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Urinary Chromium Excretion in Response to an Insulin Challenge Is Not a Biomarker for Chromium Status

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Abstract Over 50 years ago, chromium (Cr) was proposed to be an essential trace element; however, recent studies indicate that this status should be removed as the effects of Cr supplementation appear to be pharmacological rather than nutritional. The pharmacological basis for Cr's effects can explain the inability of investigators to discover a biomarker for Cr status. One potential biomarker has not been examined to date. Cr is known to be mobilized in the body in response to insulin (or insulin release in response to a glucose challenge), resulting in an increase in urinary Cr excretion. The magnitude of increase in urinary Cr loss as a function of dietary Cr intake was tested as a potential biomarker for Cr. Zucker lean rats housed in carefully controlled metal-free conditions were provided a series of purified diets containing variable Cr contents (from 16 μg/kg diet to 2,000 μg/kg) for 23 weeks. The 16 μg/kg diet contained less Cr than any diet examined to date. Urine samples were collected before and after insulin and glucose challenges (0, 2, 6, and 12 h postinjection). Urinary Cr levels were analyzed by the standard method of addition using graphite furnace atomic absorption. The rate of urinary Cr loss after a glucose or insulin challenge was found to not be dependent on the Cr content of the rats' diets. Blood iron levels of the rats were also measured to determine if the addition of Cr to the diet altered iron status. The Cr content of the diet was found to have no affect on blood iron levels. Overall, the study demonstrated that insulin-stimulated urinary Cr excretion cannot be used as a biomarker for Cr status.

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Introduction

Despite chromium (Cr) being assumed to be an essential element for over 50 years, the evidence for Cr's status is ambiguous at best, particularly as no biomarker for Cr status exists [1, 2]. Thus, one of the key concerns in the field of Cr nutrition is the identification of a specific clinical measure of the Cr nutritional status of an individual [1]. For example, the summary of the NIH Office of Dietary Supplement's Chromium and Diabetes Workshop identified three research questions/directives that needed to be addressed. One was "development and refinement of methodologies for assessing Cr status" [3]. Studies of the supplementation of human diets with Cr have led to disparate results. The differing results have been postulated to reflect the variation in initial Cr status of individuals in each study [1]. This Cr status refers to the pool of utilizable Cr, not simply total Cr in the blood or tissues. Previously, no procedure has been examined to test the pool of utilizable Cr, which limits the interpretation of any study of Cr supplementation of human subjects. For example, urinary and plasma Cr do not correlate with tissue Cr concentrations nor with plasma glucose, insulin, or lipid levels [1]. In fact, recent studies have shown that Cr cannot currently be considered an essential trace element for mammals [4]. If Cr is not essential, then no biomarker for Cr status should exist.

If Cr is an essential element, the lack of correlation found in previous studies could result from the measurement of urinary Cr levels rather than the extent of Cr mobilization in response to an insulin or glucose challenge. This laboratory's studies with low-molecular-weight Cr-binding substance (LMWCr) may suggest a probe of the utilizable pool of Cr (if such exists). LMWCr is a small, Cr-binding

peptide that carries Cr from the tissue to the urine via the bloodstream [5, 6]. According to our model for the role of LMWCr in Cr transport [7], urinary LMWCr output should change in response to increases in the blood concentration of insulin or glucose, and this change should be a marker for Cr status if Cr is an essential element. For example, insulin and glucose challenges result in a movement of Cr from Cr bound in the bloodstream to the protein transferrin to the tissues, after which Cr is ultimately lost in the urine as LMWCr [8–10]. Cr lost in response to an insulin or glucose challenge is Cr that is being mobilized and should, therefore, reflect utilizable Cr. Hence, this laboratory has examined changes in the rate of urinary Cr excretion in response to an insulin or glucose challenge.

Methods

Chemicals and Blood Variable Assays

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 125I RIA kit from MP Biomedicals. Gamma counting was performed using a Packard Cobra II autogamma counter. Blood glucose levels were measured using a OneTouch glucose meter. Iron content was determined using a modified colorimetric method for determining nonheme iron concentration in biological samples [11].

Animals

Thirty-two male Zucker lean rats were obtained from Charles River Laboratories International at 6 weeks of age. (Male rats were chosen for consistency with previous studies, while the use of Zucker lean rats would allow for the effects of health condition to subsequently be examined by comparison of results with those of Zucker obese and Zucker diabetic fatty rats if urinary Cr loss would prove to be a potential biomarker for Cr status.) 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 Cr into their diets. Rats were fed specific diets and distilled water ad libitum for a 23-week period prior to glucose and insulin challenges. Rats were weighed, and food consumption was measured twice weekly; these results have been reported previously [4]. All diet groups averaged approximately 225 g in body mass at the start of the study; the average body masses of the groups at the end of the study were all between 400 and 500 g and statistically equivalent [4]. Diet did not affect body mass [4]. 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 randomly separated into four treatment groups, each containing eight rats as follows: (1) rats on a purified AIN-93 G Cr-sufficient diet [Cr as KCr$(SO_4)_2 \cdot 12H_2O$, the Cr source designated for the AIN-93 G diet], (2) rats on the AIN-93 G diet, with Cr not included in the mineral mix, (3) rats on the AIN-93 G Cr-sufficient diet with an additional 200 μg Cr/kg [Cr as $KCr(SO_4)_2 \cdot 12H_2O$], and (4) rats on the AIN-93 G Cr-sufficient diet with an additional 1,000 μg Cr/kg [Cr as $KCr(SO_4)_2 \cdot 12H_2O$]. Purified AIN-93 G rodent diets and modified AIN-93 G diets were obtained from Dyets (Bethlehem, PA, USA). Diets were received in powder form. Cr content of the diets was determined by graphite furnace atomic absorption spectroscopy utilizing a PerkinElmer Analyst 400 atomic absorption spectrometer equipped with an HGA-900 graphite furnace and an AS-800 autosampler using a Cr hollow cathode as previously described [4]. The values were AIN-93 G, 1,135 μg Cr/kg; AIN-93 G without mineral mix, 16 μg Cr/kg; AIN-93 G+200 μg Cr/kg, 1,331 μg Cr/kg; and AIN-93 G+1,000 μg Cr/kg, 2,080 μg Cr/kg [4]. All values were close to anticipated values.

Housing

Housing in metal-free caging was performed as described previously [4]. Briefly, Iris Buckle Up boxes were obtained from Target; the boxes were approximately 18 cm high, 45 cm wide, and 28 cm long. 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.

Urine Collection

After 23 weeks on the diets, the rats were placed in metabolic cages for 6 h prior to and removed 12 h after an intravenous glucose challenge (1.25 mg glucose/kg body mass). Urine was collected prior to injection and 2, 6, and 12 h post injection. The first 8 h of the urine collection occurred during the dark period with the remainder occurring during the photoperiod. The urine was transferred to preweighed disposable centrifuge tubes and stored at −20 °C. After continuing on the diet another 2 weeks, the rats were placed in metabolic cages for 6 h prior to and removed 12 h after an intravenous insulin (five insulin units (bovine, zinc) per kilogram of body mass) [12] challenge. Urine was collected as described for the glucose challenge. Rats had unrestricted access to food and water during the urine collection period.

Cr Concentration in Urine

Each urine sample was digested with a mixture of ultra high purity concentrated HNO_3 (99.99 % trace element free) and 30 % H_2O_2. The digestion was continued with controlled heating (subboiling) for 15 h. All glassware was acid washed. Blank digestions were carried out in the same fashion. Cr concentrations were determined utilizing a PerkinElmer Analyst 400 atomic absorption spectrometer equipped with HGA-900 graphite furnace and an AS-800 autosampler using a Cr hollow cathode lamp operating at 8 mA; a spectral bandwidth of 0.8 nm was selected to isolate the light at 353.7 nm, with operating conditions (temperature (degrees Celsius), ramp time (seconds), hold time (seconds)): drying 1 (90, 45, 20), drying 2 (140, 20, 20), ashing (800, 15, 15), atomization (2,500, 0, 5), and cleaning (2,700, 1, 5). Other instrumental parameters include cuvette: pyrolytic, carrier gas: argon (flow 250 mL/min), sample volume: 20 μL, and measurement mode: peak area. Urine Cr concentrations were calculated using the method of standard additions with samples spiked with 10, 20, 30, and 50 μg/L of PerkinElmer Pure Atomic Spectroscopy Standard 1,000 μg Cr/mL in HNO_3. Fits of the standard addition lines had r^2 values >0.98, while each triplicate point generally had standard deviations less than 2 %.

To test whether urine could be contaminated by feces in the metabolic cages, ^{51}Cr-containing rat feces (available from previous work [13]) were used to line the urine and feces collection component of the metabolic cage; a rat was housed in the cage (with food and water) and urine was collected. ^{51}Cr content of the urine was then determined by gamma counting. Contamination of the urine with Cr from the feces was insignificant.

Statistical Analysis

Statistical analyses were performed using SPSS (SPSS, Chicago, IL, USA). Data are represented graphically as average values with standard error (SE) bars. Data were calculated independently, tested for homogeneity of variance with Levene's test and analyzed using univariate analysis of variance and descriptive statistics. For eight animals per group, an expected difference between two means would be significant at the α=0.05 level and $1-\beta$=0.01 if the difference between the means is twice the standard deviation; these values are reasonable based on the effects of insulin on urinary Cr in Sprague–Dawley and Zucker obese rats [10]. 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 ($p \leq 0.05$). Area under the curve was calculated using the trapezoid rule.

Results

Testing Urinary Cr Loss as a Biomarker

To better test whether urinary Cr in responses to a glucose or insulin challenge can reflect Cr status, animals with a carefully controlled diet and environment need to be utilized.

For example, one needs to be certain that the carbohydrate content is consistent across diets, and the Cr content needs to be known to a reasonable degree of accuracy. Thus, the current study has been performed with rats on purified diets which Cr contents have been measured by graphite furnace atomic absorption and kept in metal-free cages. Only the Cr content of the diets was varied. The diets range from a diet with as low a Cr content as reasonably possible, 16 μg/kg diet, to over 2,000 μg/kg diet, a pharmacologically relevant amount of Cr. If Cr mobilized in response to insulin is to be a biomarker, then the amount of Cr mobilized should be saturable. In other words, the amount of mobilized Cr should be comparable for rats on a standard diet to those on a Cr-supplemented diet. However, if Cr is essential, Cr-sufficient rats theoretically should be able to mobilize Cr more efficiently than Cr-deficient rats. Thus, rats receiving less Cr in their diet than required to maintain a proper pool of utilizable Cr should excrete less Cr in the urine in response to a glucose or insulin challenge. Whether Cr supplementation of Cr-sufficient rats increases the amount of readily available/mobilizable Cr or just increase Cr quantities in longer term stores has not been examined previously.

Urine samples after the rats were maintained for over 23 or 25 weeks on the diet reveal a distinct trend in the rates of urinary Cr loss; as the Cr content of the diet increases, the rate of Cr loss (expressed in ng Cr lost per hour) increases, reflecting an increase in the Cr concentration of the urine (Figs. 1 and 2). In response to glucose, urinary Cr loss rates tend to increase for rats on the AIN-93 G diet with no added Cr, the diet supplemented with the recommended amount of Cr, and the diet supplemented with an additional 200 Cr μg/kg diet (Fig. 1). This is the anticipated result based on the previous studies with humans and rats described above. The urinary Cr rates tend to return to baseline values by 12 h after the challenge. However, the glucose challenge had no response on the rates of urinary Cr loss in the rats receiving the largest amount of Cr, the AIN-93 G diet with an additional 1 mg Cr/kg diet (Fig. 1). This situation appears to be reminiscent of the study of Anderson et al. [14], where an increase in urinary Cr loss was observed in individuals on a self-selected diet but not for individuals supplemented with 200 μg Cr daily. Thus, a Cr intake apparently can be achieved in humans and rats where the Cr transport system is overwhelmed so that an insulin or glucose challenge results in no apparent increase in urinary Cr loss. Over the course of 12 h from the challenge, the rate of urinary Cr loss is identical for all groups, as shown from identical areas under the curve (AUC) (Fig. 3).

The response to the insulin challenge is nearly identical. In response to insulin, urinary Cr loss rates tend to increase for rats on the AIN-93 G diet with no added Cr, the diet supplemented with the recommended amount of Cr, and the diet supplemented with an additional 200 μg Cr/kg (Fig. 2). This, again, is the anticipated result based on the previous studies with humans and rats described above. The urinary Cr rates actually tend to dip below baselines from 2 to 6 h after the challenge, before tending to return to baseline values by 12 h after the challenge. However, the insulin challenge, again, had no response on the rates of urinary Cr loss in the rats receiving the largest amount of Cr, the AIN-93 G diet with an additional 1 mg Cr/kg diet (Fig. 2). Over the course of 12 h from the challenge, the rate of urinary Cr loss is identical for all groups, as shown from identical AUC (Fig. 4).

The Cr content of the diets needs to be put in perspective. For the diet with the lowest Cr content, 16 μg Cr/kg diet corresponds to about 2.4 μg Cr/kg body mass/day (assuming that a 100 g rat eats 15 g of food a day [15]). Assuming an average body mass for a human of 65 kg, this corresponds to approximately 1.6×10^2 μg Cr/day. Thus, the diet would be Cr-sufficient diet for humans, based on the AI of 30 μg/day [16]. The diet supplemented with 2 mg Cr/kg corresponds to about 20 mg Cr/day for a 65-kg human in this simple analysis (without potential corrections for surface area, metabolism, etc.). Curiously, humans supplementing their diet with 200 μg Cr/daily appear to have the Cr transport system saturated with Cr so that no increase in urinary Cr loss occurs with a glucose challenge while the transport system in rats is overwhelmed by a proportionally smaller Cr intake.

The SE in Figs. 1 and 2 are surprisingly large. However, the magnitude of the error does not arise from the determination of the Cr concentrations but rather from the range of individual response to the challenges. (One can readily note in Fig. 1 the change in the standard deviation from the baseline values to values after the challenges.) Figure 5 displays the effects of the insulin challenge on the eight individual rats on the AIN-93 G diet containing the standard quantity of Cr in the mineral mix. Note the wide variation in responses between rats. This wide range in individual response mirrors that observed for humans on self-selected diets and self-selected diets supplemented with Cr reported by Anderson et al. [17]; subjects on supplemented diets displayed a much greater range of urinary Cr loss so that "urinary Cr excretion after a glucose challenge was not predictable" [14].

Effect of Supplemental Cr on Blood Plasma Iron Concentrations

Given that Cr in the bloodstream can compete with ferric ions for the metal-binding sites of transferrin, the effects of Cr on the nonheme iron content of the blood plasma of the rats were analyzed. The concentration of nonheme iron in the blood plasma was not significantly affected by the addition of Cr to the diet (Fig. 6). Given the AIN-93 G diet

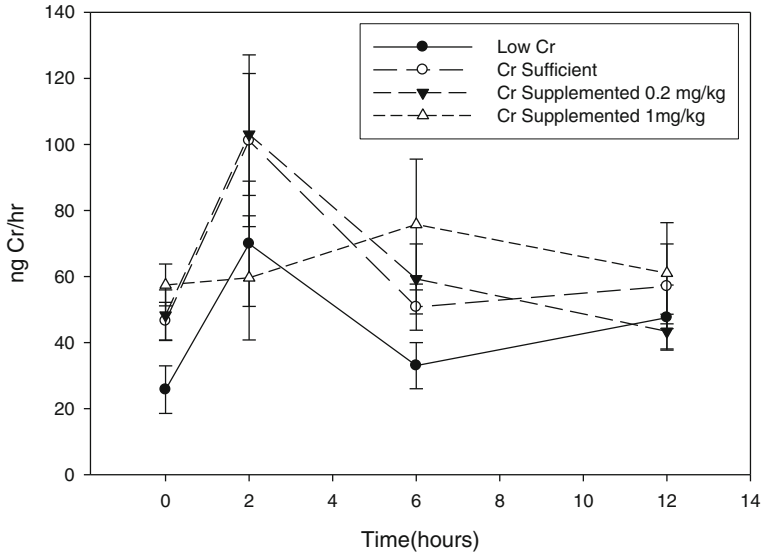

Fig. 1 Rate of urinary Cr loss (ng Cr/h) for Zucker lean rats on purified diets over 6 h before a glucose challenge (1.25 mg glucose/kg body mass), over 2 h after a glucose challenge, between 2 and 6 h after a glucose challenge, and between 6 and 12 h after a glucose challenge

provides 35 mg of Fe per kilogram diet from its mineral mix [18] (in addition to the Fe contents of the other components) while the diet with the most Cr in this study contained ~2 mg Cr/kg diet, a significant effect on plasma iron would not be expected.

Discussion

The amount of Cr released in direct response to an insulin or glucose challenge theoretically should reflect whether an adequate amount of Cr is available for insulin potentiation, i.e., reflect Cr status, assuming Cr is an essential element. In humans, urine Cr levels have been shown to reflect Cr intake [1, 14, 17]. Human serum Cr levels also reflect Cr intake [19]; serum levels of Cr 90 min after a glucose challenge correlate with Cr levels before the challenge [19]. Human urine Cr concentrations do not correlate with serum glucose, insulin, or lipid parameters or with age or body mass [17]. Similarly, human urine Cr concentrations after a glucose challenge have been reported to correlate with Cr concentrations before the challenge for individuals not taking a Cr supplement, although the data were not presented [17]. 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) [14]. Urinary Cr loss after a glucose challenge was found not to be predictable and suggested to not reflect Cr status [14]. Yet, the extent of movement of Cr 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

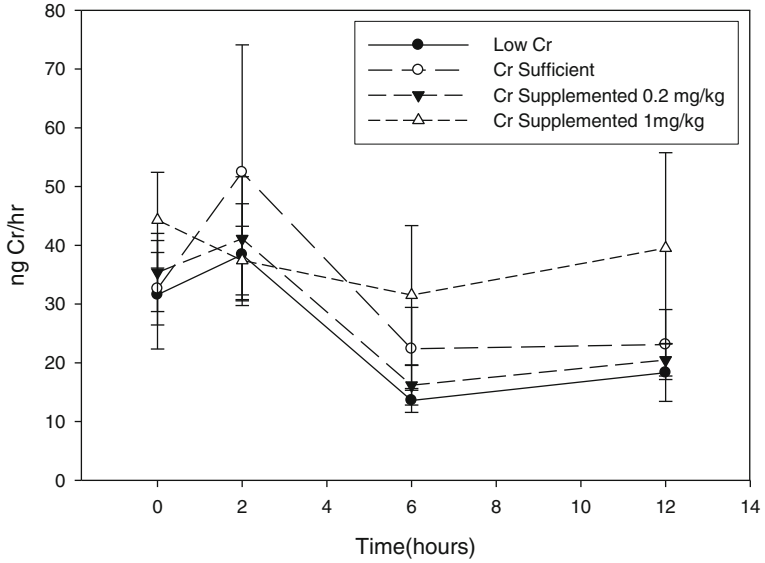

Fig. 2 Rate of urinary Cr loss (ng Cr/h) for Zucker lean rats on purified diets over 6 h before an insulin challenge (5 insulin units (bovine, zinc)/kg body mass), over 2 h after an insulin challenge, between 2 and 6 h after an insulin challenge, and between 6 and 12 h after an insulin challenge

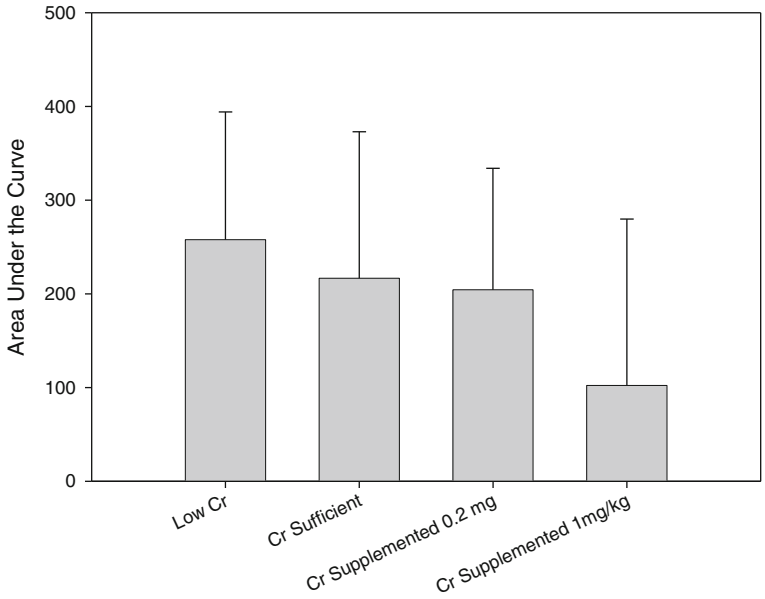

Fig. 3 Area under the curve (AUC) for the rate of urinary Cr loss in the glucose tolerance tests. The area is the total area minus the area where no change occurred from baseline values

study was not established. Notably, self-selected American diets have been shown to provide on average about 30 μg Cr daily, a quantity that has been deemed an adequate intake (AI) so that >98 % of individuals receiving this quantity cannot be considered Cr deficient [16]. (This discussion assumes that experiments were performed after circa 1978 when improvements in graphite furnace atomic absorption techniques for determining tissue and body fluid concentrations of Cr were improved and demonstrated that previous values were orders of magnitude too high [20]).

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 [21]. 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 [21]. 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 [21]. 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 [21, 22]. Urinary Cr excretion after a glucose tolerance test does

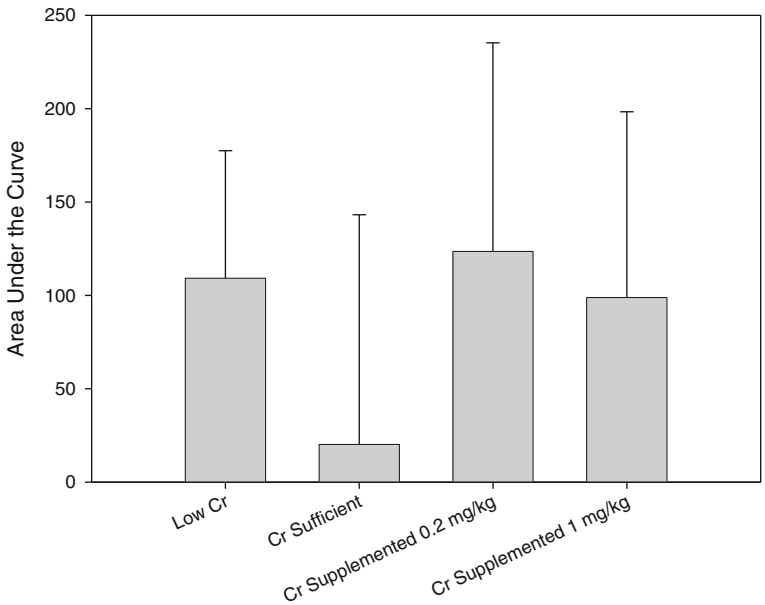

Fig. 4 Area under the curve (AUC) for the rate of urinary Cr loss in the insulin tolerance tests. The area is the total area minus the area where no change occurred from baseline values

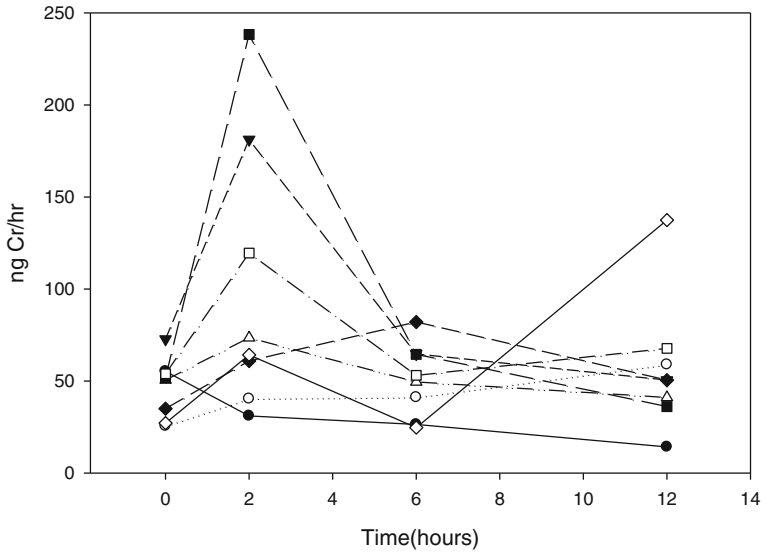

Fig. 5 Rate of urinary Cr loss (ng Cr/h) for individual Zucker lean rats on AIN-93 G diet containing the standard quantity of Cr in the mineral mix over 2 h before an insulin challenge (5 insulin units (bovine, zinc)/kg body mass), over 2 h after an insulin challenge, between 2 and 6 h after an insulin challenge, and between 6 and 12 h after an insulin challenge

not differ between control men or hyperinsulinemic men or differ between men on diets with differing high amylase cornstarch contents [23]. Eight of 10 healthy individuals have been found to have increased urinary Cr loss (nanogram of Cr per minute) for 4 h after an oral glucose tolerance test compared to the 4 h 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 [24]. Finally, Morris et al., 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 increases in urinary Cr loss [25], consistent with their earlier studies demonstrating increased urinary Cr loss after an oral glucose challenge [26]. 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. Cr supplementation may eliminate the effect, although the change from supplementation has been noted in a single study.

Rats have been conclusively shown to increase Cr excretion in response to an insulin or glucose challenge despite one claim (where no data was presented) [27]. Nearly all Cr (III) in the bloodstream is in the form of Cr transferrin [28]. Using radiolabeled ^{51}Cr transferrin, Cr has been shown to be transferred from the bloodstream to the tissues to the bloodstream (presumably bound to the peptide LMWCr) and then lost in the urine, again presumably as LMWCr [8–10]. This movement of Cr(III) from the bloodstream

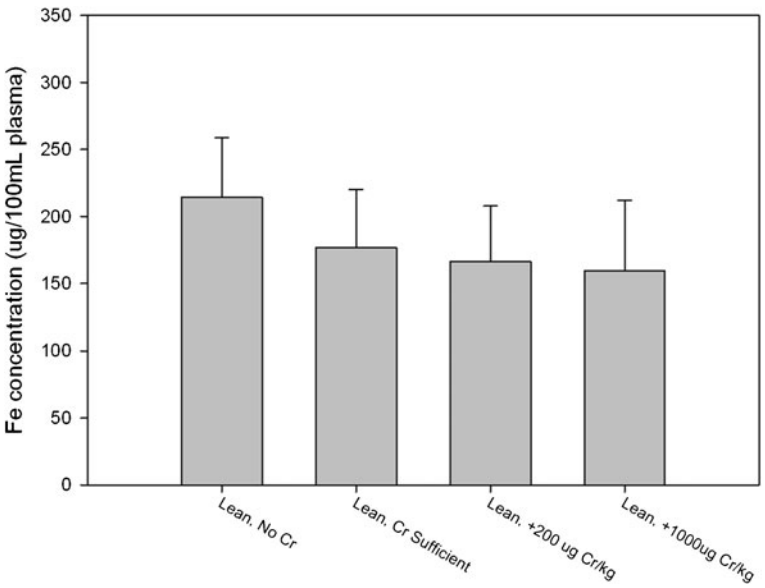

Fig. 6 Blood plasma iron concentrations. All values are statistically equivalent

to the urine is enhanced by insulin [8–10]. Insulin does not appear to change the mechanism of Cr transfer or rate of transfer but changes the amount of Cr mobilized from the bloodstream [10].

Previously, the effects of these diets on the insulin sensitivity of Zucker lean rats have been examined [4]. Rats on the three AIN-93 G diets with added Cr had greater insulin sensitivity than rats on the AIN-93 G diet without any added Cr; insulin sensitivity increased as the amount of Cr in the diet increased [4]. Given the large quantities of Cr involved, this was clearly a pharmacological effect. Notably, the results of this study cannot be correlated with the insulin sensitivity of the rats. Rats with the greatest insulin sensitivity (AIN-93 G diet+1 mg Cr/kg diet) had no tendency toward increased urinary Cr loss in response to an insulin or glucose challenge. In contrast, these rats appear to be receiving a quantity of Cr that overwhelms the system responsible for increasing urinary Cr loss in response to either challenge.

Given that the urinary Cr loss in response to glucose or insulin is not decreased in the AIN-93 G diet with no added Cr compared to the diet with the added Cr, this study provides no evidence that using a diet with as low a Cr content as reasonably possible generates Cr deficiency. This is consistent with the associated study [4] that demonstrated that this diet provided no observable ill health effects. Thus, as no diet has been found that can actually generate Cr deficiency in rats (or in humans [2, 5]), and no nutritional evidence exists that Cr is an essential element. Additionally, no biomolecules that bind Cr has been shown to perform an essential function in a biological system. Thus, Cr cannot be considered an essential trace element for mammals [2, 5]. Even if evidence existed that Cr was an essential element, the range of individual responses to insulin and glucose challenges in rats (this study) and humans [14] would preclude the use of urinary Cr loss as a biomarker for Cr status. Consequently, the establishment of a biomarker for Cr status is probably impossible as the element cannot be shown to be essential for health.

Conclusions

These studies demonstrate that urinary Cr excretion in response to a glucose or insulin challenge cannot be used as a biomarker for Cr status. Combined with previous studies that have demonstrated the inability of tissue or body fluid levels of Cr or any other experimental parameter to be correlated with beneficial effects of Cr administration (so that no biomarker for Cr status exists) and the failure of a diet with as little Cr as reasonably possible to have any deleterious effects, Cr can no longer be considered an essential element.

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References

1. Lukaski HC (1999) Chromium as a supplement. Ann Rev Nutr 19:279–301
2. Vincent JB (2010) Chromium: celebrating 50 years as an essential element? Dalton Trans 39:3787–3794
3. National Institutes of Health (1999) Office of Dietary Supplements Chromium and Diabetes Workshop Summary, November 4, 1999, Natcher Conference Center, National Institutes of Health. http://ods.od.nih.gov/News/chromium_diabetes.aspx (accessed December 13, 2012).
4. Di Bona KR, Love S, Rhodes NR, McAdory D, Sinha SH, Kern N, Kent J, Strickland J, Wilson A, Beaird J, Ramage J, Rasco JF, Vincent JB (2011) Chromium is not an essential trace element for mammals: effects of a "low-chromium" diet. J Biol Inorg Chem 16:381–390
5. Vincent JB (2013) The bioinorganic chemistry of chromium (III). Wiley, Chichester
6. Vincent JB (2000) Elucidating a biological role for chromium at a molecular level. Acc Chem Res 33:503–510
7. Vincent JB (2000) Quest for the molecular mechanism of chromium action and its relationship to diabetes. Nutr Rev 58:67—72
8. Clodfelder BJ, Emamaullee J, Hepburn DD, Chakov NE, Nettles HS, Vincent JB (2001) Trail of chromium from the blood to the urine: the roles of transferrin and chromodulin. J Biol Inorg Chem 6:608–617
9. Clodfelder BJ, Upchurch RG, Vincent JB (2004) A comparison of the insulin-sensitive transport of chromium in healthy and model diabetic rat. J Inorg Biochem 98:522–533
10. Clodfelder BJ, Vincent JB (2005) Time-dependent transfer of chromium in adult rats from the bloodstream to the urine. J Biol Inorg Chem 10:383–393
11. Fish FW (1988) Rapid colorimetric micromethod for the quantitation of complexed iron in biological systems. Meth Enzymol 158:357–364
12. Cefalu WT, Wang ZQ, Zhang XH, Baldor LC, Rusell JC (2002) Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. J Nutr 132:1017–1114
13. Rhodes NR, NcAdory D, Love S, Di Bona KR, Chen Y, Ansorge K, Hira J, Kent J, Lara P, Rasco JF, Vincent JB (2010) Urinary chromium loss associated with diabetes is offset by increases in absorption. J Inorg Biochem 104:790–797
14. Anderson RA, Polansky MM, Bryden NA, Roginski EE, Patterson KY, Veillon C, Glinsmann W (1982) Urinary chromium excretion of human subjects: effects of chromium supplementation and glucose loading. Am J Clin Nutr 36:1184–1193
15. Anderson RA, Bryden NA, Polansky MM (1997) Lack of toxicity of chromium chloride and chromium picolinate in rats. J Am Coll Nutr 16:273–279
16. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. (2002) A Report of the Panel on Micronutrients, Subcommittee on Upper Reference Levels of Nutrients and of Interpretations and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific

Evaluation of Dietary Reference Intakes, National Academy of Sciences, Washington, D. C.
17. Anderson RA, Polansky MM, Bryden NA, Patterson KY, Veillon C, Glinsmann WH (1983) Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected urine parameters. J Nutr 113:276–281
18. Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127:838S–841S
19. Anderson RA, Bryden NA, Polansky MM (1985) Serum chromium of human subjects: effects of chromium supplementation and glucose. Am J Clin Nutr 41:571–577
20. Veillon C, Patterson KY (1999) Analytical issues in nutritional chromium research. J Trace Elem Exp Med 12:99–109
21. Anderson RA, Bryden NA, Polansky MM, Reiser S (1990) Urinary chromium excretion and insulinoegnic properties of carbohydrates. Am J Clin Nutr 51:864–868
22. Reiser S, Powell AS, Yang C-Y, Canary JJ (1987) An insulinogenic effect of oral fructose in humans during postpradial hyperglycemia. Am J Clin Nutr 45:580–587
23. Behall KM, Howe JC, Anderson RA (2002) Apparent mineral retention is similar in control and hyperinsulinemic men after consumption of high amylase cornstarch. J Nutr 132:1886–1891
24. Gurson CT, Saner G (1978) The effect of glucose loading on urinary excretion of chromium in normal adults, in adults from diabetic families and in diabetics. Am J Clin Nutr 31:1158–1161
25. Morris BW, Mac Neil S, Stanley K, Gray TA, Fraser R (1993) The inter-relationship between insulin and chromium in hyperinsulinaemic euglyceamic clamps in healthy volunteers. J Endocrin 139:339–345
26. Morris BW, Blumsohn A, Mac Neil S, Grat TA (1992) The trace element chromium—a role in glucose homeostasis. Am J Clin Nutr 55:989–991
27. Anderson RA, Polansky MM (1995) Dietary and metabolite effects on trivalent chromium retention and distribution in rats. Biol Trace Elem Res 50:97–108
28. Hopkins LL Jr, Schwarz K (1964) Chromium(III) binding to serum proteins, specifically siderophilin. Biochim Biophys Acta 90:484–491