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Anti-Hyperglycemic Activity of Zinc Plus Cyclo (His-Pro) in Genetically Diabetic Goto-Kakizaki and Aged Rats

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We previously reported that treatment of streptozotocin-induced diabetic rats with zinc plus cyclo (his-pro) (CHP) decreased fed blood glucose levels and water intake. The present study was conducted to examine the dose-dependent, acute, and chronic treatment effects of CHP on oral glucose tolerance (OGT), fed blood glucose levels, water intake, and plasma insulin levels in young and aged Sprague-Dawley (S-D) rats, nondiabetic Wistar rats, and genetically diabetic Goto-Kakizaki (G-K) rats. Acute gastric gavage of 10 mg zinc plus 1.0 mg CHP/kg body weight significantly improved OGT in 4- and 13-month-old nondiabetic S-D rats and in 2-month-old diabetic G-K rats. Young S-D and G-K rats returned to pretreatment OGT values 1 week after acute gavage of zinc plus CHP (ZC), but improved OGT values persisted for at least 1 week after gavage in aged S-D rats. OGT values and fed blood glucose decreased to the greatest extent among other treatments when G-K rats were given free access to drinking water containing 1.0 to 1.5 mg CHP/L plus 10 mg zinc/L for 2 weeks. Although food and water intake showed a tendency to decrease, no statistically significant differences were observed in young G-K rats. Plasma insulin levels and blood glucose levels in both normal and diabetic G-K rats decreased with 2-week treatment with ZC. To test the direct effects of ZC on muscle tissue, we observed the effect of various doses of ZC on normal and G-K rat muscle slices. The optimal level of CHP alone for maximal muscle glucose uptake in muscle slices from normal rats was 10 µg/mL and 5.0 µg/mL in G-K rats, and ZC stimulated glucose uptake. However, no statistically significant difference was demonstrated between normal and G-K rat tissues in this study. These results indicate that oral intake of an optimal dose of ZC stimulates blood glucose metabolism, probably by stimulating muscle glucose utilization. Exp Biol Med 228:1338–1345, 2003

Key words: cyclo (his-pro); zinc; Goto-Kakizaki rats; diabetes mellitus; glucose uptake; aged rats

The present study was designed to determine the effects of zinc plus cyclo (his-pro) (CHP) on the improvement of glucose metabolism in Type II diabetic, Goto-Kakizaki (G-K) rats and in insulin-resistant, aged, Sprague-Dawley (S-D) rats. In previous studies, we demonstrated that treatment of streptozotocin-induced diabetic rats with zinc plus CHP (ZC) significantly improved indices of diabetes, such as hyperglycemia (1, 2). This diabetic rat model mimics human Type I diabetes mellitus (IDDM), a genetic disorder of pancreatic β-cell destruction evoked by certain environmental factors. Although ZC may affect insulin secretion, it appears that the effect of ZC on the improvement of blood glucose metabolism is likely due to improved insulin sensitivity for glucose utilization in muscle and adipose cells, as well as possible diminished glucose output in liver cells.

More than 90% of diabetic patients are afflicted with Type II diabetes (NIDDM). A hallmark of this disease is insulin resistance, whereby the primary insulin target organs—adipose, muscle, and liver tissues—are poorly responsive to insulin action (3). It is therefore necessary to determine whether ZC improves blood glucose metabolism by stimulating insulin sensitivity or insulin secretion in a Type II diabetic animal model. The strain we chose is the G-K rat, which are nonobese and nonketotic and exhibit mild fasting hyperglycemia. The G-K rat was first generated by Drs. Goto and Kakizaki in Japan by inbreeding Wistar rats (4). Since the G-K rat is a nonobese, mildly diabetic animal model that shows no co-segregation of hyperglycemia and obesity with insulin resistance (5), it was the best animal model for our study. Hence, we studied the effects of ZC treatment on glucose metabolism in these diabetic G-K rats.

Cyclo (his-pro) is a metabolite of thyrotrophin-releasing hormone (TRH), but is also produced directly from amino acid or peptide sources (6). Metabolism of this peptide may be related to diabetes, since CHP levels in both animal and human diabetic subjects are significantly lower than in nondiabetic subjects (7, 8). The fact that pancreatic TRH levels decrease after streptozotocin injections further supports this hypothesis (9). Although infusion of TRH increases insulin secretion in chickens (10), TRH levels are not affected by hyperglycemia (11) and do not directly stimulate glucose utilization by muscle or adipocytes in humans (12). Elevated blood glucose levels induced by oral or intravenous glucose infusion significantly increased peripheral CHP concentrations (13), in contrast with TRH levels, and orally administered glucose is more effective than intravenous infusion in increasing peripheral CHP concentrations (14). In another study (15), CHP levels in the circulatory system and tissue such as brain striatum also increased after induction of hyperglycemia by streptozotocin injection, and were reversed when insulin was administered to lower glucose levels. These phenomena suggest that blood glucose metabolism is directly related to CHP concentrations in the gut (16), and that CHP metabolism is directly related to glucose metabolism.

The possible biological effects of CHP on diabetes can be explained by the fact that CHP stimulates intestinal zinc absorption and muscle glucose uptake (17), and that zinc is involved in glucose uptake and utilization in insulin-requiring cells (18). These data support the possibility that CHP is directly or indirectly involved in blood glucose control mechanisms. More interestingly, CHP concentrations in the brains of these obese mice are also elevated compared with levels in lean mice (19). Obese, aged rats express leptin resistance accompanied by insulin resistance (20). These facts suggest that ZC treatment may also affect leptin-mediated signal transduction mechanisms in the hypothalamus of obese animals. Although Mizuma *et al.* (21) suggest that CHP actually increases insulin availability by decreasing the hepatic insulin clearance rate, it appears that ZC is involved in stimulating muscle glucose uptake (22, 23) rather than reactivating defective β-cells or decreasing hepatic insulin clearance rate. Present studies demonstrate that ZC regulates blood glucose metabolism by stimulating glucose uptake in muscle cells.

Research Design and Methods

Materials. Zinc and L-histidine were purchased from Sigma Chemical Co. (St. Louis, MO). CHP was obtained from Dr. Ke Won Kang, Hans Biomed Corp., Seoul, Korea. A rat-specific insulin assay kit was purchased from American Laboratory Products Co. (Windham, NH). Accu-Chek Glucometer and strips were purchased from Roche Diagnostics Corp. (Indianapolis, IN), and 1,2-^{3}H-2-deoxy-D-glucose and 1-^{14}C-D-mannitol were purchased from Perkin-Elmer Life Sciences (Boston, MA).

Animals. Four- and 13-month-old male Sprague-Dawley (S-D) rats, and 2-month-old Wistar rats were purchased from Charles River Laboratories, Indianapolis, IN. Five 1-month-old male and five age-matched female stock of Goto-Kakizaki rats were purchased from the University of South Florida, Comparative Medicine Department (Dr. Robert V. Farese), and the colony was expanded at the Animal Facility of the VA Greater Los Angeles Healthcare System, Los Angeles, CA. G-K rats were used for experiments at 6 to 8 weeks of age. These studies were conducted with the approval of the Institutional Animal Care and Use Committee of the VA Greater Los Angeles Healthcare System.

Methods. *Measurement of blood glucose and insulin levels.* Glucose in whole blood was measured by Accu-Chek Glucometer, using one drop of blood obtained from a cut on the underside of the rat's tail. Plasma insulin levels were determined at the end of each experiment, using a rat-specific ELISA kit per manufacturer instructions.

Measurement of acute effects of cyclo (his-pro) on oral glucose tolerance. Twelve 4-month-old and twelve 13-month-old S-D rats were fasted overnight to lower blood glucose levels and deplete liver glycogen stores, and ten 3-month-old normal and ten age-matched male G-K rats were fasted for 4 hrs to lower blood glucose levels but maintain diabetic pathology. Half of the rats in each group received 1.0 mg CHP/kg, 10 mg zinc/kg, and 0.5 mg L-histidine/kg plus 1.0 g of glucose/kg body weight (BW) *via* gastric gavage, while the remaining rats were given 1.0 g glucose/kg BW only. Three-hr average area-above-fasting glucose concentrations (TAFGC; an index of oral glucose tolerance (OGT)) were determined for all rats. TAFGC were measured by determining blood glucose levels every 30 min for 3 hrs, following gastric gavage of 1.0 g glucose/kg BW to rats fasted 4 hrs or overnight. TAFGC for an individual animal was calculated by subtracting the fasting blood glucose level from each value obtained at the 30-min intervals over 3 hrs. The area under the curve of blood glucose levels was then integrated.

Measurement of cyclo (his-pro) dose-dependent effects on TAFGC values in acute treatment of Goto-Kakizaki rats with zinc plus cyclo (his-pro). Two-month-old G-K rats were divided into six groups of either 5 or 10 rats. Before the start of testing, all the rats were deprived of food for 16 hrs, at which time blood glucose concentrations were measured. These values were used as the fasting blood concentration of each rat. Each rat was given 1.0 g/kg BW glucose together with the test solution. The test solution for the first group was distilled water (DW); the second group, DW containing 10 mg zinc/kg BW; the third, 1.0 mg CHP/kg BW; the fourth, 10 mg zinc plus 0.25 mg CHP/kg BW; the fifth, 10 mg zinc plus 0.5 mg CHP/kg BW; and the sixth group, 10 mg zinc plus 1.0 mg CHP/kg BW. Blood concentrations in each rat were measured immediately after gavage of glucose plus test solution, and every 30 min for 3 hrs. The total area above fasting blood glucose concentra-

tion of each rat was integrated and divided by 3 hrs. The values presented are the average of mg glucose/dL above the fasting blood glucose concentrations during the first 3 hrs after gavage of glucose plus test solution.

Measurement of dose-dependent effects on long-term treatment with zinc plus cyclo (his-pro) on fed blood glucose concentrations and water intake. Six-week-old G-K rats were divided into six groups of either 5 or 10 rats. The first group was given drinking water with distilled water (DW) alone; the second group, DW containing 10 mg/L zinc only; the third, 1.0 mg/L CHP only; the fourth, 10 mg/L zinc plus 0.25 mg/L CHP; the fifth, 10 mg/L zinc plus 0.5 mg/L CHP; and the sixth group, 10 mg/L zinc plus 1.0 mg/L CHP. Every other day for 2 weeks, fed blood glucose levels were measured in the morning, and rates of decrease in blood glucose of each rat group were calculated by regression analysis. Average water intake of each group was determined by dividing the total amount of water consumed by total rat weight every 2 to 3 days for 3 to 4 weeks.

Measurement of plasma insulin levels. After 6 weeks of ZC treatment, diabetic G-K rats and young and aged nondiabetic S-D rats were anesthetized by intraperitoneal (i.p.) injection of 100 mg pentobarbital, followed by heparin infusion through the tail vein. Blood samples were collected via heart puncture through a 5.0-mL syringe, transferred into a 15-mL cell culture tube, and immediately centrifuged to separate plasma. Plasma samples from each rat were frozen at −80°C until analysis. Plasma insulin levels were measured by RIA by Dr. Yong Liu at the Center for Human Nutrition, UCLA School of Medicine, Los Angeles, CA.

Measurement of cyclo (his-pro) effects on glucose uptake in muscle tissues. The epitrochlearis muscle was removed from three 4-month-old, nondiabetic Wistar rats, and from three age-matched, diabetic G-K rats after euthanasia by i.p. injection of 100 mg pentobarbital/kg BW. Muscles were finely sliced with a razor blade in Krebs Henseleit Buffer (KHB) solution. [10× KHB stock solution contains 118.5 mM NaCl; 4.7 mM KCl; 3.4 mM $CaCl_2$; 1.2 mM KH_2PO_4; 1.2 M $MgSO_4$; 25 mM $NHCO_3$; 32 mM mannitol; 8 mM glucose; 0.1% w/v BSA.]. Approximately 10 muscle slices of 1 to 3 mm^3 each were incubated in 15-mL polypropylene test tubes containing 2.0 mL Eagle's media with various amounts of CHP (0, 2.0, 5.0, 10.0, or 20.0 μg/mL) plus 15 μM zinc and 1.5 μCi 1, 2-^{3}H-2-deoxy-D-glucose (specific activity = 1.0 μCi/mg) and 0.3 μCi 1-^{14}C-D-mannitol (specific activity = 0.1 μCi/mg). Tubes were incubated at 35°C for 20 min under continuous gassing (95% carbon dioxide and 5% oxygen). We have demonstrated that muscle tissue slices mimic normal tissue activity based on zinc uptake and excretion response to varying CHP and arachidonic acid doses (17). At the end of the incubation period, tissue samples were washed twice with KHB solution, dried overnight in a 90°C oven, weighed, reconstituted in distilled water, and frozen overnight. Each

sample was then homogenized in a Polytron homogenizer and centrifuged for 5 min at 1000 × *g,* after which the supernatant was removed to a 20-mL vial for scintillation counting. The precipitate was washed with 2.0 mL KHB solution and added to the vial containing the initial 2.0 mL supernatant. Liquid scintillation cocktail (15 mL) was added, and samples were counted in a Beckman liquid scintillation counter. Total tissue glucose uptake rate was calculated by subtracting the counts in 1-^{14}C-D-mannitol (nonspecific binding to tissue) from the counts in 1,2-^{3}H-2-deoxy-D-glucose (absorbed and nonspecific binding of glucose).

Statistical analysis. Statistics were performed by one- or two-way analysis of variance (ANOVA) and multiple comparison tests, using GraphPad Prism 3.02 and Instat versions supplied by GraphPad Software Co, San Diego, CA. A *P* value less than 0.05 was considered statistically significant. To determine minimum sample size, we used previous data (24) to estimate the difference between means, a desired power of 0.8, and significance level of $P < 0.05$ for a two-tailed equal variance model, and calculated that a sample size of five or more would suffice to detect differences between the optimal treatment doses and control.

Results

Acute Zinc Plus Cyclo (his-pro) Treatment Improves Oral Glucose Tolerance in Aged and Goto-Kakizaki Rats. To investigate whether glucose disposal rates were increased with ZC treatment, we determined acute changes in TAFGC values in normal and G-K rats treated with ZC. As shown in Figure 1, TAFGC values in both 4-month-old nondiabetic Wistar and diabetic G-K rats were significantly lowered after 1.0 mg/kg CHP plus 10.0 mg/kg zinc administration. For 4-month-old nondiabetic Wistar rats, the average TAFGC value for ZC-treated rats was 28.5 ± 8.9 mg/dL vs 10.4 ± 5.4 mg/dL for DW-treated
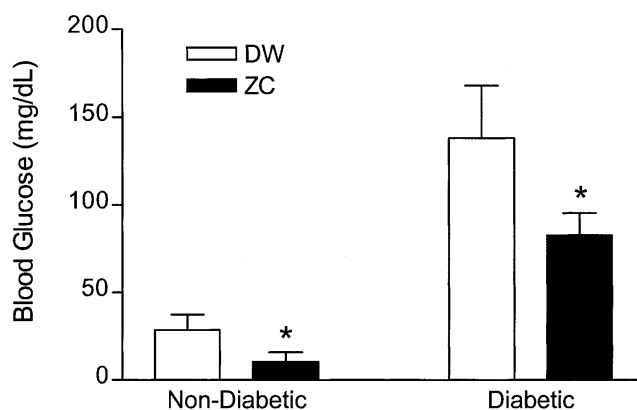

Figure 1. Acute effects of ZC on OGT (TAFGC) values in nondiabetic Wistar and diabetic G-K rats after gastric gavage of glucose. Rats were fasted for 4 hrs, and gastric gavage of 1.0 g glucose with 10 mg zinc plus 1.0 mg CHP (ZC)/kg BW or without (DW) was administered. Blood glucose levels were measured every 30 min for 3 hours (n = 5–7 for each treatment group). Three-hour average area-above-fasting glucose concentrations (TAFGC) of ZC-treated rats were significantly different from the values of untreated rats in each group (*P < 0.05, as evaluated by two-way ANOVA).

rats ($P < 0.05$). TAFGC values in G-K rats treated with ZC were also significantly lower than those treated with DW (138.0 ± 29.9 mg/dL vs 82.6 ± 12.7 mg/dL; $P < 0.05$). In a similar study using six groups of 5 to 10 G-K rats, we determined acute CHP dose dependency of TAFGC values (Fig. 2). The most effective dose of CHP was 1.0 mg CHP/kg BW; the mean TAFGC of this treatment group was significantly different ($P < 0.01$) from that of the DW-treated rats.

The 1.0 mg CHP/kg BW dose also improved OGT in 4- and 13-month-old nondiabetic S-D rats (Fig. 3). Mean TAFGC values were significantly different between ZC-treated and control rats in both rat groups, as determined by two-way ANOVA ($P < 0.05$). Interestingly, 1 week after acute CHP treatment, the improved TAFGC values in the 13-month-old rats were sustained or further decreased, while TAFGC in the 4-month-old rats returned to pretreatment levels, as indicated in Figure 4. The difference in TAFGC values between treated and untreated aged rats was highly significant, as evaluated by two-way ANOVA ($P < 0.01$). However, plasma insulin levels tended to decrease with ZC treatment, though the difference was marginally significant ($P < 0.1$ by t test) only in 4-month-old rats (Table I). However, the overall effect of ZC treatment on plasma insulin levels was decreased when evaluated by two-way ANOVA ($P < 0.05$).

Long-Term Zinc Plus Cyclo (his-pro) Treatment Decreases Blood Glucose and Water Intake in Goto-Kakizaki Rats. If ZC improves OGT in an acute test, it is expected that ZC should lower fed blood glucose levels with long-term treatment. To test this hypothesis, 6-week-old G-K rats were divided into six groups of 5 to 10 rats and treated with various doses of zinc and/or CHP. As depicted in Figure 5, ZC treatment decreased fed blood
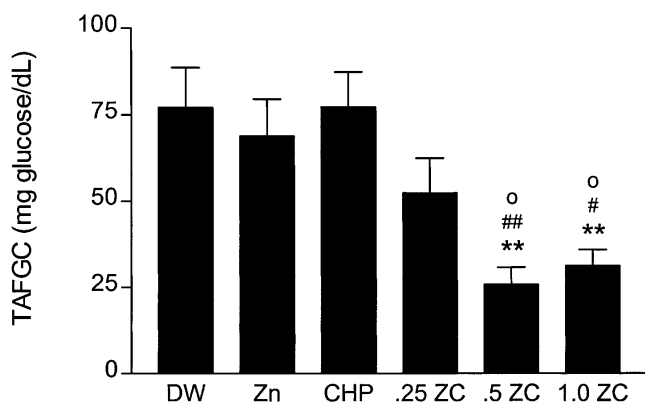

Figure 3. Acute effects of ZC on OGT in 4- and 13-month-old Sprague-Dawley rats after glucose gastric gavage. Blood glucose levels were measured every 30 min for 3 hrs after gastric gavage with 1.0 g glucose plus DW or 1.0 mg CHP/kg BW and 10 mg zinc/kg BW (ZC) ($n = 6$ for each treatment group). TAFGC in both 4- and 13-month-old Sprague-Dawley rats were significantly different between treated and untreated rats as evaluated by two-way ANOVA ($P < 0.05$).

glucose levels in G-K rats after 2 weeks of treatment. CHP doses of 0.5 and 1.0 mg/L plus 10 mg/L zinc led to a maximal rate of decrease in blood glucose ($P < 0.05$, as determined by one-way ANOVA and Dunnett's multiple comparison test). Similar to fed blood glucose levels, plasma insulin concentrations also significantly decreased with administration of 1.0 mg/L CHP plus 10 mg/L zinc, compared with values of CHP alone ($P < 0.01$, as evaluated one-way ANOVA and Bonferroni multiple comparison test) (Table II). Water intake in diabetic G-K rats was equivalent in all groups, compared with DW-treated rats (not significant as tested by ANOVA) (Fig. 6).

Effects of Cyclo (his-pro) on Muscle Glucose Uptake in Nondiabetic Wistar and Diabetic Goto-Kakizaki Rats. Cyclo (his-pro) concentrations of 0 to 10 μg/mL increased glucose uptake in muscle slices in a con-

Figure 2. Concentration-dependent, acute effects of ZC on TAFGC in G-K rats. TAFGC after gavage of 1.0 g/kg BW glucose with DW (DW), 10 mg/kg BW zinc only (Zn), 1.0 mg/kg BW CHP only (CHP), 10 mg/kg BW zinc plus 0.25 mg/kg BW CHP (.25 ZC), 10 mg/kg BW zinc plus 0.5 mg/kg BW CHP (.5 ZC), 1.0 mg/kg BW CHP, or 1.0 mg/kg BW CHP (1.0 ZC) were determined ($n = 5$–10 for each treatment group). **$P < 0.01$ compared to the values of DW-treated rats, ##$P < 0.01$ compared to Zn-treated rats, #$P < 0.05$ compared to Zn-treated rats, °$P < 0.05$ compared to CHP-treated rats, analyzed by one-way ANOVA and Bonferroni multiple comparison test.
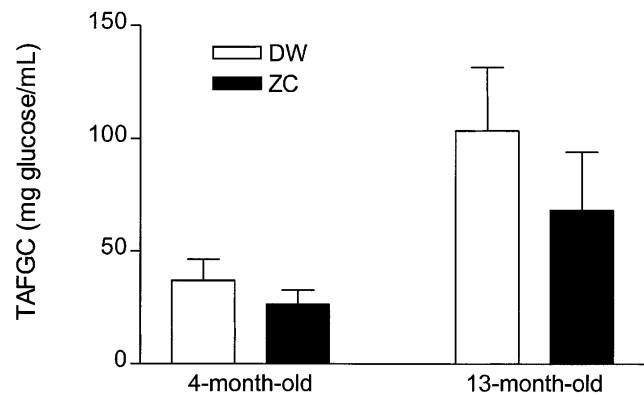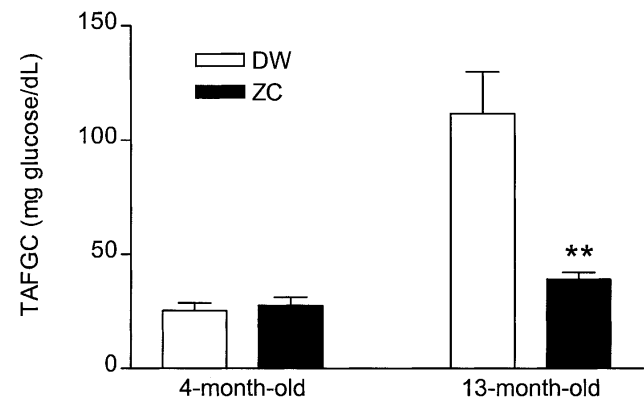

Figure 4. OGT in 4- and 13-month-old rats treated with ZC 1 week after acute bolus treatment. The same rats used in Figure 3 were tested for OGT after a 1-week washout period without ZC treatment ($n = 6$ for each treatment group). Blood glucose levels in 13-month-old rats treated with ZC, as summarized by TAFGC, were significantly different compared with levels in aged, DW-treated rats (**$P < 0.01$, as evaluated by two-way ANOVA and Bonferroni multiple comparison test).

Table I. Plasma Insulin Levels in Young and Aged Sprague-Dawley Rats After Treatment with Zinc Plus CHP

Age of rats	Treatment (n = 6)[a]	Plasma insulin (ng/mL) (mean ± SEM)	P value[b]
4 months	DW	0.152 ± 0.021	
	CHP plus zinc	0.117 ± 0.012	0.067[c]
13 months	DW	0.161 ± 0.011	
	CHP plus zinc	0.133 ± 0.011	NS

[a] Rats were treated for 2 weeks with 10 mg/L zinc plus 1.0 mg/L CHP in drinking water. [b] For t-test comparing CHP plus zinc-treated and untreated groups of rats in each age group. [c] ZC treatments significantly decreased plasma insulin levels in overall two-way ANOVA (P < 0.05).

centration-dependent manner (Fig. 7) (P < 0.001, as determined by two-way ANOVA). However, there was no significant difference in glucose uptake between muscle slices in normal versus G-K rats. Concentrations of 10 μg/mL and 5.0 μg/mL CHP evoked the greatest response in muscle slices from nondiabetic Wistar rats and diabetic G-K rats, respectively.

DISCUSSION

The major etiopathological factor for Type II diabetes in humans is insulin resistance in muscle and fat cells. This defect eventually leads to pancreatic β-cell dysfunction and insulin insensitivity in liver cells. Thus, at least three major organ abnormalities are expressed in Type II diabetic humans. Approximately 80% of Type II diabetic patients are obese. The G-K rat mimics and serves as a model of the nonobese, Type II diabetic human. One mechanism of human obesity is thought to be leptin resistance in the hypothalamus (20), which is similar to insulin resistance that is induced by dietary habits and lifestyle, resulting in impaired signal transduction mechanisms in the hypothalamus and muscle and fat cells. Therefore, the pathophysiology of obese diabetic animals poses more complicated data interpretation than nonobese diabetic animals, in the study of diabetes. However, the G-K rat model exhibits only the pathophysiology of diabetes and serves as the best model for the study of diabetes unrelated to obesity. Using this animal model, we demonstrated that ZC improved blood glucose metabolism by stimulating glucose utilization in
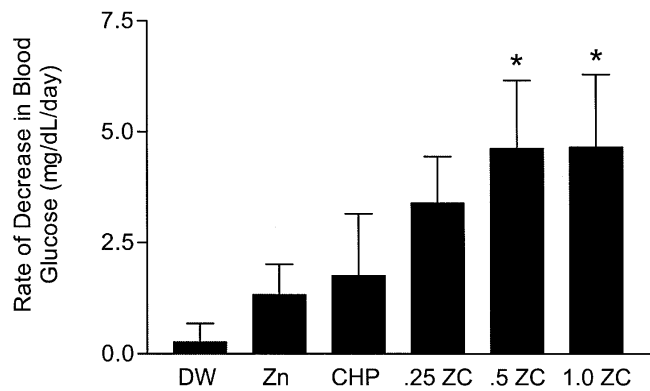

Figure 5. Rates of decrease in blood glucose levels in G-K rats treated with various doses of ZC. Six-week-old diabetic G-K rats were treated with DW (DW), 10 mg/L zinc only (Zn), 1.0 mg/L CHP only (CHP) or 10 mg/L zinc plus 0.25 mg/L CHP (.25 ZC), 0.5 mg/L CHP (.5 ZC), or 1.0 mg/L CHP (1.0 ZC) for 2 weeks. Blood glucose levels were estimated every other day to determine the rate of decrease in postprandial blood glucose levels (n = 5–10 for each treatment group). *P < 0.05, compared with DW-treated rats. Statistical significance was determined using one-way ANOVA and Dunnett's multiple comparison test.

muscle cells, not by enhancing insulin synthesis in pancreatic β-cells. Although the effects of ZC on hepatic glucose output were not determined, it appears that ZC treatment results in little inhibition of hepatic glucose output in G-K rats. Since G-K rats exhibit only mild hyperglycemia, damage to the liver and therefore diminished liver function is not expected. If the liver is intact, ZC treatment will not alter liver function. In support of this possibility, fasting blood glucose levels in the G-K rats were not high, and did not decrease after improvement of OGT in the insulin-resistant, aged S-D rats (Fig. 4).

A major biochemical abnormality in insulin resistant, noninsulin-dependent diabetes mellitus (NIDDM) is decreased responsiveness of insulin receptor β-subunit autophosphorylation (25–28). Impaired insulin receptor autophosphorylation initiates altered signal transduction mechanisms in muscle and fat cells and zinc affects insulin receptor-mediated signal transduction mechanisms of these cells via the control of glucose uptake (22, 23). Thus, it is possible that ZC stimulates glucose uptake in muscle tissues of both normal and G-K rats by affecting autophosphorylation of the insulin receptor β-subunit. The optimal dose for maximal blood glucose response with acute administration of CHP is generally five times higher than that of chronic

Table II. Plasma Insulin Levels in Six-Month-Old G-K Rats Treated with Various Doses of Zinc and CHP

Levels of zinc and CHP and in drinking water (mg/L) (n = 5–10)	Plasma insulin (ng/mL) (mean ± SEM)	P-values[a] compared to DW only	P-values[a] compared to CHP only
DW	0.457 ± 0.028	—	<0.01
Zinc only (10 mg/L)	0.637 ± 0.048	<0.05	<0.05
CHP only (1.0 mg/L)	0.907 ± 0.090	<0.001	—
Zinc plus CHP (10/0.25 mg/L)	0.817 ± 0.167	<0.01	NS
Zinc plus CHP (10/0.5 mg/L)	0.621 ± 0.079	NS	NS
Zinc plus CHP (10/1.0 mg/L)	0.552 ± 0.092	NS	<0.01

[a] One-way ANOVA with multiple comparison (Bonferroni method) was performed to compare the values of DW- and CHP-treated rats.
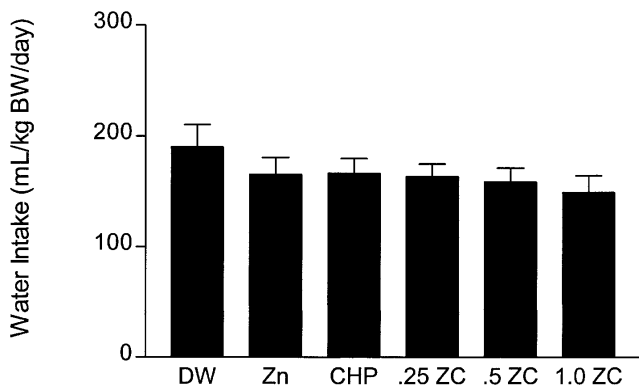
Figure 6. Water intake of G-K rats treated with increasing levels of ZC. Water intake was measured in the same rats depicted in Figure 5. The amount of water intake was measured every 2 to 3 days ($n = 5$–10 for each treatment group). There was no significant difference in water intake between treatment groups, as analyzed by one-way ANOVA and Dunnett's multiple comparison test.

administration. With acute treatment with high doses of ZC, blood glucose levels are immediately lowered and OGT is significantly improved (Figs. 1 and 3). The acute effects of high-dose CHP may be related to the autophosphorylation of the insulin receptor β-subunit, stimulating glucose uptake. However, possible cellular structural rearrangements *via* protein synthesis and degradation, as well as new enzymatic activities, may also be involved in the improvement of OGT.

The gradual decrease of blood glucose levels with chronic ZC treatment of diabetic G-K rats in a CHP concentration-dependent manner (Fig. 5) further supports the possible restructuring of cellular components. This function may occur over an extended period of time, and its effects are gradual. In this case, ZC may improve insulin resistance by supplying new zinc, which becomes an integral part of
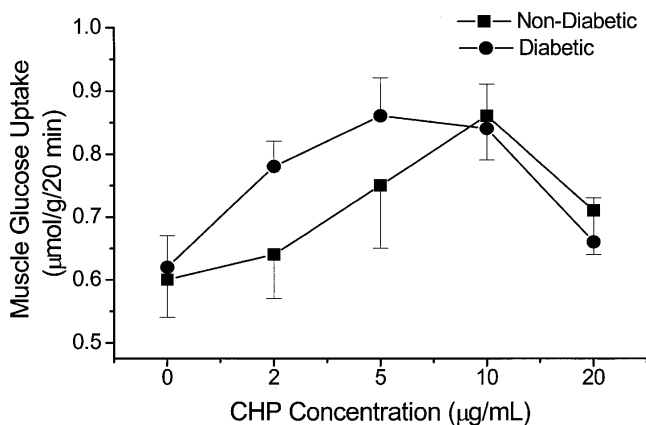

Figure 7. CHP concentration-dependent glucose uptake capacity of rat muscle tissue. Two-month-old, age-matched, nondiabetic Wistar and diabetic G-K rat muscle tissue samples from the epitrochlears were removed and incubated at 35°C for 20 min with 1,2-³H-2-deoxy-D-glucose and 1-¹⁴C-mannitol to determine rate of glucose uptake, as described in Materials and Methods ($n = 3$ rats, 10 tissue slices each, for each concentration of CHP). ($P < 0.001$, as evaluated by two-way ANOVA between ZC-treated and untreated tissues, but not significant values between tissues sources.)

new enzymes and proteins that remove partially digested junk proteins to increase glucose transporter-4 (GLUT4) synthesis and translocation. Accumulation of incompletely digested protein fragments in the cells can impair cellular signal transduction mechanisms (29–32). CHP is capable of chelating zinc, which stimulates intestinal zinc absorption and uptake in muscle tissues (17). Thus, increased zinc metabolism, which is defective in the diabetic condition, improves insulin receptor-mediated signal transduction mechanisms. Improvement of OGT and blood glucose levels in G-K rats is CHP dose dependent. If ZC activates only autophosphorylation of the insulin receptor β-subunit, improved OGT should return immediately to the original state. In the case of aged S-D rats, protein synthesis and digestion of junk proteins must be a factor affecting improved OGT. However, acute administration of ZC in young rats may have activated the insulin receptor β-subunit, since stimulated OGT returned to the original, nonstimulated state in less than 1 week (Fig.4). These facts suggest that adequate zinc and CHP intake is important in improving glucose metabolism in diabetic animals.

Insulin resistance leads to hyperinsulinemia, followed by the development of high blood glucose levels and resulting in Type II diabetes. ZC treatment decreased both fed blood glucose levels in G-K rats (Fig. 5) and plasma insulin levels in S-D and G-K rats (Tables I and II). Plasma insulin levels were much higher in G-K rats than in S-D rats (Tables I and II), which demonstrate that improvement in blood glucose levels by ZC treatment was not due to increased plasma insulin levels. On the other hand, ZC may affect hepatic glucose output, lowering blood glucose levels by improving insulin sensitivity of liver cells. Although insulin-mediated signal transduction mechanisms in both liver and muscle cells are defective in Type II diabetic animals, it is likely that signal transduction mechanisms in the liver cells of G-K rats are normal since G-K rats are not severely diabetic and liver function is not damaged. However, we did not study the liver in ZC-treated animals; therefore, an understanding of the mechanisms by which ZC affects insulin sensitivity in liver cells requires further studies using hyperinsulinemic euglycemic clamp techniques.

It is expected that the glucose uptake rate in muscle slices of G-K rats be lower than that of normal rats. However, the basal glucose uptake rates in muscle slices from G-K rats showed no difference to normal tissues (Fig. 7). It is also expected that the glucose uptake rate of muscle tissues in the basal condition be lower in G-K rats than in normal rats, assuming higher insulin resistance in muscle tissues from G-K rats. However, the glucose uptake rates in G-K rat tissues were either higher than in normal rat tissues or the same, depending on CHP dose. Although the exact mechanisms are unclear, it appears that certain biological factors such as ZC are lacking in the diabetic muscle tissues, and addition of ZC significantly increased glucose uptake rates. Ivy *et al.* (33) reported that there was essentially no difference in the glucose uptake rate of skeletal muscles

from relatively young and aged rats, although exercise and dietary restriction did affect muscle glucose uptake rate. Since G-K rats are mildly diabetic and muscle tissues from these rats were sliced and washed several times, glucose uptake capacities of muscle tissue from G-K rats may have been normalized. Further studies with muscle tissues from several diabetic models under different experimental conditions may explain why there were no differences between glucose uptake rates in muscle tissue from G-K rats and normal rats.

If ZC increases glucose utilization, it may improve other biochemical parameters of diabetes in insulin-resistant, Type II diabetic animals and humans. When rats were treated with streptozotocin to induce diabetes, water intake was 3-fold higher than that of normal rats (1). However, young G-K rats are not severely diabetic, and development of polydipsia and polyphagia is not apparent. Thus, water intake was not significantly reduced with ZC treatment in G-K rats. Water intake was measured because most diabetic animals and human subjects develop polydipsia as well as polyphagia, which are considered to be indices of clinical diabetes (34–37). Although no significant differences in food intake were exhibited in the present study (data not shown), a statistically significant difference in food intake may be apparent with a larger number of animals.

In conclusion, ZC treatment resulted in marked antidiabetic activity in aged, insulin-resistant, and genetically diabetic G-K rats. ZC appears to increase glucose utilization in muscle tissues (22, 23), though the exact mechanism by which ZC lowers blood glucose levels is not clearly established. Further research elucidating the insulin-mediated signal transduction mechanisms in muscle and fat cells as well as insulin sensitivity in the regulation of hepatic glucose output in the presence of CHP and zinc will lead to a better understanding of the beneficial effects of these agents in diabetic animals and humans.

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