Chromium Improves Insulin Response to Glucose in Rats

J.S. Striffler, J.S. Law, M.M. Polansky, S.J. Bhathena, and R.A. Anderson

The effects of chromium (Cr) supplementation on insulin secretion and glucose clearance (K_G) during intravenous glucose tolerance tests (IVGTTS) were assessed in rats with impaired glucose tolerance due to dietary Cr deficiency. Male Wistar rats were maintained after weaning on a basal low-Cr diet containing 55% sucrose, 15% lard, 25% casein, American Institute of Nutrition (AIN)-recommended levels of vitamins, no added Cr, and an altered mineral content as required to produce Cr deficiency and impaired glucose tolerance. The Cr-supplemented group ([+Cr] n = 6) were provided with 5 ppm Cr as $CrCl_3$ in the drinking water, and the Cr-deficient group ([−Cr]n = 5) received purified drinking water. At 12 weeks on the diet, both groups of rats were hyperinsulinemic (+Cr, 103 ± 13; −Cr, 59 ± 12 μU/mL) and normoglycemic (+Cr, 127 ± 7; −Cr, 130 ± 4 mg/dL), indicating insulin resistance. After 24 weeks, insulin levels were normal (+Cr, 19 ± 5; −Cr, 21 ± 3 μU/mL) and all rats remained normoglycemic (+Cr, 124 ± 8; −Cr, 131 ± 6 mg/dL). K_G values during IVGTTs were lower in −Cr rats (K_G = 3.58%/min) than in +Cr rats (K_G = 5.29%/min), correlating with significantly greater 40-minute glucose areas in the −Cr group ($P < .01$). Comparisons of 40-minute insulin areas indicated marked insulin hyperresponsiveness in the −Cr group, with insulin-secretory responses increased nearly twofold in −Cr animals ($P < .05$). Chromium deficiency also led to significant decreases in cyclic adenosine monophosphate (cAMP)-dependent phosphodiesterase (PDE) activity in spleen and testis ($P < .01$). In these studies, Cr deficiency was characterized by both β-cell hypersecretion of insulin and tissue insulin resistance that were associated with decreased tissue levels of cAMP PDE activity.

IMPAIRED GLUCOSE TOLERANCE and altered pancreatic β-cell function showing similarities to non–insulin-dependent diabetes in humans are observed in animals with dietary deficiency of chromium (Cr) and other minerals, including copper (Cu), zinc (Zn), manganese (Mn), and other micronutrients.[1] Glucose tolerance is also influenced by dietary iron (Fe) and is impaired in patients with hemochromatosis.[2-4] High Fe levels may interfere with transport of Cr, since both Fe and Cr are transported via transferrin.[5] In many of these patients, insulin secretion is decreased, indicating that impaired glucose tolerance may be secondary to Fe-induced changes in the endocrine pancreas. Insulin secretion is also impaired in rats fed diets containing inadequate levels of Cu.[6,7] In addition, recent studies indicating that Cu deficiency effects are exacerbated by Fe[8,9] suggest that changes in endocrine pancreas function in Cu-deficient rats also involve Fe.

In contrast to other mineral-deficiency states, Cr deficiency is difficult to produce in animals. Since Cr deficiency is not readily expressed in animals fed diets high in starch, diets high in sucrose need to be used.[10] Contaminating Cr in the basal diets, animal housing conditions, use of starch instead of sucrose, and undefined dietary effects may explain the absence or marginal responses to Cr supplementation in some studies.[11-15] In addition, most investigators have examined effects of Cr deficiency and supplementation on glucose tolerance in relation to insulin effectiveness, and less attention has been given to the associated changes in insulin-secretory responsiveness, even though it was suggested that insulin levels should be monitored in studies examining the biological action of Cr.[16] This is particularly true in light of studies demonstrating that significant changes in functioning of the endocrine pancreas occur in animals fed diets containing either excess or inadequate amounts of various minerals.[1]

The objective of these studies was to assess the influence of dietary Cr on both glucose tolerance and insulin-secretory responsiveness measured in vivo in rats. Cr deficiency and insulin resistance were produced by feeding a basal sucrose diet containing no added Cr and an altered content of other minerals to induce glucose intolerance. The basal diet used in these studies was low in Cu during the initial 6-week period of rapid growth to impair β-cell function,[6,7] and dietary-induced insulin resistance was enhanced by increasing Fe content of the basal diet.[2-4] The effects of dietary Cr were assessed by comparing glucose and insulin responses during intravenous glucose tolerance tests (IVGTTs) in rats maintained on the basal diet with responses in animals fed the same diet supplemented with Cr as $CrCl_3$ added to the drinking water. In contrast to recent studies from other laboratories,[11-15] the observations shown here confirm findings of early investigators[16] indicating that Cr is an essential dietary nutrient for maintenance of normal glucose tolerance in the rat. In addition to enhancement of insulin effectiveness,[16] the data suggest that Cr may be required for normal functioning of the endocrine pancreas.

MATERIALS AND METHODS

The study protocol and care of animals were approved by the US Department of Agriculture Beltsville Area Research Animal Care Committee. Weanling male Wistar rats (Charles River Laboratories, Wilmington, MA) were housed on a 12-hour light/dark cycle with constant ambient temperature (25°C) and environmental conditions that minimize external Cr contamination from air, dust, cages, etc.[17] Fourteen weanling animals were randomly assigned to either a Cr-deficient (−Cr) diet or Cr-supplemented (+Cr) diet. There were technical problems with three animals, resulting in six

From the Department of Nutrition and Food Science, University of Maryland, College Park; and US Department of Agriculture, Beltsville Human Nutrition Research Center, Metabolism and Nutrient Interactions Laboratory, and Nutrient Requirements and Functions Laboratory, Beltsville, MD.

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Address reprint requests to R.A. Anderson, PhD, USDA, ARS, BHNRC, NRFL, Bldg 307, Room 224, BARC-East, 10300 Baltimore Ave, Beltsville, MD 20705-2350.

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+Cr and five −Cr animals for final analysis. +Cr rats were provided with 5 ppm Cr as $CrCl_3$ in the drinking water.[18] Chromium was added to water rather than to the diet to minimize cross-contamination. Any spills of water were readily contained on absorbent paper. The basal low-Cr diet was 55% sucrose, 15% lard, and 25% casein (33 ± 14 ng Cr/g diet). Standard reference materials and methods of Cr analyses have been previously described.[19] Recommended levels of vitamins were present in the diet,[20] and to minimize Cr contamination, the macroelement content of the diet was decreased marginally. The diet contained (in milligrams per kilogram of diet) 1,400 sodium, 3,026 potassium as chloride, 3,000 calcium as carbonate, 2,400 phosphorus as potassium phosphate, and 400 magnesium as sulfate. The following trace elements (in milligrams per kilogram of diet) were also added: 10 zinc as carbonate, 400 iron as sulfate, 100 manganese as carbonate, 0.2 iodine as potassium iodate, 0.5 selenium as selenite, 6 nickel as acetate, 5 molybdenum as molybdate, 5 tin as chloride, and 5 vanadium as vanadate. To compromise functioning of the endocrine pancreas,[6,7] Cu 1 mg/kg was fed during the initial 6 weeks of rapid growth. Cu was present in the diet at recommended levels (6 mg/kg) as carbonate salt after the sixth week. High Fe was added to enhance signs of Cr deficiency, since Fe competes with Cr during absorption and transport.[4,5]

Experimental Protocol

Longitudinal studies in the basal state. Fasting plasma insulin and glucose levels were measured as indicators of the diet's effect on insulin resistance. Blood samples for baseline measurements were drawn by heart puncture under pentobarbital anesthesia (40 mg/kg body weight) at 12 and 24 weeks. Following collection, blood samples were transferred to polypropylene tubes containing EDTA (12 mg/mL). Plasma samples were harvested by centrifugation and stored frozen until assay.

Insulin and glucose responses during IVGTT. The effects of Cr deficiency and supplementation on insulin responsiveness of the pancreas and sensitivity to endogenous insulin during an IVGTT were assessed after 24 weeks on the basal low-Cr diet. IVGTTs were performed in overnight-fasted rats anesthetized with pentobarbital (40 mg/kg). The right jugular vein was exposed via a neck incision and cannulated (PE-50 connected to a 1-mL plastic syringe fitted with a 23-gauge needle) for collection of blood samples. Heparin 10 U in saline was injected. The left saphenous vein was exposed for intravenous glucose administration. Each animal was stabilized for approximately 45 minutes to allow recovery from hypotension.[21] A baseline sample of blood was drawn, and a bolus of glucose 1.25 g/kg body weight (50% dextrose solution) was injected into the saphenous vein over a 30-second interval. Following glucose injection (time 0), blood samples (0.50 to 0.80 mL) were collected at 3, 5, 7, 10, 13, 15, 17, 20, 25, 30, 40, 50, and 60 minutes and processed as indicated earlier. Each sample was replaced with an equal volume of warmed electrolyte solution (Normosol-R; Abbott Laboratories, Abbott Park, IL) containing 4% dextran (Clinical Grade; Sigma, St Louis, MO) and glucose 100 mg/dL. All animals were kept warm during the procedure using an incandescent lamp. Animals were in a semiawake state, and all showed normal skin coloration around the nose and mouth. Immediately after collection of the 60-minute blood sample, animals were euthanized by decapitation and the spleen, testis, and brain were removed for measurement of phosphodiesterase (PDE) activity. IVGTTs were performed on two animals each day (one of each group).

Assay Procedures

Plasma glucose and insulin. Glucose concentrations were measured in a Centrifichem System 600 using the hexokinase method (Serono-Baker Diagnostics, Allentown, PA). Insulin concentrations were measured using the radioimmunoassay procedure reported by Albano et al.[22] Samples were assayed in triplicate using standards prepared from rat insulin (kindly provided by Eli Lilly & Co, Indianapolis, IN). Gamma-counting and data reduction using iterative smoothed spline functions were performed with an LKB gamma counting system (Model 1282; Wallac, Gaithersburg, MD).

Tissue PDE activity. The spleen, testis, and brain were quickly removed and transferred to ice-cold 50 mmol/L Tris hydrochloride buffer, pH 7.4. The spleen and testis were used as representative insulin-sensitive tissues, and the brain as insulin-insensitive tissue. Following a 10-fold dilution with the same buffer, each tissue was homogenized twice at 0°C with a Brinkman Polytron for 10 seconds. Tissue suspensions were centrifuged (35,000 × *g* for 20 minutes at 4°C), and the resultant supernatants were assayed for cyclic adenosine monophosphate (cAMP)-dependent PDE activity as previously described.[23]

Data Analyses

Glucose clearance (K_G) was calculated from semilog plots of plasma glucose concentration (corrected for basal) using the 10-, 20-, 30-, and 40-minute time points.[24,25] Areas under response patterns recorded between 0 and 40 minutes for insulin (Fig 2) and glucose (Fig 3) were calculated after subtraction of baseline concentration using the trapezoidal rule. All values are the mean ± SEM unless otherwise noted. Differences were determined using Student's *t* test.

RESULTS

Both groups of rats showed normal growth rate and body weight gain after the sixth week when Cu content of the diet was increased to recommended levels (Fig 1). As is characteristic of trace-element deficiency,[16] +Cr animals showed a small but consistently greater weight gain than −Cr rats. Final body weights (mean ± SD) were 506 ± 47 (+Cr, n = 6) and 475 ± 76 g (−Cr, n = 5).

After 12 weeks on the low-Cr diet, both groups of rats showed a pronounced fasting hyperinsulinemia (+Cr, 103 ± 13; −Cr, 59 ± 12 μU/mL) with normoglycemia (+Cr, 127 ± 7; −Cr, 130 ± 4 mg/dL), indicating the presence of insulin resistance induced by the experimental diet. As compared with chow-fed animals, plasma insulin levels were essentially normal in both groups of rats after 24 weeks (+Cr, 19 ± 5; −Cr, 21 ± 3 μU/mL). All animals remained normoglycemic at 24 weeks (+Cr, 124 ± 8; −Cr,

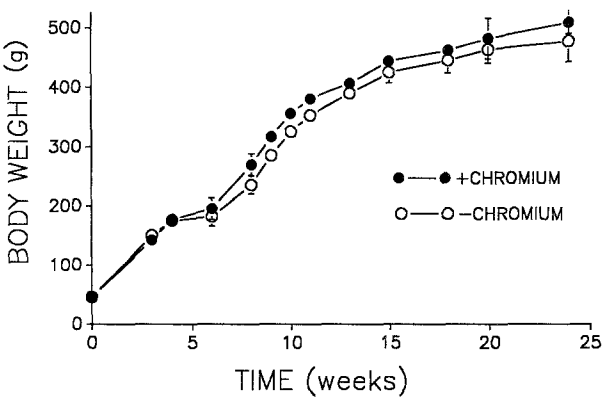

Fig 1. Body weight gain in −Cr and +Cr rats.

131 ± 6 mg/dL). Fasting insulin and glucose values for chow-fed animals were 35 ± 9 μU/mL and 135 ± 7 mg/dL, respectively.

Although insulin levels were normal in the basal (unstimulated) state, the insulin-secretory response to glucose was exaggerated in −Cr rats after 24 weeks on the diet (Fig 2). Insulin levels in +Cr rats were approximately 50% lower than in −Cr animals. The presence of a late insulin response (second-phase) commencing at 7 minutes is apparent in rats fed the high-sucrose diet. These observations are in contrast to responses in chow-fed rats, in which insulin levels continue to decline with absence of a late insulin response (data not shown). At all time points before minute 40, levels of insulin remained well above 100 μU/mL, indicating that hepatic glucose output was fully suppressed in all animals studied.[26] Therefore, differences in plasma glucose responses measured in these animals (Figs 3 and 4) represent differences in glucose utilization and peripheral tissue insulin sensitivity, and not altered hepatic glucose production.

Plasma glucose concentrations decayed exponentially following glucose administration at time 0 (Fig 3). Levels of glucose in +Cr rats tended to be lower than in −Cr animals, but differences were not significant. Although the observed Cr effects on glucose level are relatively small, they are comparable to differences recorded after intravenous glucose injection in glucose-intolerant obese human subjects, in whom blood glucose levels average 5% to 10% higher than levels in non-obese control subjects (see Fig 6 in Seltzer et al[27]).

More efficient utilization of glucose in +Cr rats is illustrated in Fig 4. The K_G for +Cr rats of 5.29%/min was 48% higher than that of −Cr animals, 3.58%/min. The mean K_G measured in −Cr rats is 16% to 20% lower than values (between 4% and 5%/min) recorded in normal animals fed stock diet.[24] In accordance with the differences in K_G, significantly larger 40-minute glucose areas were recorded in −Cr animals (Table 1).

In association with the observed modulatory effects on pancreatic insulin-secretory response and increased tissue

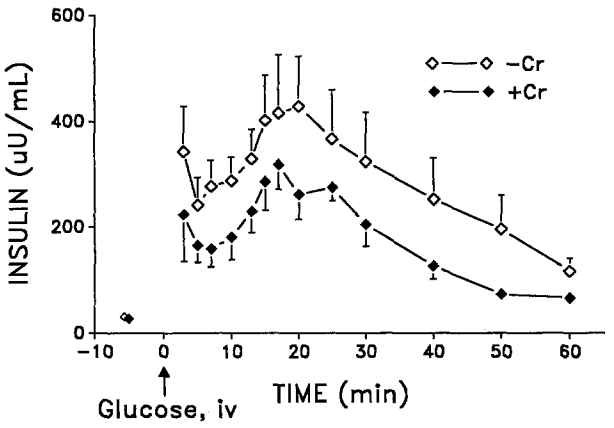

Fig 2. Plasma insulin responses during an IVGTT in −Cr and +Cr rats. Preglucose baseline insulin levels are shown.

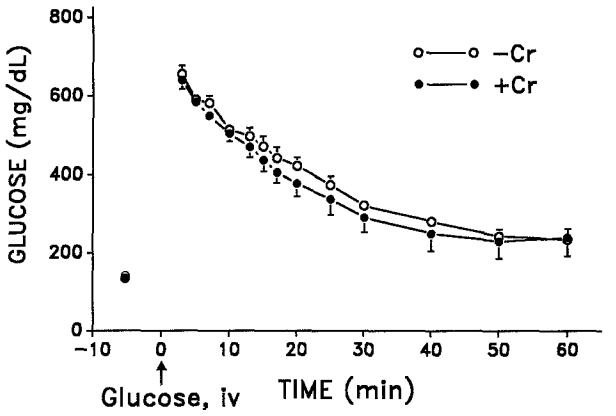

Fig 3. Plasma glucose responses during an IVGTT in −Cr and +Cr rats. Baseline glucose levels are shown.

sensitivity to endogenous insulin in +Cr animals, cAMP-dependent PDE activity was significantly enhanced in the spleen and testes from +Cr rats (Table 2). PDE in brain tissue was not altered by Cr.

DISCUSSION

In this study, the basal diet was high in sucrose to increase insulin resistance in the experimental animals. The use of high sucrose levels (55%) is based on numerous studies examining effects of dietary carbohydrate on metabolic responses and demonstrating that sucrose-fed rats are less sensitive to insulin and have significantly higher fasting insulin levels than rats fed diets high in starch.[28-31] The relatively high percent fat of the diet would also lead to decreased insulin sensitivity.[32] The decreased concentra-

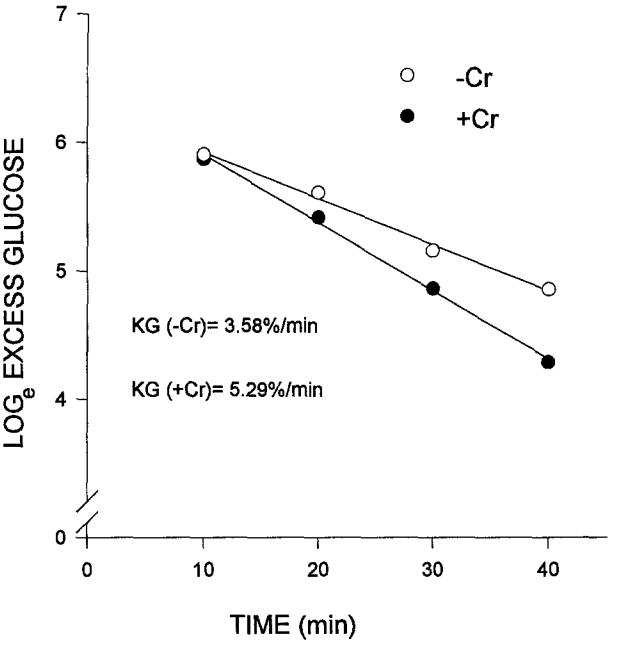

Fig 4. K_G during an IVGTT in −Cr and +Cr rats. Excess glucose level corrected for basal.

Table 1. Plasma Insulin and Glucose Responses in −Cr and +Cr Rats During an IVGTT

Parameter	−Cr (n = 5)	+Cr (n = 6)	Difference Due to Cr (%)	P*
Insulin area (μU/mL per 40 min)†	12,142 ± 2,358	6,971 ± 841	43 (↓)	.05
Glucose area (mg/dL per 40 min)‡	10,829 ± 277	8,916 ± 617	18 (↓)	.01
Glucose clearance (%/min)	3.58 ± 0.50	5.29 ± 1.18	48 (↑)	NS

*Level of significance for differences between −Cr and +Cr rats determined using Student's *t* tests.
†Insulin area above basal.
‡Glucose area above basal.

tions of calcium, potassium, magnesium, and phosphorus would have minimal effects on a growing rat, but were reduced to minimize Cr contamination. Although reduced from American Institute of Nutrition (AIN) levels,[20] concentrations of potassium and magnesium were at or above National Research Council recommendations.[33] Concentrations of trace elements, including Mn, Se, Ni, Mo, Sn, and V, were elevated as potential inhibitors of Cr. Increased levels of these micronutrients did not lead to significantly elevated dietary Cr concentrations. Vanadium, which is next to Cr in the periodic table, has been reported to compete with Cr,[34] and Mn, which is also next to Cr, displayed some competition with Cr in our diet experiments (R.A. Anderson and M.M. Polansky, unpublished observation, January 1994). Functions of the endocrine pancreas are compromised by insufficient Cu,[6,7] and high Fe was added since high Fe competes with Cr during uptake and transport.[4,5] Signs of Cr deficiency in rats are extremely marginal if Cr is simply not added to a balanced diet. Chromium seems to exert much larger effects under dietary or physical stresses.[35]

The observations reported here and in other studies from this laboratory examining intravenous glucose tolerance in Cr-deficient rats[36] are in accordance with the proposal that impaired glucose tolerance in Cr deficiency reflects defects in peripheral tissue sensitivity to insulin.[16] The presence of hyperinsulinemia in association with impaired glucose tolerance indicates decreased peripheral tissue sensitivity to insulin in the Cr-deficient state. The smaller insulin response area in the +Cr group was comparable in magnitude to the area measured in stock-fed animals (data not shown), suggesting that preservation of normal β-cell glucose sensitivity may be a significant function of dietary Cr. The insulinogenic index expressed as the ratio of incremental insulin area to the associated incremental glucose area provides a semiquantitative estimate of β-cell responsiveness to glucose.[27] A lower ratio in the +Cr group, 0.80, as compared with that in the −Cr group, 1.10, supports the conclusion that β-cell overproduction of insulin was prevented in +Cr rats. In addition, the 48% increase in K_G, which occurred at lower insulin levels in +Cr animals, indicates that dietary Cr is required for maintenance of normal tissue sensitivity to endogenous insulin.

Table 2. cAMP PDE Activity in Specific Tissues From −Cr and +Cr Rats

Tissue	−Cr (n = 7)	+Cr (n = 7)	Difference (%)	P*
Spleen	116 ± 20	151 ± 10	30	<.001
Testis	64 ± 9	86 ± 12	25	<.01
Brain	118 ± 10	108 ± 13	—	NS

NOTE. PDE activity is cAMP-hydrolyzed (pcmol/min/mg protein).
*Level of significance for differences between −Cr and +Cr rats determined using Student's *t* test.

In accordance with the findings shown here in rats, studies in human subjects demonstrate normalization of insulin responses to oral glucose as a result of supplementation with either Cr-rich brewer's yeast or $CrCl_3$.[37-42] With the exception of one study in siblings of insulin-dependent diabetics[42] reporting restorative effects on insulin comparable in magnitude to the Cr effect in rats shown here (45-minute peak insulin response to oral glucose ~25% lower), the modulatory effects of Cr on insulin concentrations in humans have been relatively small. All of the studies demonstrating normalization of insulin responses to glucose[37-42] suggest that dietary Cr may reverse compensatory changes in pancreatic β-cell sensitivity to glucose by increasing peripheral tissue insulin sensitivity and thus decreasing insulin requirements.[16] However, it should be noted that in accordance with the existence of a direct correspondence between glucose tolerance and pancreatic insulin-secretory responsiveness to glucose are studies of some investigators indicating that β-cell sensitivity to glucose may be enhanced by Cr supplementation.[43] In accordance with this, increased glucose utilization is the result of elevation of circulating insulin levels. In relation to this, we have observed enhanced first-phase insulin secretion in perfused pancreas from +Cr rats fed diets high (72%) in sucrose.[44]

In the studies described here, mild impairments of glucose tolerance and insulin resistance were present in unsupplemented −Cr animals after 24 weeks on the experimental diet. The high dietary sucrose content also contributed to the pronounced β-cell hyperactivity observed in this group of rats. In accordance with this are in vitro studies demonstrating insulin-secretory hyperresponsiveness in perfused pancreas from sucrose-fed rats.[45,46] The presence of a late insulin response observed in both groups of animals fed the same sucrose-containing diet similarly concurs with the in vitro pancreas-perfusion data of these investigators demonstrating that significant enhancement of second-phase insulin release occurs in response to sucrose feeding.[45,46] Based on measurement of systemic insulin levels and response, hyperinsulinemia may be the result of increased β-cell secretion of insulin in −Cr animals, with Cr acting to prevent β-cell hyperactivity in +Cr rats. However,

altered hepatic and renal clearance of insulin cannot be ruled out. Enhanced hepatic clearance of insulin in +Cr rats would also result in lower circulating insulin levels. Recent studies demonstrating that Cr picolinate stimulates insulin internalization in cultured muscle cells[47] support the conclusion that the insulin-lowering effect of Cr in +Cr rats may be due in part to enhanced clearance of insulin. In addition, compared with rats fed low-Cr diets containing starch, rats fed low-Cr sucrose diets show significantly greater depletion of tissue levels of Cr.[10] Similar depletion effects of dietary sucrose on the tissue status of other micronutrients have been observed.[48] This is in agreement with findings reported by Flatt et al,[15] who failed to observe Cr-deficiency symptoms in rats fed a starch diet low in Cr. Thus, based on the possibility that mobilization and depletion of bioactive Cr from tissue stores occurs during active bouts of insulin secretion,[16] β-cell hyperresponsiveness in sucrose-fed rats would promote depletion of bioactive forms of Cr. All these observations support the conclusion that depletion of tissue Cr stores and expression of Cr-deficiency effects are significantly enhanced by the presence of sucrose in the experimental diet. However, the effects of dietary sucrose on glucose tolerance are controversial.[31,45,49-53] As noted by others,[53] much of this disagreement may reflect differences in sucrose levels used by various investigators. Studies reported by some investigators indicate that high-carbohydrate and high-sucrose diets result in improved glucose tolerance at lower basal insulin levels and unchanged insulin response.[49,50,53] In such a situation (high sucrose and low fat), it is possible that Cr supplementation has minimal or no beneficial effects on glucose tolerance because peripheral tissue insulin sensitivity is already increased significantly. In agreement with this, we have observed no effects of Cr supplementation on intravenous glucose tolerance in rats fed a low-Cr diet high in carbohydrate content (72% starch), with high K_G values measured, namely 7.04% ± 0.91%/min (n = 7) in +Cr and 8.46% ± 1.13%/min (n = 7) in −Cr rats, indicating the presence of enhanced tissue insulin sensitivity in these animals (J.S. Striffler, M.M. Polansky, and R.A. Anderson, unpublished, January 1992). Similar observations (K_G > 7.0%/min) have been reported by others studying the effects of carbohydrate feeding on glucose tolerance in rats.[53] In general, diets with carbohydrate content greater than 70%[53] improve glucose tolerance by increasing carbohydrate metabolism and enhancing insulin sensitivity in peripheral tissues,[50,52] without increasing insulin responsiveness. This suggests a dissociation between insulin response and insulin sensitivity.

Comparable to exercise training,[53,54] adaptation to low temperature,[55] and feeding a very–high-sucrose diet,[49,50,53] a direct correspondence between insulin responsiveness and glucose tolerance did not exist in rats supplemented with $CrCl_3$. Thus, as compared with −Cr animals, insulin response areas were significantly decreased and glucose areas were significantly smaller, reflecting increased K_G in +Cr rats. In addition, studies in humans[28,56] and in rats[55-57] support the conclusion that a strong association between obesity and elevated basal and stimulated levels of insulin exists. In accordance with studies reported by Richard and LeBlanc,[57] Vallerand et al[55] observed a highly significant and positive correlation between incremental insulin area and body weight gain. In the current studies, −Cr rats had lower body weights and higher insulin responses, and +Cr rats were heavier and had decreased insulin responses. In summary, the absence of a direct correspondence between insulin-secretory response and glucose tolerance or body weight suggests that the two effects of Cr observed here, ie, normalization of insulin responses to intravenous glucose and increased K_G, are relatively independent and may represent distinct physiological actions of this dietary constituent.

In these studies, we observed decreased cAMP-dependent PDE activity in testes and spleen from −Cr rats versus +Cr animals. Assuming that β-cell cAMP PDE activity is also decreased in −Cr rats, high levels of cAMP in the pancreata of these animals could be responsible, in part, for the observed exaggerated insulin release. This hypothesis is based on studies suggesting that glucose-dependent insulin secretion also requires calmodulin.[58,59] Since cAMP PDE activation requires the presence of bound forms of active calmodulin, hypersecretion of insulin could be the result of defective functioning of this protein in B cells of −Cr rats. In support of this hypothesis are in vitro studies demonstrating that Cr^{+3} is bound by calmodulin with high affinity at intracellular levels of this micronutrient.[60] The proposal that the Cr effect on insulin secretion observed here may involve a Cr^{+3}-calmodulin interaction in B cells is supported by pharmacological studies using isolated rat islet preparations.[61]

Regarding the direct effects of Cr on the endocrine pancreas, Hubner et al[14] recorded a significant decrease of the 30-minute insulin response to intraperitoneal glucose administration in gravid female rats given large doses of $CrCl_3$ (1 mg Cr) by stomach tube daily for 7 weeks during and after gestation. This effect of Cr in rats fed a Cr-adequate diet was not associated with any change in glucose tolerance. These findings, suggesting direct involvement of Cr in functioning of the endocrine pancreas, are in general accordance with observations shown in this study in rats, as well as in studies using human subjects supplemented with $CrCl_3$ at nutritional levels.[38-40]

In conclusion, under the experimental conditions specified here, it is clearly demonstrated that dietary Cr is required for maintenance of normal glucose tolerance in the rat. This effect of Cr supplementation includes prevention of insulin-secretory hyperresponsiveness produced in rats fed a high-sucrose diet low in Cr. In addition to preservation of normal peripheral tissue insulin sensitivity, Cr may also have a modulatory role in maintenance of normal β-cell glucose sensitivity. These effects of Cr on glucose tolerance and β-cell functioning are associated with increased cAMP-dependent PDE activity in certain tissues of +Cr animals.

REFERENCES

1. Mooradian AD, Morley JE: Micronutrient status in diabetes mellitus. Am J Clin Nutr 45:877-895, 1987
2. Milder MS, Cook JD, Stray S, et al: Idiopathic hemachromatosis, an interim report. Medicine 59:34-49, 1980
3. Dandona P, Hussain MAM, Varghese Z, et al: Insulin resistance and iron overload. Ann Clin Biochem 20:77-79, 1983
4. Sargent T III, Lim TH, Jenson RL: Reduced chromium retention in patients with hemachromatosis, a possible basis of hemachromatotic diabetes. Metabolism 28:70-79, 1979
5. Hopkins LL Jr, Schwarz K: Chromium (III) binding to serum proteins, specifically siderophilin. Biochim Biophys Acta 90:484-491, 1964
6. Cohen AM, Teitelbaum A, Miller E, et al: Effect of copper on carbohydrate metabolism in rats. Isr J Med Sci 18:840-844, 1982
7. Hassel CA, Marchello JA, Lei KY: Impaired glucose tolerance in copper-deficient rats. J Nutr 113:1081-1083, 1983
8. Fields M, Lewis CG, Lure MD, et al: The severity of copper deficiency can be ameliorated by deferoxamine. Metabolism 40:105-109, 1991
9. Fields M, Lewis CG, Lure MD, et al: Low dietary iron prevents free radical formation and heart pathology of copper-deficient rats fed fructose. Proc Soc Exp Biol Med 202:225-232, 1993
10. Anderson RA: Effects of exercise, physical trauma, and high sugar intake on chromium, copper and zinc metabolism, in Tomita H (ed): Trace Elements in Clinical Medicine. Tokyo, Japan, Springer-Verlag, 1990, pp 185-191
11. Donaldson DL, Lee DM, Smith CC, et al: Glucose tolerance and plasma lipid distributions in rats fed high-sucrose, high-cholesterol, low-chromium diet. Metabolism 34:1086-1093, 1985
12. Davies DM, Holdsworth ES, Sherriff JL: The isolation of glucose tolerance factors from brewer's yeast and their relationship to chromium. Biochem Med 33:297-311, 1985
13. O'Flaherty EJ, McCarthy CP: Alterations of rat adipose tissue metabolism associated with dietary chromium supplementation. J Nutr 108:321-328, 1978
14. Hubner G, Noack K, Zuhlke H, et al: Influence of trivalent chromium on the beta-cell function. Exp Clin Endocrinol 93:293-298, 1989
15. Flatt PR, Juntti-Berggren L, Berggren P-O, et al: Effects of dietary inorganic trivalent chromium (Cr^{3+}) on the development of glucose homeostasis in rats. Diabete Metab 15:93-97, 1989
16. Mertz W: Chromium—An overview, in Shapcott D, Hubert J (eds): Chromium in Nutrition and Metabolism. New York, NY, Elsevier, 1979, pp 1-14
17. Polansky MM, Anderson RA: Metal-free housing units for trace element studies in rats. Lab Anim Med 29:357-359, 1979
18. Mertz W, Roginski EE, Schroeder HA: Some aspects of glucose metabolism of chromium-deficient rats raised in a strictly controlled environment. J Nutr 86:107-112, 1965
19. Anderson RA, Bryden NA, Polansky MM: Dietary chromium intake. Biol Trace Elem Res 32:117-121, 1992
20. American Institute of Nutrition: Report of the AIN Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 177:1340-1348, 1977
21. Striffler JS, Cardell EL, Cardell RR Jr: Effects of glucagon on hepatic glycogen and smooth endoplasmic reticulum. Am J Anat 160:363-379, 1981
22. Albano JDM, Ekins RP, Maritz G, et al: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol (Copenh) 70:487-509, 1972
23. Law J, Fumagalli A, Schein PS, et al: Selective inhibition of cardiac cyclic nucleotide phosphodiesterases by doxorubicin and daunorubicin. Life Sci 36:589-599, 1984
24. Mertz W, Schwarz K: Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. Am J Physiol 196:614-618, 1959
25. Kim S, Pi-Sunyer FX: Beta-cell insulin secretory response to glucose in odd-carbon number fatty acid–enriched rats. Diabetes 23:605-609, 1974
26. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979
27. Seltzer HS, Allen EW, Herron AL Jr, et al: Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 46:323-335, 1967
28. Hallfrisch J, Lazar F, Jorgensen C, et al: Insulin and glucose responses in rats fed sucrose or starch. Am J Clin Nutr 32:787-793, 1979
29. Vrana A, Slabochova Z, Kazdova L, et al: Insulin sensitivity of adipose tissue and serum insulin concentration in rats fed sucrose or starch diets. Nutr Rep Int 3:31-37, 1971
30. Wright DW, Hansen RI, Mondon CE, et al: Sucrose-induced insulin resistance in the rat: Modulation by exercise and diet. Am J Clin Nutr 38:879-883, 1983
31. Gutman RA, Basilico MZ, Bernal A, et al: Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically an isocaloric sucrose-rich diet. Metabolism 36:1013-1020, 1987
32. Hallfrisch J, Cohen L, Reiser S: Effects of feeding rats sucrose in a high fat diet. J Nutr 111:531-536, 1981
33. National Research Council: Nutrient Requirements of Laboratory Animals, vol 10 (ed 2 rev). Washington, DC, National Academy of Sciences, 1972
34. Hill CH: Mineral interrelationships, in Prasad AS (ed): Trace Elements and Human Disease, vol 2. New York, NY, Academic, 1975, pp 281-300
35. Anderson RA: Stress effects on chromium nutrition of humans and farm animals, in Lyons TP, Jacques KA (eds): Biotechnology in the Feed Industry. Proceedings of Alltech's Tenth Annual Symposium. Nottingham, England, Univ Press, 1994, pp 267-274
36. Striffler JS, Polansky MM, Anderson RA: Dietary chromium decreases insulin resistance in rats. Proceedings of the 75th Annual Meeting of the Endocrine Society, Las Vegas, NV, June 15, 1993
37. Offenbacher EG, Pi-Sunyer FX: Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. Diabetes 29:919-925, 1980
38. Uusitupa MIJ, Kumpulainen JT, Voutilainen EV, et al: Effect of inorganic chromium supplementation on glucose tolerance, insulin response, and serum lipids in non–insulin-dependent diabetics. Am J Clin Nutr 38:404-410, 1983
39. Anderson RA, Polansky MM, Bryden NA, et al: Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. Metabolism 36:353-355, 1987
40. Liu VJK, Abernathy RP: Chromium and insulin in young subjects with normal glucose tolerance. Am J Clin Nutr 35:661-667, 1982
41. Riales R, Albrink MJ: Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high density lipoprotein of adult men. Am J Clin Nutr 34:2670-2678, 1981

42. Doisy RJ, Streeten HP, Freiberg JM, et al: Chromium metabolism in man and biochemical effects, in Prasad AS, Oberleas D (eds): Trace Elements in Human Health and Disease, vol 2. New York, NY, Academic, 1976, pp 79-104

43. Potter JF, Levin P, Anderson RA, et al: Glucose metabolism in glucose-intolerant older people during chromium supplementation. Metabolism 34:199-204, 1985

44. Striffler JS, Polansky MM, Anderson RA: Dietary chromium enhances insulin secretion in perfused rat pancreas. J Trace Elem Exp Med 6:75-81, 1993

45. Kergoat M, Bailbe B, Portha B: Effect of high sucrose diet on insulin secretion and insulin action: A study in the normal rat. Diabetologia 30:252-258, 1987

46. Hara SL, Ruhe RC, Curry DL, et al: Dietary sucrose enhances insulin secretion of aging Fischer 344 rats. J Nutr 122:2196-2203, 1992

47. Evans GW, Bowman TD: Chromium picolinate increases membrane fluidity and rate of insulin internalization. J Inorg Biochem 46:243-250, 1992

48. Fields M, Ferretti RJ, Smith JC Jr, et al: Effect of copper deficiency on metabolism and mortality in rats fed sucrose or starch diets. J Nutr 113:1335-1345, 1983

49. Anderson JW, Herman RH, Zakim D: Effect of high glucose and high sucrose diets on glucose tolerance of normal men. Am J Clin Nutr 26:600-607, 1973

50. Brunzell JD, Lerner RL, Hazzard WR, et al: Improved glucose tolerance with high carbohydrate feeding in mild diabetes. N Engl J Med 284:521-524, 1971

51. Reiser S, Bohn E, Hallfrisch J, et al: Serum insulin and glucose in hyperinsulinemic subjects fed three different levels of sucrose. Am J Clin Nutr 34:2348-2358, 1981

52. Kolterman OG, Greenfield M, Reaven GM, et al: Effect of a high-carbohydrate diet on insulin binding to adipocytes and on insulin action in vivo in man. Diabetes 28:731-736, 1979

53. Vallerand AL, Lupien J, Bukowiecki LJ: Synergistic improvement of glucose tolerance by sucrose feeding and exercise. Am J Physiol 250:E607-E614, 1986

54. Kemmer FW, Berger M: Therapy and better quality of life: The dichotomous role of exercise in diabetes mellitus. Diabetes Metab Rev 2:53-68, 1986

55. Vallerand AL, Lupien J, Bukowiecki LJ: Cold exposure reverses the diabetogenic effects of high fat feeding. Diabetes 35:329-334, 1986

56. Pfeiffer EF: Obesity, islet cell function, and diabetes mellitus, in Gretin H, Levine R, Pfeiffer EF, et al (eds): Lipid Metabolism, Obesity and Diabetes Mellitus: Impact Upon Atherosclerosis. Stuttgart, Germany, Thieme, 1974, p 143

57. Richard D, LeBlanc J: Effects of physical training and food restriction on insulin secretion and glucose tolerance in male and female rats. Am J Clin Nutr 33:2588-2594, 1979

58. Henequin JC: Effects of trifluoperazine and pimozide on stimulus-secretion coupling in pancreatic B cells. Biochem J 196:771-780, 1981

59. Niki H, Niki A, Hidaka H: Effects of a new calmodulin inhibitor (W-7) on glucose induced insulin release and biosynthesis. Biomed Res 2:413-417, 1981

60. MacNeil S, Dawson R, Lakey T, et al: Activation of calmodulin by the essential trace element chromium. Cell Calcium 8:207-216, 1987

61. Ghafghazi T, McDaniel ML, Lacy PE: Chromium-induced inhibition on insulin secretion from isolated islets of Langerhans. Diabetologia 18:229-232, 1980