Effects of Chromium Picolinate Supplementation on Insulin Sensitivity, Serum Lipids, and Body Weight in Dexamethasone-Treated Rats

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Chromium (Cr) is essential for the regulation of insulin action, and Cr supplementation has been studied as a potential therapy of insulin resistance and lipid abnormalities. Corticosteroid treatment is well known to cause the abnormality of carbohydrate metabolism. Recently, it has been reported that corticosteroid increases urinary loss of Cr, and Cr supplementation recovers steroid-induced diabetes mellitus. In this experiment, rats were treated daily with dexamethasone (DEX) (0.2 mg/kg, intraperitoneal [IP]) for the first 7 days and were further treated with DEX plus either chromium picolinate (CrP, 30 mg/kg/d) orally or a placebo for a period of 14 days. At the end of experiment (D21), the control rats, which were treated only with DEX weighed 320 g (80% of initial weight) on average, but CrP-treated rats weighed 364 g (91% of initial weight. $P < .05$). Glucose tolerance tests (GTTs) and insulin sensitivity tests were conducted. During insulin sensitivity tests, the area under the curve ($AUC_{0\rightarrow120}$) of the time-glucose concentrations curves in CrP-treated group were decreased compared with those in the control group (271.4 ± 74.9 *v* 1,097.4 ± 722.2 mmol/L/min, $P < .01$). Fasting serum insulin levels in CrP-treated rats were clearly decreased by 46.9% compared with those in the control group (0.52 ± 0.19 *v* 0.98 ± 0.36 nmol/L, $P < .05$). During the GTTs, the $AUC_{0\rightarrow120}$ for time-glucose concentrations curves in CrP-treated group was not significantly different from the control group, but the $AUC_{0\rightarrow120}$ of serum insulin concentrations in the CrP-treated group were 55.8% lower than those in the control group (123.1 ± 42.5 *v* 278.2 ± 59.1 nmol/L/min, $P < .01$). The mean $AUC_{0\rightarrow120}$ of time-cholesterol concentration curves during GTTs did not significantly differ between the 2 groups (867.6 ± 155.2 *v* 827.7 ± 94.3 mmol/L/h, P = not significant [NS]). In contrast, 1-hour and 2-hour plasma triglycerides were significantly lower in the CrP-treated group, and the mean AUC of the time-triglyceride curve was significantly lower in CrP-treated group than in the control group (3.4 ± 0.5 *v* 5.9 ± 1.3 mmol/L/h, $P < .05$). We suggest that Cr supplementation in DEX-treated rats can relatively reverse a catabolic state and increase insulin sensitivity. Our results support the hypothesis that Cr supplementation can be considered to improve carbohydrate and lipid metabolism in patients receiving corticosteroid treatment.

CHROMIUM (Cr) is an essential trace element and exists in several oxidative states. Trivalent Cr is the most stable form of Cr and has never been shown to cause clinical cancer unlike the hexavalent form.[1] It has been reported that associations of Cr deficiency include not only abnormalities of glucose and lipid metabolism,[2] but cases of impaired nerve function. Severe Cr deficiency in patients receiving total parenteral nutrition caused peripheral neuropathy, brain dysfunction, and glucose intolerance requiring increasing amounts of insulin.[3-5] Cr deficiency is also associated with parity, gestational diabetes, diabetes mellitus, and coronary heart disease.[6] Thiazide diuretics and stress increases Cr losses.[7,8]

Corticosteroid treatment is known to increase urinary Cr losses.[9] Corticosteroids have also been known to induce insulin resistance and a catabolic state in rats.[10] Recently Ravina et al[9] reported that Cr supplementation resulted in marked decrease of fasting blood glucose in 3 cases of diabetic patients who were treated with steroids. They also showed that within 1 week of supplemental Cr, blood glucose concentrations of 47 of 50 patients with uncontrolled steroid-induced diabetes markedly decreased.[11] However, there are no other reports that prove the beneficial effect of Cr on steroid-induced diabetes. Therefore, this study was undertaken to determine if Cr can improve insulin sensitivity and glucose intolerance in insulin-resistant rats induced by dexamethasone (DEX) treatment.

MATERIALS AND METHODS

The animals (male Sprague-Dawley rats, 400 g) were housed in a ventilated (10 to 15 times/hour), temperature-controlled (20 to 23°C), humidity-controlled (50% to 70%), noise-controlled (below 60 dB), and luminous intensity-controlled (200 lux) room with a 12-hour light and 12-hour dark (6 AM to 6 PM) cycle. All rats were started on DEX (0.2 mg/kg, by mouth). When 1 week had elapsed, the rats were treated with DEX combined with chromium picolinate (CrP) (30 mg/kg/day, CrP-treated group, n = 7) or a placebo (control group, n = 7) for 2 weeks. At the end of the experiment (D21), insulin sensitivity tests were performed intraperitoneal (IP) injection of glucose (2 g/kg) in conjunction with subcutaneous injection of insulin (5 U/kg, Daewoong-Lilly Co, Seoul, Korea; administered 30 minutes before the glucose load), and blood glucose concentrations were measured at 0, 30, 60, 90, and 120 minutes after the glucose load. The next day, glucose tolerance tests (GTTs) were performed by IP injection of glucose (2 g/kg) alone. Blood samples were drawn at 0, 30, 60, 90, and 120 minutes after the glucose load from the tail vein, and plasma glucose and insulin levels were measured. The area under the curve (AUC; mmol/L/min) of time-concentration during insulin sensitivity test or GTT was calculated for the glucose and insulin levels according to the trapezoidal rule.[12] The blood glucose level was measured with an automated blood glucose analyzer (GLUCOSCOT; Daiichi Co, Kyoto, Japan). The plasma insulin level was determined by a rat insulin enzyme immunoassay kit (SPI-BIO Co, Paris, France). Results are expressed as means ± SD. Significance was assessed with a nonpaired *t* test, and the level of significance was chosen at the 5% level.

RESULTS

Reversal of the Catabolic Effect of Dexamethasone

The changes in body weight at the 4 time points during the study are shown in Table 1. As expected, the DEX treatment

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Table 1. Effect of Cr on Body Weight in the DEX-Treated Rats

Day	0	7	14	21
CrP-not treated group				
BW (g)	400 ± 17	349 ± 11	334 ± 14	320 ± 13
DEX/CrP	DEX		DEX alone	
CrP-treated group				
BW (g)	401 ± 31	338 ± 28	337 ± 32	364 ± 36*
DEX/CrP	DEX		DEX + CrP	

NOTE. Values are mean ± SD. All rats were treated with DEX for 21 days, and CrP was added from the 8th day of the treatment in the CrP-treated group.

*$P < .05$ *v* CrP-not treated group.

induced a catabolic state. After the first 7 days of the DEX treatment, the mean body weights in both the CrP-treated and control groups similarly decreased by 16.0% and 12.8%, respectively. In the control group, the mean body weight continued to decrease during the treatment with DEX alone, and the weight measured 320 g (80% of the baseline weight) at the end of the experiment (D21). By contrast, the mean body weight in the CrP-treated rats was recovered with CrP supplementation, and the mean weight slightly increased to 364 g at the end of the experiment (91% of the baseline weight, $P < .05$ *v* control).

Insulin Sensitivity Test

The blood glucose levels and $AUC_{0\rightarrow120}$ after the insulin and glucose load for both groups of subjects are shown in Table 2 and Fig 1. Compared with those observed in the control group, the plasma glucose concentrations were markedly decreased in the CrP-treated group during insulin sensitivity tests. Consequently, the AUCs, calculated from 0 to 120 minutes, were significantly lower in the CrP-treated than the control group (271.4 ± 74.9 *v* 1,097.4 ± 722.2 mmol/L/min, $P < .01$).

GTT and Serum Insulin Level

Figures 2 and 3 show the glucose and insulin levels during the GTTs for the CrP-treated and control groups. At baseline, fasting glucose levels in the CrP-treated rats were not different from those in the control rats, but fasting serum insulin levels of the CrP-treated rats were clearly decreased by 46.9% compared with those of the control group (0.52 ± 0.19 *v* 0.98 ± 0.36 nmol/L, $P < .05$). The $AUC_{0\rightarrow120}$ of glucose in the CrP-treated group was not significantly different from the control group. The $AUC_{0\rightarrow120}$ of the glucose concentrations in the control rats was 867.6 ± 155.2 mmol/L/min and the $AUC_{0\rightarrow120}$ in the CrP-treated rats was 827.7 ± 94.3 mmol/L/min. By contrast, the $AUC_{0\rightarrow120}$ of serum insulin concentrations during GTT in the CrP-treated group were 55.8% lower than those in the control group (123.1 ± 42.5 *v* 278.2 ± 59.1 nmol/L/min, $P < .01$). When the insulin response was analyzed with respect to the glucose response, the ratio of insulin to glucose at 30 minutes and 60 minutes significantly decreased in the CrP-treated group ($P < 0.05$), and the ratio of the AUCs was significantly lower in the CrP-treated group than in the control group (4.7 ± 1.8 *v* 10.2 ± 4.1; $P < .05$) (Table 3).

Table 2. Blood Glucose Concentrations During Insulin Sensitivity Tests

	Blood Glucose (mmol/L)				
(min)	0	30	60	90	120
Control	3.2 ± 0.4	12.8 ± 8.4	11.1 ± 8.0	7.8 ± 6.3	6.5 ± 5.1
CrP-treated	3.1 ± 0.9	4.4 ± 1.6*	1.8 ± 0.5†	0.9 ± 0.3†	1.0 ± 0.4†

NOTE. Values are the mean ± SD.

*$P < .05$ *v* control group.

†$P < .01$ *v* control group.

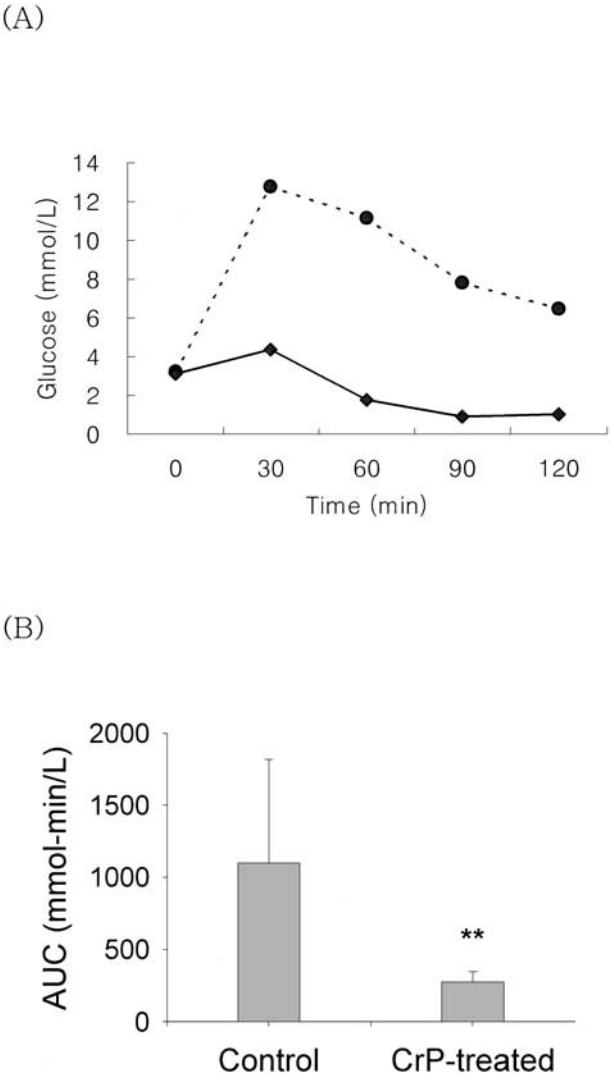

Fig 1. (A) Blood glucose response to insulin (5 U/kg, subcutaneous) plus glucose (2 g/kg, IP) in the control group (●) and the CrP-treated group (◆). (B) The box shows the AUCs of control and CrP-treated groups together with SD.

Serum Lipid Profile

The fasting plasma concentrations of cholesterol and triglycerides showed no differences between the control and the CrP-treated groups. The mean area under the cholesterol curve during GTTs did not significantly differ between the 2 groups (3.7 ± 0.4 *v* 3.6 ± 0.3 mmol/L/h, P = not significant [NS])

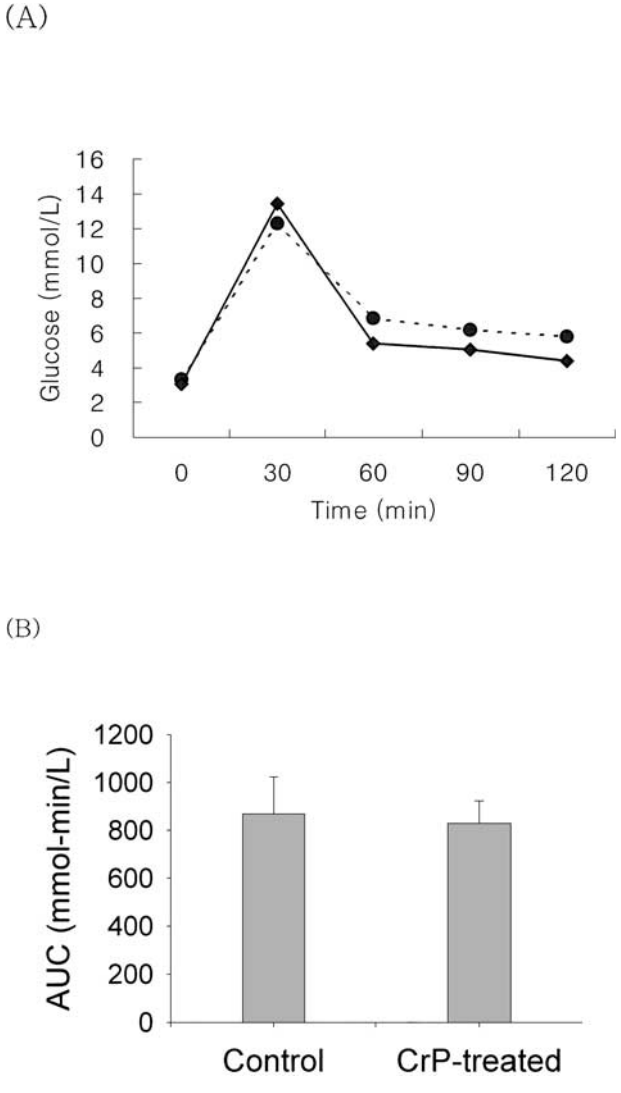

Fig 2. (A) Blood glucose concentrations during GTT (2 g/kg glucose, IP) in the control (●) and the CrP-treated groups (◆). (B) The box shows the AUCs of control and CrP-treated groups together with SD.

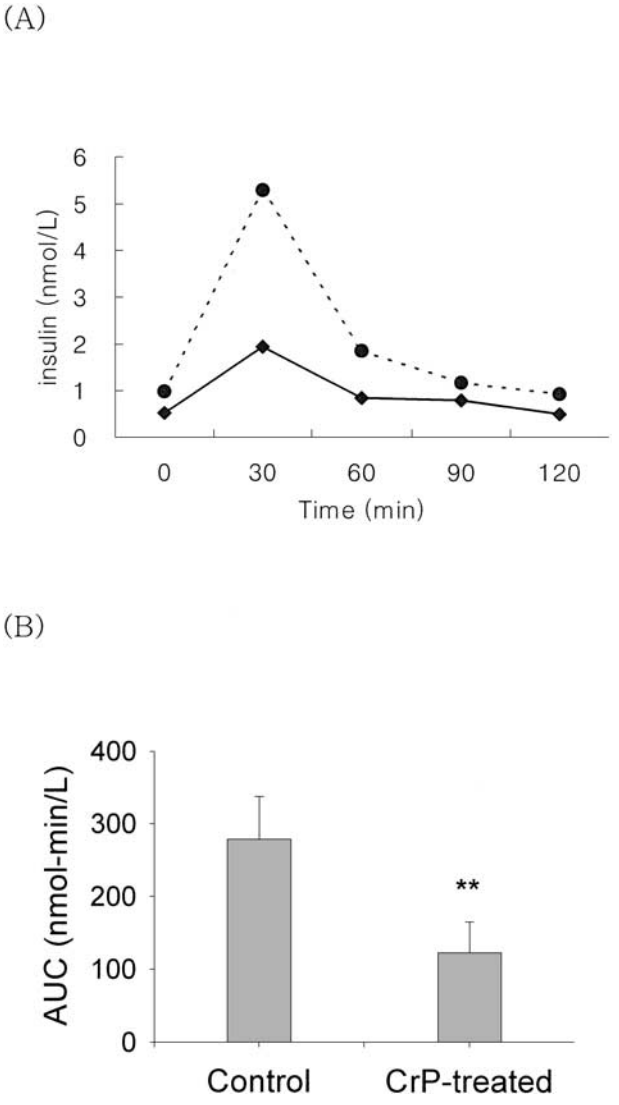

Fig 3. (A) Blood insulin variations during GTT in the control (●) and the CrP-treated groups (◆). (B) The box shows the AUCs of control and CrP-treated groups together with SD.

(Fig 4). In contrast, 1-hour and 2-hour plasma triglycerides were significantly lower in the CrP-treated group (Fig 5), and the mean area under the triglyceride curve was significantly lower in the CrP-treated group than in the control group (3.4 ± 0.5 *v* 5.9 ± 1.3 mmol/L/h, $P < .05$).

DISCUSSION

Since Schwarz and Mertz[13] demonstrated the existence of a new dietary factor (Cr) in 1959, it has been known that Cr is an essential nutrient involved in normal carbohydrate and lipid metabolism. There have been several studies involving Cr supplementation in healthy or diabetic humans. Most scientific evidence has not supported benefits of Cr supplementation in healthy subjects with good glucose tolerance,[14,15] but there are some reports that showed the beneficial effects.[16] Before a recent large, prospective, placebo-controlled, blinded study of Cr supplementation in 180 patients with type 2 diabetes in Beijing, China,[17] the 2 well-designed placebo-controlled studies of Cr supplementation in type 2 diabetes showed negative results.[18,19] Recently, Trow et al[20] reported no beneficial effects of dietary Cr supplementation on glucose tolerance, plasma insulin, and lipoprotein levels in diabetes, However, in roughly half the diabetic patients supplemented with Cr, improvement

Table 3. Ratios of Glucose to Insulin Levels During the GTTs

	Insulin/Glucose [(nmol/L)/(mmol/L)]					AUC (Insulin)
(min)	0	30	60	90	120	AUC (Glucose)
Control	9.6	13.7	8.6	6.0	5.1	10.2 ± 4.1
CrP-treated	6.7	4.6*	5.0*	5.1	3.6	4.7 ± 1.8*

*$P < .05$ *v* control group.

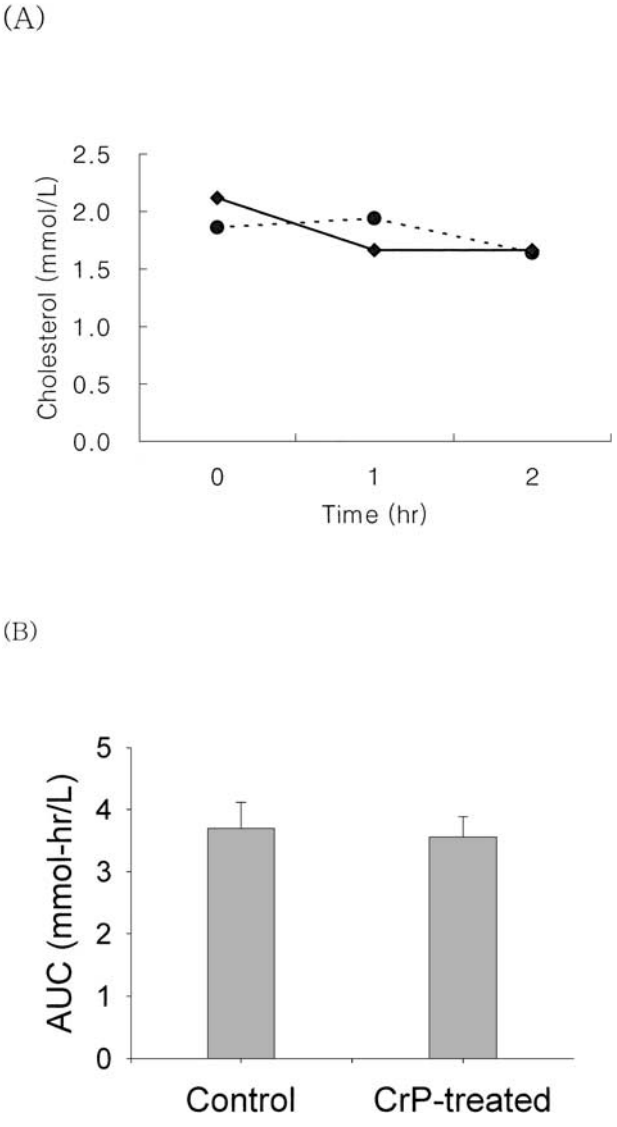

Fig 4. (A) Plasma cholesterol concentrations during GTTs in the control (●) and the CrP-treated groups (◆). (B) The box shows the AUCs of control and CrP-treated groups together with SD.

of fasting blood glucose, glycosylated hemoglobin, total cholesterol, and low-density lipoprotein (LDL) cholesterol was reported. Results from the recent study by Anderson et al[17] indicate that Cr may be efficacious in treating type 2 diabetes, and results from Cefalu et al[21] in 29 obese individuals show that CrP can increase insulin sensitivity independent of a change in weight or body fat percentage. These reports imply that the metal was having a physiologic rather than a pharmacologic effect. To date, no unequivocal pharmacologic effects have been observed with Cr supplementation in humans.

Cr deficiency has been demonstrated in a patient who had long-term parenteral nutrition and who developed glucose intolerance and peripheral neuropathy that responded dramatically to infusion of Cr.[3-4] Although Rabinowitz et al[22] did not find any relative lack of body Cr stores in an adult American diabetic population, Doisy et al[23] reported that diabetes might result in higher rates of Cr excretion. According to recent studies, loss of Cr balance would appear possible, because the mean levels of plasma Cr were approximately 33% lower and urine levels almost 100% higher in 93 type 2 diabetic patients compared with a group of healthy individuals.[24]

The mechanism of action of Cr on the control of blood glucose concentrations is the potentiation of insulin action. Studies with epididymal fat tissue from Cr-deficient rats showed that near maximal insulin response could be achieved by adding Cr.[25,26] Supplemental Cr leads to increased insulin binding to cells due to increased insulin receptor number.[27] Cr was also shown to affect cell sensitivity measured in euglycemic clamp studies.[28] A breakthrough in establishing the mechanism of Cr action at the molecular level occurred in the 1980s. Yamamoto and Wada[29] reported the isolation and characterization of a unique Cr-binding oligopeptide from liver and kidney

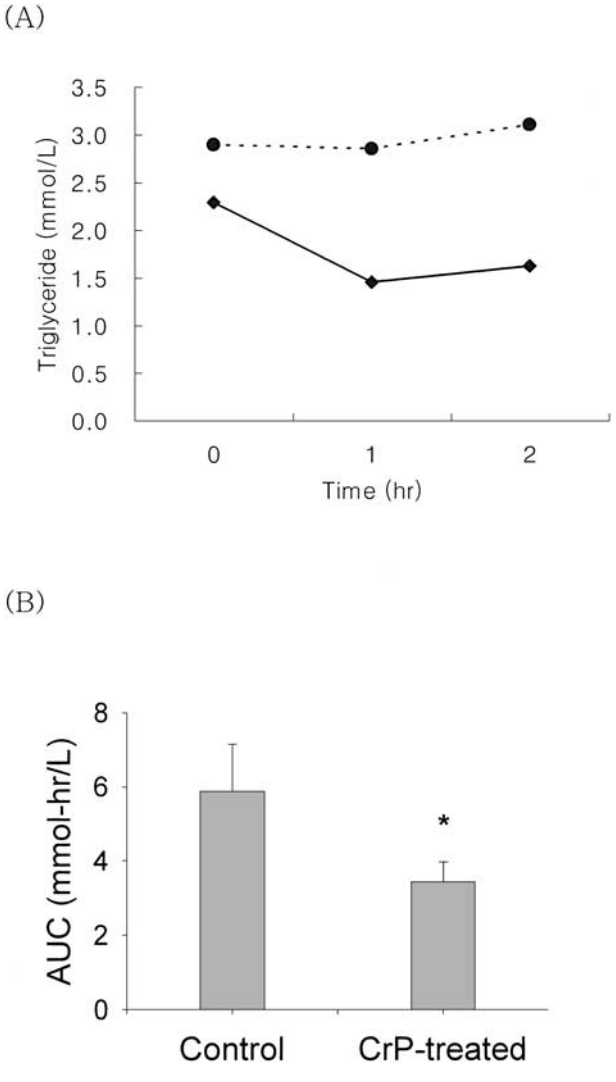

Fig 5. (A) Plasma triglyceride concentrations during GTTs in the control (●) and the CrP-treated groups (◆). (B) The box shows the AUCs of control and CrP-treated groups together with SD.

in animals, named low-molecular-weight Cr-binding substance (LMWCr) or chromodulin. This 1,500 dalton peptide binds 4 chromic ions in response to an insulin-mediated chromic ion flux, and the metal saturated oligopeptide can bind to the insulin receptor, thereby stimulating insulin receptor's tyrosine kinase activity up to several fold.[30,31] Thus, chromodulin appears to play a role in an autoamplification mechanism in insulin signaling. Cr is now recognized as an insulin-sensitizing cofactor for a low-molecular-weight protein and is known to be associated with decreased glucose intolerance, decreased risk factors associated with cardiovascular diseases, improved immunity, and increased life span.

Urinary Cr losses can be used as a measure of mobilized Cr, because mobilized Cr is not reabsorbed by the kidney, but is excreted in the urine.[8] The stress of corticosteroid treatment increases Cr losses. Because Cr intake is normally marginal, corticosteroid administration may lead to marginal Cr deficiency.[2] Ravina et al[9] demonstrated continual supplementation of CrP reduced the dose of insulin and or hypoglycemic drugs in 3 patients with corticosteroid-induced diabetes, and they also demonstrated the similar effects of Cr supplementation in 47 of 50 patients with uncontrolled steroid-induced diabetes in another study.[11] In the present study, we observed increased insulin sensitivity in the insulin sensitivity test, decreased plasma insulin concentration, and decreased level of triglycerides by Cr supplementation in DEX-treated rats. These findings provide support for the hypothesis that Cr deficiency could lead to insulin resistance and elevated insulin secretory response to glucose[32] and are in agreement with the findings described by Striffler.[5] Triglyceride levels were markedly reduced in CrP-treated rats, whereas cholesterol levels were not. These results are consistent with those reported by Lee and Reasner,[33] who observed similar results in Hispanic patients with type 2 diabetes mellitus.

In this study, CrP supplementation was also shown to be effective in recovering catabolic effects of DEX. It has been reported that whereas the mean body weight in the rats normally increased by 23% during a 7-day period, mean body weight in DEX-treated rats decreased by 18% and 25% at doses of 0.1 and 1 mg/day, respectively.[10] Our data demonstrated that treatment with DEX also induced body weight loss, but Cr supplementation lead to a significant reversal of DEX-induced weight loss.

In summary, our data indicate that Cr supplementation in DEX-treated rats can relatively reverse a catabolic state, decrease serum triglyceride levels, and increase insulin sensitivity. This raises the possibility that Cr supplementation can be beneficial for patients with steroid-induced diabetes. Therefore, Cr supplementation can be considered to improve carbohydrate and lipid metabolism in patients receiving corticosteroid treatment. Additional studies are needed to establish the amount and the form of supplemental Cr in people receiving corticosteroids or in patients with steroid-induced diabetes.

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