Overproduction of Insulin in the Chromium-Deficient Rat

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The hypothesis that the insulin secretory hyperresponsiveness observed in rats with diet-induced insulin resistance may be a basic characteristic of dietary chromium (Cr) deficiency was evaluated. Two groups of weanling rats were fed ad libitum a purified diet containing 64% sucrose, 20% casein, 5% corn oil, and the recommended levels of vitamins and minerals without added Cr. Cr-deficient (−Cr) rats were provided with distilled drinking water only, while Cr-supplemented (+Cr) rats received water containing 5 ppm Cr as $CrCl_3$. A third group of rats fed a commercial chow diet served as sucrose controls. Effects of Cr deficiency were assessed by comparing fasting levels of glucose, insulin, and plasma lipids in blood samples collected biweekly from the −Cr and +Cr groups over a 3-month period. Both groups of rats fed the low-Cr sucrose diet developed a transient hyperinsulinemia and hyperlipidemia relative to the chow-fed control rats. There were significant effects of Cr supplementation on plasma triglycerides during the initial 2 weeks of dietary adaptation. Effects of the low-Cr diet were evaluated after the 12-week period by comparing the insulin response area and glucose clearance during a 40-minute intravenous glucose tolerance test (IVGTT). The rates of glucose clearance (K_G) in −Cr and +Cr rats were similar (4.2 ± 1.0 and 4.3 ± 0.8 %/min, respectively) and were comparable to the K_G in chow-fed rats (4.6 ± 0.8). In contrast, insulin secretory responses in −Cr rats were exaggerated (area, 14,083 ± 3,399 μU/mL · min), being twofold greater ($P < .05$) relative to the +Cr group (6,183 ± 864). The insulin secretory response area in chow-fed rats (7,081 ± 408 μU/mL · min) was similar to the value in the +Cr group. These observations provide support for the hypothesis that Cr deficiency can lead to elevated insulin secretory responses to glucose.

TYPE 2 diabetes mellitus (type 2 DM) has been described as a metabolic disorder characterized by both insulin resistance and relative insulin deficiency. However, whether the primary defect is a decreased tissue sensitivity to insulin or an abnormal functioning of the pancreas has yet to be determined.[1-3] Although the initial events resulting in altered glucose metabolism probably involve genetic factors, environmental factors also exert a decisive influence on the development of type 2 DM.[4,5] A major environmental factor known to have significant effects on the development and severity of type 2 DM is diet. Animals with a dietary deficiency of chromium (Cr), as well as other micronutrients, have impaired glucose tolerance and abnormal pancreatic β-cell functioning showing similarities to type 2 DM in humans.[6]

Studies in rats indicate that the severity of Cr deficiency is also influenced by the level of other micronutrients present in the diet.[7,8] Clinical studies in humans with either mild or severely impaired glucose tolerance have demonstrated that blood glucose regulation, elevated blood lipid content, and the need for exogenous insulin therapy can be partially or even completely normalized by Cr supplementation.[9-13] In addition to the diabetogenic effects of inadequate dietary intake of Cr, impaired glucose tolerance can also be produced in animals by altering the level of dietary macronutrients, particularly sucrose and fat.[14-19] In addition, the effects of Cr deficiency are more apparent in animals fed low-Cr diets that are high in sucrose.[8,19] Similarly, a large number of studies examining metabolic effects of dietary fat have demonstrated a marked insulin resistance and glucose intolerance in rats fed diets high in saturated fat.[20-25] Similar to the effects of feeding a low-Cr diet containing sucrose, insulin resistance and Cr deficiency are more severe in animals adapted to a low-Cr diet containing a high level of saturated fat.[26]

In this study, a highly significant effect of Cr deficiency on insulin secretion in vivo was demonstrated using rats adapted to a diet containing micronutrients at levels recommended by the American Institute of Nutrition (AIN) for maintenance of optimal growth in rodents, termed the AIN-76A diet.[27,28] The mineral content of the diet used in this study differed from the AIN-76A formulation only in that no Cr was added. Dietary Cr effects were assessed by comparing glucose and insulin responses to intravenous administration of glucose in rats fed the basal low-Cr sucrose diet versus rats fed the same diet and supplemented with Cr as $CrCl_3$ added to the drinking water.[8,26] In addition, changes in plasma levels of glucose, insulin, and lipids were also monitored during the 3-month period of adaptation to the low-Cr diet prior to performing glucose tolerance tests. Comparisons to Cr-adequate rats fed a commercial rat diet are also provided. The observations recorded here and previously[8,26] provide additional support for the conclusion that Cr is an important dietary nutrient required for maintenance of normal glucose tolerance in the rat.[7,29]

MATERIALS AND METHODS

Animals

Weanling male Wistar rats (Charles River Laboratories, Wilmington, MA) were maintained for 12 to 14 weeks prior to study on a basal low-Cr diet prepared at the US Department of Agriculture (USDA) Beltsville facility. The animals were housed on a 12-hour light-dark cycle with constant ambient temperature (25°C) and environmental conditions that minimize external Cr contamination from the air, dust, cages, etc.[30] At the time of weaning, each animal was randomly assigned to either of two groups. Cr-deficient (−Cr) or Cr-supplemented (+Cr). Both groups of rats were adapted to the same basal

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Submitted December 16, 1998; accepted February 3, 1999.

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0026-0495/99/4808-0023$10.00/0

low-Cr diet, which was fed ad libitum. +Cr rats were provided with 5 ppm Cr as $CrCl_3$ in the drinking water, while the −Cr group received purified drinking water only. Responses in stock-fed animals were also assessed for comparison using a third group of rats maintained under the same housing conditions and provided with regular rat chow and purified drinking water ad libitum.

Diet

The macronutrient content of the basal diet was modified from the AIN-76A diet in that sucrose content was 64% and no starch was added. Other macronutrients were as recommended and included corn oil (5%) as dietary fat, casein (20%) as dietary protein, and cellulose (5%) as nonnutritive fiber (AIN-76A). The diet contained 0.2% choline and 0.3% methionine, and recommended levels of vitamins were added. The AIN-recommended levels of minerals were present, except that no Cr was added. The study protocol and animal care were approved by the USDA, Beltsville Area Research Animal Care Committee.

Experimental Procedures

Longitudinal studies in the basal state. Longitudinal studies were performed using 12 rats per group. Plasma insulin, glucose, triglyceride, and cholesterol levels were measured as indicators of the dietary effects on insulin resistance. Fasting blood samples were drawn by heart puncture under pentobarbital anesthesia (40 mg/kg body weight) at biweekly intervals using six animals from each group so that individual animals were bled once per month. Using this approach, six blood samples were obtained at 2, 4, 6, 8, and 12 weeks after the low-Cr diet was initiated.

Insulin and glucose responses during an intravenous glucose tolerance test. The effects of Cr deficiency and supplementation on in vivo insulin responsiveness of the pancreas and tissue sensitivity to endogenous insulin during an IVGTT were assessed in six animals from each group after 12 weeks on the basal diet using the following protocol. Studies were performed in overnight-fasted rats anesthetized with pentobarbital (40 mg/kg intraperitoneally). 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. Ten units of heparin 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.[31] A baseline blood sample 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. 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 Chemical, St Louis, MO) and 5.6 mmol/L glucose. All animals were kept warm during the procedure by an ordinary incandescent lamp. Most animals were in a semi-awake state, and all showed normal skin coloration around the nose and mouth. Immediately after collection of the 60-minute blood sample, the animals were euthanized with pentobarbital. Tolerance tests were performed on two animals (one from each group) each day, and the final body weight (mean ± SD) was 437 ± 42 g (+Cr, n = 6) and 456 ± 32 g (−Cr, n = 5). The body weight of the chow-fed control group was 424 ± 53 g (n = 6).

Assay Procedures for Plasma Glucose, Triglyceride, Cholesterol, and Insulin

Following collection, blood samples were transferred to polypropylene tubes containing EDTA (12 mg/mL) and stored on ice. Plasma samples were harvested by centrifugation and stored frozen until assay. Glucose, triglyceride, and cholesterol concentrations were measured in a Centrifichem 600 apparatus (Baker Instruments, Allentown, PA) using enzymatic methods. Insulin concentrations were measured using the radioimmunoassay procedure of Albano et al.[32] Samples were assayed in triplicate against 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).

Data Analysis

Results are expressed as the mean ± SEM. Cr deficiency and dietary sucrose effects in the basal state (Table 1) were compared among groups using a one-way ANOVA. Differences between groups were tested using Dunnet's test, and were considered significant at a P value of less than .05. The glucose clearance rate (K_G) was obtained from semilogarithmic plots of the plasma glucose concentration (corrected for basal) using four time points (post–glucose administration) and computer-generated regression lines. The areas under the response patterns recorded between 0 and 40 minutes (post-glucose) for insulin and glucose (Table 2) were calculated after subtraction of the baseline concentration. Differences between −Cr and +Cr groups were determined using Student's t test.

RESULTS

Both groups of rats fed the low-Cr sucrose diet (modified AIN-76A diet) showed an essentially normal growth rate and body weight gain over the 12-week period of study. Cr supplementation did not alter these parameters. Growth patterns in the two groups of rats fed the low-Cr modified AIN-76A diet were similar to those in chow-fed rats. Changes in plasma insulin, glucose, and lipid levels during the 12-week period of adaptation to the low-Cr modified AIN-76A and chow diets are listed in Table 1. Fasting plasma insulin tended to increase in all three groups during the period of rapid growth, ie, the initial 8

Table 1. Longitudinal Changes in Fasting Plasma Insulin (pmol/L), Glucose (mmol/L), and Lipids (mmol/L) in −Cr, +Cr, and Chow-Fed Rats

	Weeks on Diet				
Parameter	1-2	3-4	5-6	8-9	11-12
−Cr					
Ins	65 ± 22	65 ± 14	172 ± 50	187 ± 14[a]	93 ± 22
Gluc	4.9 ± 0.2	5.3 ± 0.4[b]	5.3 ± 0.3	5.8 ± 0.3[c]	6.4 ± 0.2[b]
Chol	2.2 ± 0.2[a]	2.0 ± 0.3	IS	2.4 ± 0.2	1.8 ± 0.2
Trig	1.9 ± 0.3[a]	0.7 ± 0.1[a]	IS	1.1 ± 0.2	0.7 ± 0.1
+Cr					
Ins	57 ± 22	43 ± 7	179 ± 29	194 ± 36[a]	129 ± 22
Gluc	4.7 ± 0.2	5.0 ± 0.2[bc]	6.1 ± 0.2	6.3 ± 0.2[b]	7.6 ± 0.2[a]
Chol	2.3 ± 0.5[a]	1.8 ± 0 1	2.2 ± 0.2	2.1 ± 0.2	2.0 ± 0.1
Trig	1.1 ± 0.2[b]	0.7 ± 0.1[a]	0.8 ± 0.1	1.1 ± 0.11	0.9 ± 0.1
Chow-fed					
Ins	36 ± 7	57 ± 7	108 ± 22	86 ± 14[b]	129 ± 21
Gluc	4.9 ± 0.2	6.1 ± 0.2[a]	6.1 ± 0.3	6.9 ± 0.2[a]	7.3 ± 0.1[a]
Chol	1.6 ± 0.1[b]	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	IS
Trig	0.5 ± 0.1[c]	0.4 ± 0.1[b]	0.6 ± 0.1	0.7 ± 0.2	IS

NOTE. Blood samples were collected by heart puncture in rats (12 per group) under pentobarbital anesthesia. Biweekly sampling was performed on 6 animals from each group on an alternating basis, with each animal sampled at monthly intervals. The same variables in each column with different superscripts are significantly different ($P < .05$).

Abbreviations: Ins, insulin; Gluc, glucose; Chol, cholesterol; Trig, triglyceride; IS, insufficient sample.

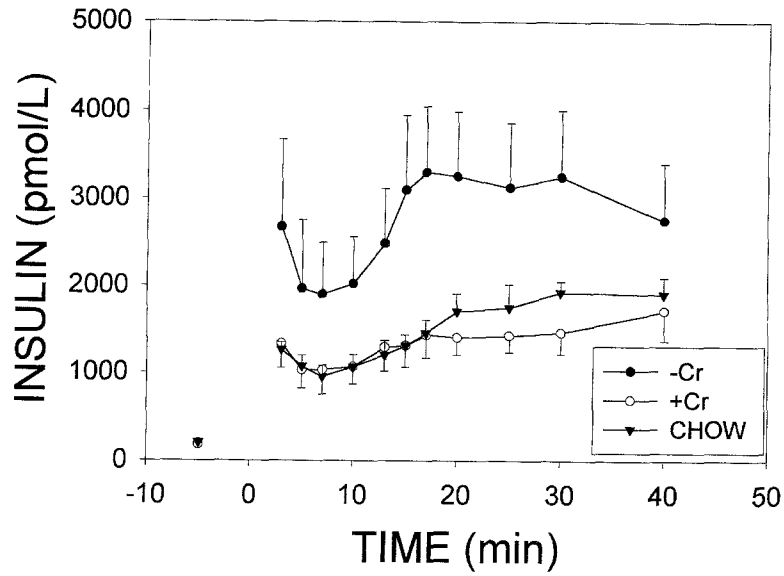

Fig 1. Plasma insulin response during an IVGTT in −Cr and +Cr rats fed a low-Cr sucrose diet and in chow-fed rats.

weeks of dietary adaptation, when the animals were 12 weeks old. Insulin levels were twofold higher in rats fed the low-Cr diet relative to the stock-fed animals at 6 and 8 weeks, and this difference was significant at 8 weeks. Insulin levels were similar in all three groups at 12 weeks. There were no effects of Cr supplementation in +Cr rats compared with the −Cr group. Fasting plasma glucose also increased in all three groups, with the increase in +Cr rats tending to parallel that of the chow-fed animals. After 12 weeks on the diet, −Cr animals had significantly lower fasting plasma glucose levels relative to +Cr rats. Fasting plasma lipids were significantly higher in the +Cr and −Cr groups fed the modified AIN-76A diet compared with the chow-fed rats at 2 and 4 weeks, although the differences were not significant at 8 weeks. After 2 weeks on the low-Cr modified AIN-76A diet, plasma triglyceride levels were significantly lower in +Cr versus −Cr rats, which were markedly hypertriglyceridemic. This beneficial effect of Cr supplementation was not observed at subsequent time points. In contrast to the lack of effect of Cr deficiency on the basal (fasting) insulin concentration, insulin responses to intravenous glucose injection were increased more than twofold in −Cr animals compared with the +Cr group (Fig 1). Note that the insulin levels attained in +Cr rats were comparable to the response levels measured in chow-fed rats.

The effects of Cr supplementation on glucose utilization in rats fed the low-Cr modified AIN-76A diet are compared in Fig 2. The glucose clearance rates (K_G) represented by the slopes of the lines in Fig 2 show complete overlap, indicating the lack of any Cr effects in rats fed the low-Cr diet. Glucose clearance rates in the two groups of animals fed the low-Cr diet were nearly identical to the rates measured in the chow-fed animals. Table 2 compares insulin and glucose response areas during the 40-minute glucose tolerance test in −Cr and +Cr rats fed the low-Cr modified AIN-76A diet and chow-fed rats. The insulin hyperresponsiveness measured in −Cr rats (Table 2) is clearly evident, with the decrease due to Cr deficiency being greater

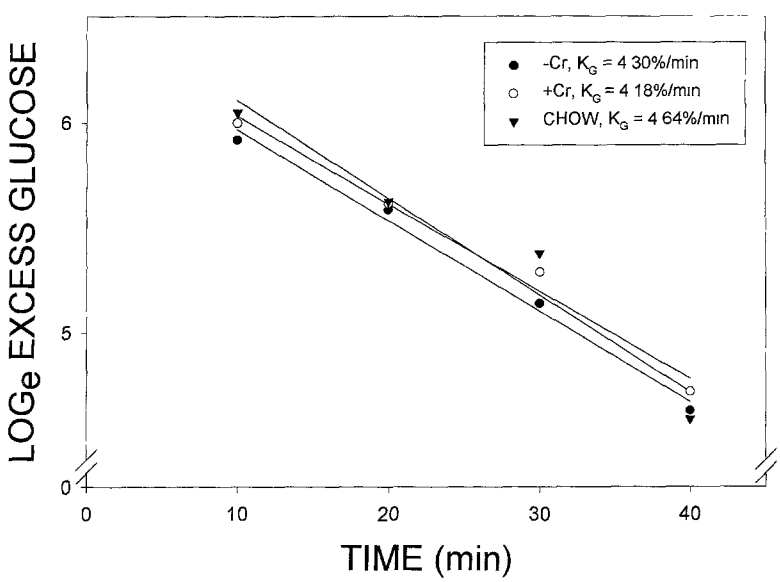

Fig 2. Glucose clearance (K_G) during an IVGTT in −Cr and +Cr rats fed a low-Cr sucrose diet and in chow-fed rats. Excess glucose represents glucose levels corrected for basal glucose concentrations.

Table 2. IVGTT Plasma Insulin and Glucose Responses in −Cr and +Cr Rats Fed a 63% Sucrose Diet Low in Cr (modified AIN-76A diet)

Response	Chow-fed (n = 6)	−Cr (n = 6)	+Cr (n = 5)	P*
Insulin area				
(μU/mL · min)†	7,081 ± 408	14,083 ± 3,399	6,183 ± 864	<.05
Glucose area				
(mg/dL · min)†	12,179 ± 619	11,042 ± 790	11,492 ± 812	NS
K_G (%/min)	4.64 ± 0.79	4.30 ± 0.78	4 18 ± 1.0	NS

*Level of significance for +Cr and −Cr groups using Student's *t* test.
†40-minute area above basal.

than 50%. These observations are similar to previous observations in −Cr rats fed a low-Cr diet containing high levels of iron and ultratrace nutrients.[8] There were no Cr supplementation effects on glucose utilization in animals fed the low-Cr diet. Our previous observations demonstrated enhanced glucose clearance (utilization) in +Cr rats fed low-Cr diets high in saturated fat[26] or containing high levels of iron and ultratrace nutrients.[8]

DISCUSSION

In previous studies examining the effects of Cr deficiency in rats, the interactive influence of other dietary micronutrients[8] and macronutrients[26] was used to enhance the development of Cr deficiency in rats fed a low-Cr diet. It should be noted that the mineral contents of the low-Cr torula yeast/sucrose diets used in the early studies[33-35] and in more recent studies[36-40] on Cr deficiency were not completely defined and differed considerably from diets prepared using purified materials or commercial rat chow. Diets used in the early studies resulted in undefined dietary stresses.

After 3 months on the low-Cr sucrose diet containing the recommended levels of micronutrients, plasma lipid levels were comparable in all three groups of rats. However, prior to 3 months, the lipogenic effects of sucrose were apparent in rat groups fed the sucrose diet compared with those fed chow (Table 1). Animals fed sucrose-rich (63%) diets containing 5% corn oil and no animal fat, similar to the diet used here, have been reported to show multiphasic changes in plasma lipid levels and intravenous glucose tolerance.[41] According to these investigators,[18,41] plasma and tissue triglyceride levels are significantly elevated if rats are maintained long-term on the sucrose-rich diet for more than 3 months, although fasting (basal) insulin levels are normal. In contrast, studies by Reaven et al[20] using sucrose (or fructose) diets containing animal fat (12% lard) demonstrated significant fasting hyperinsulinemia and an elevation of plasma lipid levels in young rats fed a sucrose-lard diet for 1 month. Similar observations were reported in studies comparing the use of starch versus sucrose (54% carbohydrate) in the metabolic response to intraperitoneal glucose in rats fed diets containing high levels of saturated fat (12%), as well as corn oil (4%), for 2 months prior to study.[14] In addition to the transient hypertriglyceridemia, the sucrose-fed rats used in our study tended to be hyperinsulinemic during the first 2 months of dietary adaptation. In accordance with our previous observations,[8] this relative fasting hyperinsulinemia was not present after 3 months on the sucrose diet (Table 1). Significant Cr effects on plasma triglycerides were transient, and no effects of supplementation on fasting insulin levels were observed. Comparable observations indicating no Cr supplementation effects on plasma insulin were recorded in studies using squirrel monkeys fed commercial monkey chow, which also contains no added animal fat.[42] In contrast, in our previous study using rats fed a 40% fat (lard) low-Cr diet for 4 months, fasting hyperinsulinemia and an enhanced insulin response to glucose were present and glucose clearance was severely depressed, with these indices of insulin resistance being improved in +Cr rats.[26] In addition, beneficial effects of Cr supplementation on plasma triglyceride levels were also observed in high-fat–fed rats. These observations underscore this apparent dietary requirement for saturated fat in producing rats with more severe insulin resistance in studies on Cr deficiency and supplementation effects. In light of the lipogenic effects of sucrose shown here (Table 1) and by others,[14-16,18,41] as well as the apparent need to include saturated fat in the diet for the production of Cr deficiency in rats,[26] it is expected that no deficiency effects on fasting plasma glucose, lipids, and other metabolic variables are observable in rats fed a low-Cr starch diet for only 1 month.[43] In accordance with our observations in rats[26] are clinical studies reporting that the lipid-lowering effects of Cr supplementation are greater in subjects with elevated plasma lipids compared with subjects with lower blood lipids.[44]

Hypersecretion of insulin has been directly measured in the perfused pancreas of rats fed diets high in sucrose, which may, in part, contribute secondarily to insulin resistance in these animals.[45,46] Using a 63% sucrose diet similar to the diet used here, Lombardo et al[41] observed an enhanced insulin response to intravenous glucose in animals fed the diet for 45 days. However, in subsequent longitudinal studies by these same investigators,[18] insulin responses were normalized and comparable to the responses measured in chow-fed rats if the animals were maintained on the diet for 3 months or longer. These observations are in accordance with the data shown here indicating that insulin responses in +Cr rats fed the low-Cr sucrose diet are similar to the responses in chow-fed rats. The observations reported here indicate that fasting insulin levels are normal and similar in both +Cr and −Cr groups and are comparable to findings from previous studies using rats fed a low-Cr sucrose diet (55%) for 6 months.[8] Insulin responses to intravenous glucose were significantly elevated in −Cr rats used here, with levels of the hormone being more than 50% lower in the +Cr animals. Thus, as shown here and previously,[8] there was no direct correspondence between the basal fasting insulin level and insulin responsiveness to glucose or the effects of Cr on these variables. This is in contrast to obesity, in which a direct association between basal insulin and the insulin response to glucose has been demonstrated.[47,48] Basal insulin levels have been reported to be elevated in patients with type 2 DM, although some investigators have not observed this.[49,50] The relationship between basal insulin and the insulin response to glucose in type 2 DM has not been clearly established.

Recent clinical studies assessing the effect of supplementation with Cr picolinate in patients with type 2 DM have demonstrated a significant decrease of both fasting insulin and the 2-hour insulin response to an oral glucose load.[11] These effects have also been observed in subjects with normal glucose

tolerance and insulin resistance.[44] Cr losses are also greater in humans consuming diets high is sucrose and other simple sugars compared with diets high in complex carbohydrates, and may explain the larger effects of Cr observed using high-sucrose diets.[51] Cr losses are related to circulating insulin levels, and increases in insulin are greatest due to the intake of sucrose and combinations of simple sugars plus fructose.[52]

The presence of a marked post-glucose hyperinsulinemia in association with impaired glucose tolerance[8] or, as seen here, normal glucose tolerance indicates the presence of decreased peripheral tissue sensitivity to insulin in the −Cr state. In addition, the smaller insulin response area in the +Cr group was similar in magnitude to the area measured in the chow-fed rats (Table 2), indicating that preservation of normal β-cell sensitivity to glucose may be a significant function of dietary Cr, as previously proposed.[8] The insulinogenic index expressed as the ratio of the incremental insulin area to the associated incremental glucose area provides a semiquantitative estimate of β-cell responsiveness to glucose.[53] A lower ratio in the +Cr group (0.559) relative to the −Cr group (1.420) supports the conclusion that normal insulin responsiveness to glucose was maintained in the supplemented rats. In these studies using rats fed a sucrose diet containing optimal levels of micronutrients,[27,28] the extra insulin secreted by the pancreas in −Cr animals was able to compensate for peripheral insulin resistance. As a result, −Cr rats had normal glucose tolerance and cleared the injected glucose as efficiently as +Cr and chow-fed animals. In contrast, animals fed a low-Cr sucrose (55%) diet high in iron have lower rates of glucose clearance than supplemented rats fed the same diet.[8] Despite an exaggerated insulin response comparable in magnitude to the response shown here, only partial compensation was observed, suggesting that Cr deficiency in the high-iron/sucrose–fed rats was more severe. It remains to be shown whether this large effect of Cr deficiency on the post-glucose insulin response is entirely or only partly the result of β-cell oversecretion of insulin, or whether decreased hepatic removal of insulin also occurs in −Cr animals. This is based on the well-documented "single-pass effect" of the liver on circulating insulin levels,[54] as well as the variable nature of the hepatic insulin removal process.[55] Assuming that hepatic insulin sensitivity is also influenced by Cr, the amply demonstrated direct relationship between insulin sensitivity of the liver and hepatic clearance of insulin[56] suggests that hepatic removal of insulin may be enhanced in +Cr rats and thus contribute to the lower insulin levels in +Cr animals.

In summary, we have demonstrated a Cr-deficiency effect in a non–diet-stressed rat model. Animals with this mild form of Cr deficiency have normal glucose tolerance in which the peripheral effect of Cr deficiency is compensated by oversecretion of insulin. The insulin hyperresponsiveness in −Cr rats is likely the result of increased β-cell sensitivity to glucose.

ACKNOWLEDGMENT

Mention of trade name, proprietary product, or vendor does not constitute a warranty of the product by the US Department of Agriculture or imply its approval to the exclusion of other products or vendors that may also be suitable.

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