with glass tubes. The University of Alabama glass shop cut and bent glass tubing of the appropriate diameter to match the length and shape of the stainless steel tubes. To prevent potential injury, the end of the tubing exposed to the rats was fire-polished.

Data collection

Rats were weighed, and food consumption was measured twice weekly. At 23 and 25 weeks, respectively, rats were fasted for between 10 and 12 h then given an intravenous glucose challenge (1.25 mg glucose/kg body mass) or an intravenous insulin challenge (5 insulin units/kg body mass). Blood was collected in EDTA-lined capillary tubes by a tail vein prick. Blood was collected before intravenous challenges and 30, 60, 90, and 120 min after the challenge injections. Area under the curve was calculated using the trapezoid rule.

Chromium concentration determinations

Samples of each powdered diet (200 mg) were digested with a 30:1 mixture of ultra-high-purity concentrated HNO_3 (99.99% trace-element free) and ultra-high-purity concentrated H_2SO_4 (99.99% trace-element free). The digestion was continued with controlled heating (subboiling) until the samples had been heated to dryness. Then, the residue was diluted to 10 mL with doubly deionized water (Milli-Q, Millipore). All glassware was acid-washed. Blank digestions were carried out in the same fashion. Chromium concentrations were determined utilizing a PerkinElmer Analyst 400 atomic absorption spectrometer equipped with an HGA-900 graphite furnace and an AS-800 autosampler using a chromium hollow cathode lamp operating at 10 mA; a spectral bandwidth of 0.8 nm was selected to isolate the light at 353.7 nm. The operating conditions were as follows (temperature, ramp time, hold time): drying 1 (100 °C, 5 s, 20 s), drying 2 (140 °C, 15 s, 15 s), ashing (1,600 °C, 10 s, 20 s), atomization (2,500 °C, 0 s, 5 s), and cleaning (2,600 °C, 1 s, 3 s). Other instrumental parameters included the following: pyrolytic cuvette, argon carrier gas (flow rate 250 mL/min), 20-μL sample volume, and peak area measurement mode. The digestion and atomic absorption methods were verified by analysis of a certified reference material, 1573a Tomato Leaves (NIST).

Statistical analysis

Statistical analyses were performed using SPSS (SPSS, Chicago, IL, USA). Data are represented graphically as average values with standard error bars. Data were calculated independently, tested for homogeneity of variance with Levene's test, and analyzed using univariate analysis of variance and descriptive statistics. Blood insulin and blood glucose tolerance tests were further analyzed for the area under each curve. Post hoc least significant difference analyses were used to indicate significant differences at a 95% confidence level ($P \leq 0.05$).

Results and discussion

Metal-free caging

Dietary studies examining the roles of trace metals in the diet require animals be in an environment free from access to extraneous metal, including all cage components and bedding. Stainless steel, used in almost all small animal housing, in addition to being composed of iron has significant quantities of other metals, most notably chromium. Bedding, made of natural materials, contains a variety of metal ions and is unsuitable for use in many such studies. The removal of metal housing components and bedding can be challenging as replacement components must be stable under the experimental conditions (including those used to wash the housing) and not be hazardous to the animals or susceptible to damage by the animals (e.g., by gnawing). Metal-free caging has been described previously [14, 15]; however, at the times such caging was described, the assortment of commercial plastic products was limited in comparison with the variety of products currently available. Consequently, developing suitable, metal-free housing that should require less labor and construction should be possible.

Conventional shoe-box-type caging utilizes a plastic container for the base and walls of the housing and a stainless steel metal mesh lid. The lid allows for plenty of circulation of air in the cage; the metal mesh is the only surface the rats can chew. Replacement of the metal housing component must be accomplished so that adequate ventilation is maintained and no plastic material is available for the rats to chew. Thus, for example, a simple plastic mesh lid would not suffice. As an alternative, the use of plastic storage boxes was examined. The boxes utilized were Iris Buckle Up boxes with a volume of

Fig. 1 The metal-free housing unit with the antifatigue matting inside the cage

approximately 22 L (Fig. 1). The similar size to shoe-box-type housing allows these cages to be placed on regular racks for shoe-box-type housing. Latches on two sides of the lid allowed the box to be securely closed so that animals could not escape. The only modification of the boxes required was the drilling of numerous small holes, which could be performed with a standard electric hand drill; this greatly simplifies construction compared with previous reported metal-free housing. The pattern of 4-mm holes drilled on all sides of the box and the lid provided ventilation, and the holes on the floor allowed urine to flow out of the box onto absorbent paper. This pattern of holes with 5-cm spacing does not compromise the integrity of the sides of the boxes, so the boxes are sturdy and do not break easily. One additional hole was required to accommodate the tube from the water bottle. As The University of Alabama Animal Care Facility normally utilizes water bottles with stainless steel tubes for rodents, replacement of the tubes was readily accomplished by using glass tubing of the same diameter cut to the same length and bent to the same shape as the original steel tube. The end of the glass tubing utilized by the rats was fire-polished.

To prevent rats from walking in fluids draining from the bottom of the cage, a piece of vinyl antifatigue matting was placed in the bottom of each cage. The matting comes in rolls, from which pieces of the desired size can readily be cut. The rats had surprisingly little desire to chew on the matting. A rat placed in the box without the matting would rapidly climb on top of its food container to avoid the liquid on the bottom of the cage; the addition of the mat immediately eliminated this problem. The back of the cage bottom was elevated by approximately 1 cm to facilitate drainage of liquid from the box.

The metal-free housing required cleaning every 2 days as hair and dander accumulated in the absence of bedding. The cages could be readily cleaned using a standard mechanical cage washing system. The high-temperature and high-pressure water had no observable effects on the plastic boxes or mats, even after 6 months of use. In some areas of the animal care facility rooms where the metal-free cages were kept, moisture accumulated near the top of the housing initially, indicating that sufficient circulation of air was not present in all parts of the room to allow the metal-free cages to vent moisture from the rats. This was readily fixed by placing a household circulating fan in each room. The cages only have sufficient ventilation to house one rat per cage. Measurement of the temperature in the cages indicated that the temperature in a shoe-box-type cage with bedding stayed 1–2 °C higher than the temperature in the metal-free housing.

The metal-free housing, as an accidental consequence of its design, is readily capable of acting as a metabolic cage. If the housing is placed over another container, urine can readily be collected. Feces falls between the treads of the duckboard design of the antifatigue mat pieces. After the rat and mat have been removed, the feces can readily be collected from the storage box. The empty storage boxes and lids are designed to stack conveniently, so little space is required to store the boxes when they are not in use.

Zucker lean rats with the same birth date and shipping date were maintained in either regular shoe-box-type housing with conventional bedding or in metal-free housing. The rats kept in the shoe-box-type housing were obtained for a different study but provided data useful for this comparison. Over the course of 3 months, both groups had identical body masses (data not shown) and were identical in appearance. No differences were observed in the rate or type of health issues between rats in conventional shoe-box housing and rats in the metal-free housing; no behavioral differences were noted as well.

Diet results

The AIN-93 diet is a purified diet for experimental rodents reported by the American Institute of Nutrition [16]. It comes in two forms: the AIN-93G diet, designed for early phase growth and reproduction, and the AIN-93M diet, designed for animal maintenance [17]. Consequently, the AIN-93G standard purified diet was chosen for use with the young rats utilized in this study. When chromium is omitted from the mineral mix, the diet is low in chromium compared with standard chow diets. Analysis of the diet revealed only 16 μg Cr/kg diet; this is as low a chromium content as can reasonably be provided to rodents. Although this diet contains about half the chromium concentration of that in the purified diets used by Anderson and coworkers [9, 11, 12], this concentration is actually within error equivalent to that of the other purified diets. Note (as

described earlier) that despite the low concentration of chromium, this diet should be considered chromium-sufficient on the basis of comparisons with the human AI. Consequently, if no adverse effects from this diet are observed in the rats, then producing a diet that is "deficient" in chromium but sufficient in other dietary requirements is probably not possible, indicating that no nutritional methodology could possibly demonstrate that chromium is an essential trace element for mammals. The diets that were supplemented with chromium were also analyzed for their chromium content by graphite furnace atomic absorption spectrometry: AIN-93G, 1,135 μg Cr/kg; AIN-93G + 200 μg Cr/kg, 1,331 μg Cr/kg; AIN-93G + 1,000 μg Cr/kg, 2,080 μg Cr/kg. All values were close to anticipated values.

As shown in Fig. 2, the chromium content of the diets had no effect on the body mass of the rats throughout the course of the study. On only 3 days during the course of the study were the body masses of any of the groups of rats statistically different from those of the others. This is not unexpected as chromium supplementation has been shown numerous times to not influence body mass [18, 19]. The chromium content also had no effect on food intake (data not shown). Similarly, the rats receiving the different diets were identical in appearance. No differences were observed in the rate or type of health issues between rats on the various diets.

Chromium deficiency has been reported to lead to alterations in glucose metabolism and insulin insensitivity (for reviews, see [4, 20]). More specifically, mammals with chromium deficiency reportedly respond less efficiently to insulin and glucose challenges in terms of maintaining and restoring normal blood plasma glucose and insulin levels. Thus, after 23 and 25 weeks, respectively, the rats were fasted for 10–12 h and given an intravenous glucose (1.25 mg glucose/kg body mass) or insulin [5 units insulin (bovine, zinc) per kilogram body mass] challenge. Blood samples were collected immediately before the challenges and 30, 60, 90, and 120 min after the challenges. In the glucose challenge, as shown in Fig. 3, the plasma glucose levels of the rats on the various diets were equivalent at all time points except 60 min after the challenge; at this time, the plasma glucose levels of the rats on the AIN-93G diet without chromium and the AIN-93G diets supplemented with 200 or 1,000 μg Cr/kg were statistically equivalent. The glucose concentrations for the rats on the AIN-93G diet were statistically higher than those for the rats on the diet without added chromium and the AIN-93G diet supplemented with 1,000 μg Cr/kg. Thus, only the rats on the chromium-sufficient AIN-93G diet appeared to have elevated glucose levels. This is also reflected in the areas under curves (AUCs) for the glucose tolerance tests (Fig. 4). The AUC for the rats on the diet without added chromium is statistically equivalent to the AUCs for all the diets with added chromium. As the glucose concentrations are statistically equivalent for the rats on the diet without added chromium and the diets supplemented with 200 and 1,000 μg Cr/kg at all time points after the challenge, the rates of glucose clearance for these rats are by necessity

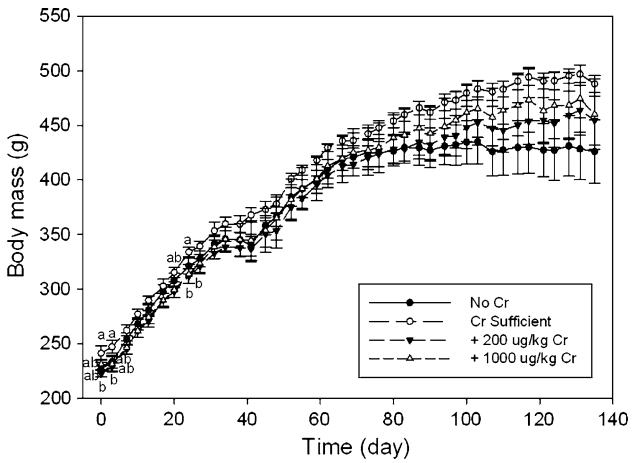

Fig. 2 Body mass of Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

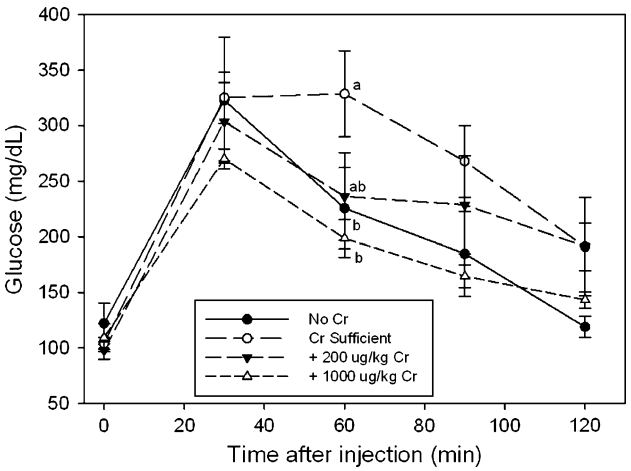

Fig. 3 Plasma glucose levels in glucose tolerance tests for Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

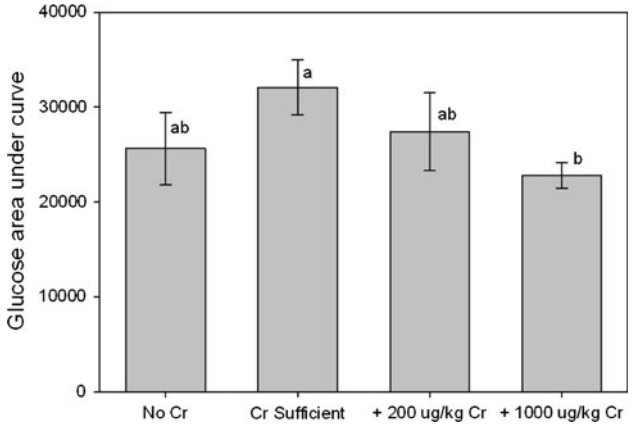

Fig. 4 Area under the curve for plasma glucose concentrations in glucose tolerance tests for Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

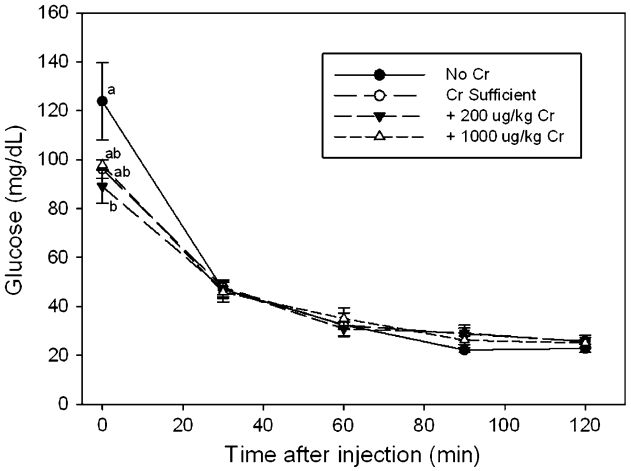

Fig. 5 Plasma glucose levels in insulin tolerance tests for Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

statistically equivalent. The glucose clearance (K_G) [1, 9] was calculated for rats on each diet; K_G is equal the slope of the best fit line of a plot of ln(percentage of baseline glucose) versus time × 100%, where percentage of baseline glucose = (glucose concentration at a given time/glucose concentration at time zero) × 100% (this procedure avoids the use of excess glucose following Woolliscroft and Barbosa [5]). K_G was 1.07% per minute for the rats without chromium in the mineral mix of their diet, 0.600% for the rats on the AIN-93G diet with chromium in the mineral mix, 0.473% for the rats on the AIN-93G diet with 200 μg Cr/kg added, and 0.696% for the rats on the AIN-93G diet with 1,000 μg Cr/kg added. As the K_G for the rats on the standard AIN-93G diet is within the range of the other three K_G values, all the K_G values are statistically equivalent. Thus, the glucose tolerance tests indicate that rats on the diet without added chromium could handle increases in blood glucose concentration equally efficiently as rats on chromium-sufficient or chromium-supplemented diets, providing no evidence that chromium is an essential dietary component.

In insulin tolerance tests, the plasma glucose concentrations of the rats on all the various diets were statistically equivalent; however, prior to the insulin challenge, the rats on the AIN-93G diet supplemented with 200 μg Cr/kg had statistically lower plasma glucose concentrations than the rats on the diet without added chromium (Fig. 5). All glucose levels of the rats on the other diets were statistically equivalent prior to the challenge. It should be noted that this difference was not observed prior to the glucose challenge. Not surprisingly, this results in the AUCs in the insulin tolerance tests for the rats on all the various diets being statistically equivalent (Fig. 6). Thus, the rats on the diet without added chromium could manage their blood glucose after an insulin challenge equally well as rats on chromium-sufficient or chromium-supplemented diets, providing no evidence that chromium is an essential dietary component.

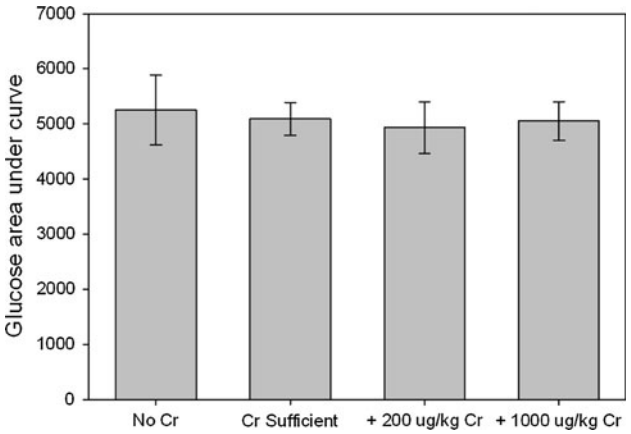

Fig. 6 Area under the curve for plasma glucose concentrations in insulin tolerance tests for Zucker lean rats on the AIN-93G diets. No significant differences between groups were observed. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

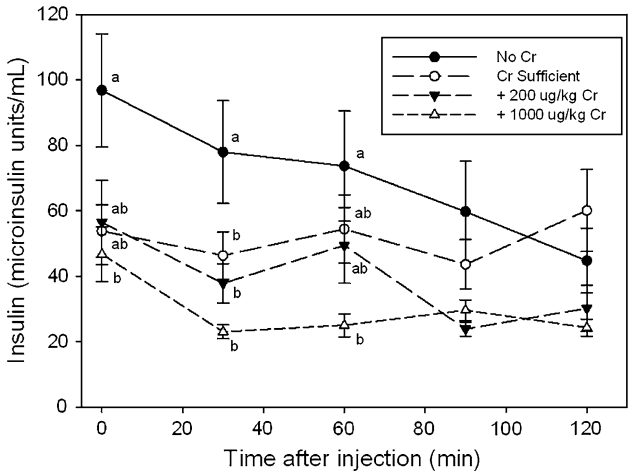

Fig. 7 Plasma insulin levels in glucose tolerance tests for Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

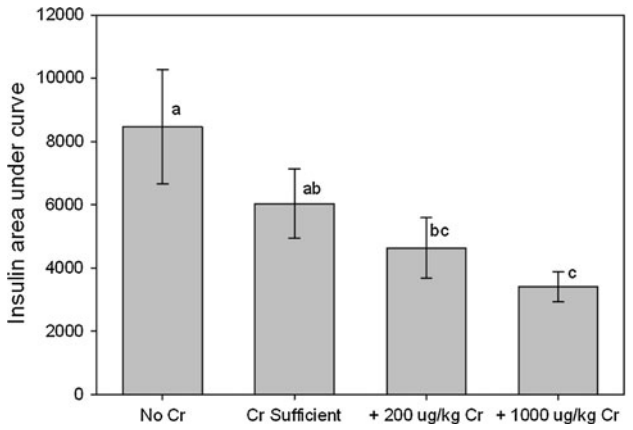

Fig. 8 Area under the curve for plasma insulin concentrations in glucose tolerance tests for Zucker lean rats on the AIN-93G diets. *Different letters* indicate significant differences between groups. No Cr, rats on the AIN-93G diet, with Cr not included in the mineral mix; Cr sufficient, rats on a purified AIN-93G Cr-sufficient diet [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +200 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]; +1,000 μg/kg Cr, rats on the AIN-93G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2{\cdot}12H_2O$]

The effects of the glucose challenge on plasma insulin levels were also examined (Fig. 7). Prior to the glucose challenge, a distinct trend was observed in the blood plasma insulin levels as the insulin concentrations dropped as the amount of chromium in the diet increased. The difference is only statistically significant between the rats on the diet without added chromium and the rats on the diet with the most added chromium (AIN-93G + 1,000 μg Cr/kg). As a consequence, 30 min after the challenge, the blood plasma insulin concentration of the rats on the diet without added chromium was statistically greater than that of the rats on all the other diets or on the diet with the most added chromium (no increase in plasma insulin concentration in response to the glucose challenge is observed in Fig. 7 as the levels of insulin return to the baseline in approximately 30 min, the time of the first data point after glucose administration). Sixty minutes after the challenge, the plasma insulin concentration of the rats on the diet without added chromium was greater than that of the rats on the diet with the greatest quantity of added chromium. In other words, increasing the dietary chromium intake of the rats by approximately 100-fold appears to result in a lowering of fasting plasma insulin levels. Thus, high doses of chromium appear to have a pharmacological effect on rats. The effects are best observed in the AUCs for the insulin concentration in the glucose challenge (Fig. 8). The AUCs for the rats on the diet without added chromium and on the AIN-93G diet with chromium are statistically equivalent, indicating no effect from the inclusion of chromium in the diet's mineral mix. However, supplementing the AIN-93G diet with 200 or 1,000 μg Cr/kg results in statistically lower AUCs, whereas the rats receiving the AIN-93G diet with 1,000 μg Cr/kg had areas statistically lower than those of the rats on the AIN-93G diet with the chromium in the mineral mix. Consequently, nutritionally relevant amounts of chromium in the diet seem to have no effect on plasma insulin levels in response to a glucose challenge; however, supranutritional levels of chromium lead to lower concentrations of insulin being required to restore normal glucose levels in a timely fashion. Thus, supranutritional levels of chromium appear to increase insulin sensitivity in the healthy Zucker lean rats. This laboratory has previously observed that daily supplementation of high doses of chromium(III) for 24 weeks results in lower fasting plasma insulin concentrations and lower insulin concentrations after a glucose challenge [20]. Insulin levels after the insulin challenge were also measured. The insulin levels were statistically equivalent at all time points for rats on all the various diets (data not shown).

Four types of studies are generally cited as evidence that chromium is an essential element: (1) studies of rats provided with "chromium-deficient" diets, (2) studies examining the absorption of chromium as a function of intake, (3) studies of patients on total parenteral nutrition (TPN), and (4) studies of the association between insulin response and chromium [4, 20]. All are problematic. For example, one study has reported that the absorption of chromium in humans is inversely proportional to intake [21]. However, a closer examination of the results indicates that the

statistical analysis is limited; and the conclusion only holds true for female subjects, as no effects on absorption were observed as a function of chromium intake for the male subjects. Chromium is absorbed by passive diffusion in rats (reviewed in [22]). A limited number of patients on TPN and demonstrating a variety of symptoms similar to those of type 2 diabetes have had their symptoms improve after addition of chromium to the TPN solution (reviewed in [23, 24]); however, the doses of chromium utilized were pharmacological, not nutritionally relevant. Investigation of the relationship between chromium mobilization in the body and insulin action requires studies to examine what is happening at a molecular level. Finally, as described above, studies with rats on "chromium-deficient diets" have been reported. Yet, a closer analysis of these studies reveals a number of flaws, such as failure to determine the chromium content of the diet, the use of metal components in caging that could provide a source of chromium for gnawing rats, and the use of additional stresses other than limiting the chromium content of the diet. Unfortunately, for example, the chromium content of the diet was not reported (although the experimental procedures at the time would not have likely produced the correct value). Additionally, the rats were maintained in wire mesh cages, possibly with stainless steel components, allowing the rats to obtain chromium by chewing on these components. Consequently, the actual chromium intake of the rats in these studies is impossible to gauge, putting into great question the suggestion that the rats were chromium-deficient. The use of large amounts of metal ions is also of concern. Supranutritional doses of Cr^{3+} have pharmacological effects on rodent models of altered carbohydrate and lipid metabolism including type 2 diabetes (reviewed in [4]). Additionally, Woolliscroft and Barbosa [5] have examined the effects of a normal and a *Torula* yeast diet in intravenous glucose tolerance tests in rats. They reproduced the results of Mertz and Schwarz; yet, observation of a significant difference in glucose metabolism between the two groups of rats depended on the method used to present the data, i.e., using measured plasma glucose concentrations versus using "excess" plasma glucose concentrations. The effect was only statistically significant when "excess" plasma glucose was used. As calculating the "excess" plasma introduces error, use of actual measured plasma glucose is the accepted practice. Thus, these studies do not provide evidence of chromium being an essential trace element. Subsequent studies on healthy rodents in the 1960s, 1970s, and 1980s suffer from similar methodological complications [4]. The use of these other stresses, such as diets with high sugar or fat content, can lead to alterations in carbohydrate and lipid metabolism. The effects of the addition of high concentrations of chromium to these diets could potentially be explained by pharmacological effects of chromium, rather than a nutrition effect. The current study demonstrates that low-chromium diets do not lead to observable deleterious effects and do not provide evidence that chromium is an essential trace element. In fact, no unequivocal data exist supporting an essential role for chromium. Given that currently no data confirm that chromium is an essential element, chromium should simply no longer be considered an essential element.

Conclusions

These studies clearly reveal that a diet with as little chromium as reasonably possible had no effect on body composition, glucose metabolism, or insulin sensitivity compared with a chromium- "sufficient" diet. The addition of supranutritional amounts of chromium to the diet had a pharmacological effect of increasing insulin sensitivity. These results clearly indicate that chromium can no longer be considered an essential element as nutritional studies have failed to demonstrate a deleterious effect from low chromium content in the diet and no biochemical studies have conclusively shown an essential function for chromium bound to a biomolecule. The mechanism of the pharmacological effects of chromium(III) is an area requiring continued research.

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