

Antioxidant Effects and Insulin Resistance Improvement of Chromium Combined with Vitamin C and E Supplementation for Type 2 Diabetes Mellitus

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Summary To determine the effects of combined supplementation with chromium (Cr) and vitamins C and E on oxidative stress in type 2 diabetes, adult subjects with HbA1c >8.5%. Subjects ($n = 30$) in this randomized, double blind, placebo-control study were divided into three groups (placebo, Cr or Cr + C + E) on daily treatment. The Cr group received 1000 μg of Cr (as Cr yeast); the Cr + C + E group received Cr (1000 μg as Cr yeast) together with vitamins C (1000 mg) and E (800 IU); and, a control group received a placebo. Baseline plasma Cr levels were not significant differences comparing the supplementation and placebo groups. Thiobarbituric acid reactive substances (TBARS) and total antioxidative status (TAS) were also not significant different. Following the 6-month study period, the plasma TBARS levels, fasting glucose, HbA1c and insulin resistance were significantly decreased in the Cr and Cr + C + E groups, but not for the placebo group. Plasma TAS and glutathione peroxidase were significantly higher for Cr and Cr + C + E groups relative to the placebo group. These findings suggest that Cr supplementation alone and combined of Cr together with vitamins C and E was effective for minimization of oxidative stress and improvement of glucose metabolism in type 2 DM patients.

Key Words: antioxidants, chromium, vitamin C, vitamin E, insulin resistance

Introduction

It is believed that diabetes is associated with increased oxidative stress as increased blood concentrations of thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, have been reported [1, 2]. Oxygen-derived radicals and reactive oxygen species are known to attack cell membranes, resulting in the propagation of lipid peroxidations.

Oxidative damage due to free radicals is associated with vascular disease in people with type 1 and type 2 diabetes

mellitus (DM) [3, 4]. There are several potential sources of free radical production in diabetics, including autoxidation of plasma glucose (1), leucocyte activation, and increased transition metal bioavailability [5]. The total antioxidant status (TAS) in type 1 or 2 DM is lower than that of age-matched controls, a finding which might be attributable to lower levels of vitamin C, vitamin E [4, 6, 7], or other factors including micronutrients in blood [8–12].

Anderson *et al.* [11, 13] have reported beneficial effects for supplemental Cr on plasma glucose and related variables in type 2 DM patients. Accompanying these data, there are other studies, the results of which suggest that Cr also improves cellular antioxidant capacity in rats [14–17]. Therefore, restoring Cr status in individuals with type 2 DM may counteract the deleterious effects of oxidative stress and help prevent complications associated with diabetes [11, 13, 18].

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Vitamin E is considered the most important lipid-soluble exogenous antioxidant in humans. Vitamin C serves as an antioxidant, directly by scavenging aqueous peroxy radicals, and indirectly by regenerating reduced vitamin E [19]. Vitamins C and E defend against the damaging effects of high oxidative stress in diabetes sufferers through nonenzymatic and autooxidative glycosylation, and reduction of metabolic stress [20, 21].

In Taiwan, there has been a five-fold increase in mortality from DM-related complications in the last 20 years. For type 2 DM, there is a high incidence of oxidative complications, such as retinopathies, glomerulopathies, and vascular complications. According to our previous study find that Cr supplementation was an effective treatment strategy to minimize increased oxidative stress in type 2 diabetes mellitus patients whose HbA1c level was >8.5%, but the effects of supplementation with Cr combined with the vitamins C and E are unknown. Therefore, the present study was conducted in Taipei to determine the effects of supplementation with Cr combined with the vitamins C and E, comparing the variables associated with oxidative stresses and glucose homeostasis in a sample of patients with severely hyperglycemic diabetes.

Materials and Methods

Subjects

Our sample of volunteers were adult males and females under 56 years of age who had been diagnosed with diabetes at least 5 years previously (fasting glucose >8.5 mmol/L and HbA1c >8.5%). Key exclusion criteria included: pregnancy and lactation; trace element and vitamin supplementation in the preceding 3 months; ongoing gastric or diuretic treatment; acute renal failure (creatinine <120 μ mol/L); and, recent surgery or acute infection. And individuals with chronic kidney, liver, pulmonary or cardiac disease, smoking, alcoholism were eliminated. Patients were enrolled from Taipei Medical University Hospital. Approval was obtained from the Human Studies Review Board of Taipei Medical University Hospital. Patients were fully informed with respect to the purpose of the study, were free to ask questions throughout the investigation, and signed an informed consent form witnessed by one of the investigators prior to participation. Subjects ($n = 30$) in this randomized, double blind, placebo-control study were assigned to one of three groups (placebo, Cr or Cr + C + E) based on supplementation. Patients in each group were supplemented daily with a placebo, or with 1000 μ g Cr as yeast chromium (III), alone or combined with 1000 mg vitamin C and 800 IU vitamin E (Westar Nutrition Corp., Costa Mesa, CA). The quantity of chromium was verified by chemical analysis. The subjects' fasting-glucose concentrations were >8.5 mmol/L. All subjects complete the 6-month study. All volunteers used

Metformin, Glibenclamide, Glipizide, Gliclazide to control their diabetes, and medication dosages remained the same throughout the 6-month study. All the volunteers were advised to maintain their normal dietary and exercise habits during the study. Each month volunteers were asked to return any unused supply to measure their daily compliance. Subjects were also questioned with respect to any possible side effects and their subjective assessment of compliance.

Subjects were requested to record dietary intake, including time, place, types of food or ingredients, cooking methods, and amounts at baseline and 6th month. "The Common Food Nutrition Chart in Taiwan" issued by the Department of Health, Taiwan, ROC was used as a reference to help subjects record the data. The dietary data of these subjects were collected by a 24-h dietary recall for three consecutive days (include a no-week day) and was analyzed by the food composition software (Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC) during the study.

Analytical methods

Blood samples were drawn after an overnight fast at the beginning of the study and after 6 months of daily supplementation. The blood was taken from the antecubital vein and collected in a Vacutainer trace element-free tube (Becton-Dickinson, NJ). Urine samples were collected in 100-mL polypropylene specimen containers (Falcon, OH) and stored at -20°C . All samples were run prior to the breaking of the code, which was not available to the investigators until completion of all the samples. Blood and urinary Cr levels were determined using a Hitachi Z-5000 polarized Zeeman atomic absorption spectrophotometer using standard electrothermal graphite furnace techniques [22]. The in-house urinary sample was assayed at least twice a day (for control checking) to confirm the accuracy of the urinary Cr analysis [23].

Plasma samples and erythrocytes were stored at -70°C prior to batch analyses. The autoanalyzer system (Hitachi-7170; Hitachi Ltd, Tokyo, Japan) and diagnostic kits (Shino, Tokyo, Japan) were used to determine plasma glucose concentrations; diagnostic kits (WAKO, Osaka, Japan) were used to determine plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and blood urea nitrogen (BUN) concentrations. HbA1c level was assayed with the HbA1c kit (Bayer, Kernerstville, NC); the insulin kit (DPC, LA) was used for insulin analysis.

Plasma TBARS levels were determined for assay of malondialdehyde and thiobarbituric acid complex [24] by fluorometry kit after extraction with n-butanol. The TAS was determined for assay of the peroxidation rate through fluorescence loss of the protein R-phycoerythrin induced by 2,2'-azobis(2-amidinopropane) dihydrochloride [25]. The lag phase was compared to that of 6-hydroxy-2,5,7,8-

Table 1. Effects of 6 months of chromium or combined chromium with vitamins C and E supplementation on anthropometric and laboratory variables

	Placebo (n = 10)	Cr (n = 10)	Cr + C + E (n = 10)
Men/women (n)	5/5	4/6	5/5
Age (years)	50.5 ± 1.9	53.2 ± 2.0	51.5 ± 1.7
Body mass index (BMI; kg/m ²)			
Baseline	25.8 ± 0.8	25.7 ± 0.9	25.7 ± 0.6
6 months	25.7 ± 0.7	25.6 ± 0.8	25.7 ± 1.0
Blood AST (IU/L)			
Baseline	25.7 ± 2.4	24.8 ± 3.3	25.1 ± 2.5
6 months	25.4 ± 3.1	24.1 ± 2.4	25.9 ± 3.2
Blood ALT (IU/L)			
Baseline	31.2 ± 2.8	29.1 ± 4.4	30.4 ± 2.4
6 months	32.1 ± 3.4	29.9 ± 2.7	30.8 ± 3.5
Blood creatinine (µmol/L)			
Baseline	70.7 ± 8.8	70.7 ± 10.2	71.1 ± 8.6
6 months	70.4 ± 9.8	70.6 ± 8.9	70.9 ± 9.5
Blood urea nitrogen (mmol/L)			
Baseline	5.1 ± 0.4	5.4 ± 0.5	5.3 ± 0.5
6 months	5.4 ± 0.5	5.4 ± 0.4	5.5 ± 0.5

1. Values are the mean ± SD.

2. “*” means significant difference between the placebo group and the Cr or Cr + C + E group.

3. AST: aspartate aminotransferase.

4. ALT: alanine aminotransferase.

tetramethylchroman-2-carboxylic acid (Trolox).

Red blood cell catalase activity was measured spectrophotometrically by the absorbance change at 240 nm [26]. Superoxide dismutase activity was determined according to the method of McCord and Fridovich [27]. Glutathione peroxidase activity was measured according to the method of Lawrence and Burk [28]. Activities were calculated in units per milligram of protein to normalize differences between control and diabetic blood. The Lowry method was used for protein determination [29]. Insulin resistance was calculated using the homeostasis model assessment (HOMA) index [30].

Statistical analysis

Analysis of variance was used for statistical analysis of the data. Individual means were compared using unpaired t tests (SAS, SAS Institute, Cary, NC). Values are expressed as mean ± SD. Group means were considered significantly different at $p < 0.05$.

Results

Effects of supplementation on anthropometric and laboratory variables

The group means for age, body mass index (BMI), and

blood levels of ALT, AST, creatinine and BUN were shown in Table 1. There were no significant differences at baseline and after 6 months of supplementation. In addition, we also compared these variables between three groups at the same time-point. No remarked differences were observed. These data showed that there were no obvious differences of the organs functions in these three groups. The levels of ALT, AST, creatinine and BUN were within normal range.

Dietary data in 24-h dietary recall during the experiment

The intake of energy, carbohydrate, protein, fat, vitamin C and vitamin E were shown in Table 2. There were no significant differences at baseline and after 6 months of supplementation. In addition, no remarked differences were observed between three groups at the same time-point.

Effects of supplementation on blood glucose and insulin variables

As shown in Table 3, levels of fasting glucose, HbA1c and insulin were not significantly different at baseline. Differences were noted, however, comparing the initial and post-treatment levels of fasting glucose, HbA1c and insulin resistance index. For the Cr group, fasting glucose, HbA1c and insulin resistance index decreased significantly ($p < 0.05$) from 12.5 ± 0.5 to 11.1 ± 0.4 mmol/L and from 10.2 ± 0.5 to

Table 2. Average daily intake of calorie, carbohydrate, protein, fat, vitamin C and vitamin E in 24-h dietary recall during the experiment.

	Placebo (<i>n</i> = 10)	Cr (<i>n</i> = 10)	Cr + C + E (<i>n</i> = 10)
Energy (kcal)			
Baseline	1625 ± 147	1727 ± 215	1684 ± 261
6 months	1607 ± 226	1691 ± 305	1618 ± 279
Carbohydrate (g)			
Baseline	225.1 ± 19.3	239.8 ± 33.8	230.4 ± 42.7
6 months	215.8 ± 23.1	248.7 ± 44.3	245.5 ± 35.2
Protein (g)			
Baseline	53.9 ± 6.1	55.6 ± 7.2	51.1 ± 5.4
6 months	50.2 ± 5.6	56.9 ± 6.7	53.8 ± 8.5
Fat (g)			
Baseline	58.4 ± 7.6	63.2 ± 5.2	61.5 ± 7.3
6 months	55.5 ± 8.7	59.4 ± 6.3	57.4 ± 6.2
Vitamin C (mg)			
Baseline	95.0 ± 19.2	105.2 ± 15.9	99.7 ± 16.5
6 months	90.2 ± 23.6	104.3 ± 16.1	105.1 ± 21.4
Vitamin E (IU)			
Baseline	300 ± 24	284 ± 20	296 ± 32
6 months	312 ± 36	288 ± 24	296 ± 28

1. Values are the mean ± SD.

2. “*” means significant difference between the placebo group and the Cr or Cr + C + E group.

Table 3. Effects of 6 months of chromium or combined chromium with vitamins C and E supplementation on blood glucose and insulin variables

	Placebo (<i>n</i> = 10)	Cr (<i>n</i> = 10)	Cr + C + E (<i>n</i> = 10)
Fasting glucose (mmol/L)			
Baseline	12.25 ± 0.93	12.52 ± 0.51	12.24 ± 0.45
6 months	12.23 ± 0.43	11.12 ± 0.42*	11.04 ± 0.53*
HbA1c (%)			
Baseline	10.1 ± 0.4	10.2 ± 0.5	10.0 ± 0.5
6 months	10.2 ± 0.4	9.5 ± 0.2*	9.3 ± 0.5*
Insulin (pmol/L)			
Baseline	108.38 ± 16.50	103.58 ± 12.19	106.2 ± 11.11
6 months	120.72 ± 7.12	98.74 ± 9.55*	92.07 ± 8.67*
Insulin resistance index			
Baseline	8.3 ± 0.9	8.1 ± 0.9	8.1 ± 0.8
6 months	9.2 ± 0.8	6.9 ± 0.7*	6.3 ± 0.9*

1. Values are the mean ± SD.

2. “*” means significant difference between the placebo group and the Cr or Cr + C + E group.

3. Insulin resistance index (HOMA-IR) = fasting blood glucose (mmol/L) × fasting serum insulin (μU/mL)/22.5

9.5 ± 0.2% and 8.1 ± 0.9 to 6.9 ± 0.7, respectively. Analogous significantly decreases ($p < 0.05$) for the Cr + C + E group were 12.2 ± 0.5 to 11.0 ± 0.5 mmol/L and 10.0 ± 0.5 to 9.3 ± 0.5% and 8.1 ± 0.8 to 6.3 ± 0.9, respectively.

Effects of supplementation on blood and urinary Cr

Baseline levels of plasma Cr were not significantly different demonstrated comparing the supplementation and placebo groups. After 6 months of Cr or Cr + C + E supplementation, the levels of plasma Cr were significantly

Table 4. Effects of 6 months of chromium or combined chromium with vitamins C and E supplementation on blood and urinary chromium

	Placebo (n = 10)	Cr (n = 10)	Cr + C + E (n = 10)
Blood Cr ($\mu\text{g/dL}$)			
baseline	0.16 \pm 0.02	0.18 \pm 0.07	0.18 \pm 0.08
6 months	0.15 \pm 0.05	0.57 \pm 0.15*	0.58 \pm 0.18*
Urinary Cr (ng/mg of creatinine)			
baseline	0.21 \pm 0.05	0.17 \pm 0.02	0.18 \pm 0.02
6 months	0.25 \pm 0.05	0.95 \pm 0.24*	0.93 \pm 0.17*

1. Values are the mean \pm SD.

2. "*" means significant difference between the placebo group and the Cr or Cr + C + E group.

Table 5. Effects of 6 months of chromium or combined chromium with vitamins C and E supplementation on markers of oxidative stress

	Placebo (n = 10)	Cr (n = 10)	Cr + C + E (n = 10)
TBARS ($\mu\text{mol/L}$)			
Baseline	5.43 \pm 0.16	5.42 \pm 0.11	5.52 \pm 0.11
6 months	5.58 \pm 0.09	4.41 \pm 0.10***	3.60 \pm 0.07***
TAS (mmol/L)			
Baseline	1.10 \pm 0.04	1.14 \pm 0.06	1.11 \pm 0.05
6 months	1.11 \pm 0.08	1.27 \pm 0.04*	1.31 \pm 0.07*
SOD (U/g of erythrocytes)			
Baseline	889.3 \pm 31.2	895.4 \pm 22.7	882.8 \pm 24.3
6 months	894.5 \pm 28.7	904.0 \pm 21.2	911.4 \pm 29.9
GPx (U/g of erythrocytes)			
Baseline	9.57 \pm 0.82	9.63 \pm 0.56	9.52 \pm 0.61
6 months	9.54 \pm 0.45	11.25 \pm 0.49*	11.85 \pm 0.62*
Catalase (KU/g of erythrocytes)			
Baseline	12.3 \pm 0.8	12.1 \pm 0.5	12.8 \pm 0.2
6 months	12.4 \pm 1.0	12.7 \pm 1.0	12.4 \pm 0.4

1. Values are the mean \pm SD.

2. "*" means significant difference between the placebo group and the Cr or Cr + C + E group.

3. "***" means significant difference between the Cr group and the Cr + C + E group.

increased ($p < 0.05$) in the treatment groups compared to placebo (Table 4). The baseline levels of urinary Cr were not significantly different for all groups, increasing about five-fold after Cr and Cr + C + E supplementation, respectively (Cr, Cr + C + E>placebo; Table 4).

Effects of supplementation on blood TBARS and TAS

Mean baseline TBARS levels were 5.43, 5.42 and 5.52 $\mu\text{mol/L}$ for the placebo, Cr, and Cr + C + E groups, respectively. They were not statistically different at baseline. After 6 months of supplementation; the mean plasma TBARS levels for the placebo, Cr and Cr + C + E groups were 5.58, 4.41, and 3.60 $\mu\text{mol/L}$, respectively. The differences between placebo and Cr, Cr + C + E groups were significant ($p < 0.05$). After 6 months of supplementa-

tion, plasma TBARS levels decreased significantly in Cr + C + E groups compare to Cr alone. Baseline plasma TAS levels were 1.10, 1.14 and 1.11 mmol/L for the placebo, Cr and Cr + C + E groups, respectively, rising to 1.11, 1.27, and 1.31 mmol/L, after 6 months of placebo, Cr or Cr + C + E supplementation. Plasma TAS levels significantly increased for the Cr and Cr + C + E groups relative to that in the placebo group (Table 5).

Effects of Cr or Cr + C + E supplementation on antioxidant enzyme activity

The baseline activity of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and catalase, were not significantly different demonstrated comparing the supplementation and placebo groups. After 6 months of Cr

or Cr + C + E supplementation, the activity of glutathione peroxidase were significantly increased ($p < 0.05$) in the treatment groups compared to placebo (Table 5). The activity of other antioxidant enzymes, such as superoxide dismutase and catalase, were not different before and after Cr or Cr + C + E supplementation (Table 5).

Discussion

Statistically and clinically significant effects were demonstrated related to daily supplementation of Cr (1000 μg) with or without vitamins C (1000 mg) and E (800 IU) on TBARS and TAS in patients with type 2 DM. At the onset of previous study, plasma TBARS levels in subjects with type 2 DM were significantly higher compared to apparently healthy subjects (5.41 ± 0.11 vs 2.89 ± 0.10 $\mu\text{mol/L}$), thus, apparently confirming the notion that lipid peroxidation increases in diabetes [31]. This 2.52- $\mu\text{mol/L}$ difference in TBARS reflects the increased lipid peroxidation noted in other DM studies [13, 32, 33]. After 6 months supplementation the mean TBARS levels for the placebo, Cr and Cr + C + E groups were 5.58, 4.41 and 3.60 $\mu\text{mol/L}$, respectively, with significant decreases ($p < 0.05$) demonstrated for the latter two groups. Anderson *et al.*, reported that 6 months of Cr supplementation (400 μg chromium picolinate) significantly reduced plasma TBARS in subjects with type 2 DM [34]. Our results for the Cr group were similar to analogous findings of Anderson *et al.* [34]. Further, the plasma TBARS levels decreased 19 and 35% for the Cr and Cr + C + E groups, respectively. The present results also indicate that 6 months of supplementation with Cr in combination with vitamins C and E may significantly decrease the end products of plasma lipid peroxidation compared to Cr alone.

It has been proposed that lipid peroxide levels, which are elevated in DM, are the end products of membrane damage. These elevated peroxide levels may result from the hyperglycemic state, specifically in relation to autoxidation of plasma glucose and other small autoxidizable molecules [35], and they are associated with poor metabolic control of plasma glucose [36]. In diabetes, the vulnerability to oxidative damage may be partly attributed to lower antioxidative micronutrient status, with this diminution including trace elements. Impairments in Cr, vitamin C or vitamin E status are reportedly aggravating factors in the progression of diabetes [6, 10, 37]. Further, the increase in lipid peroxidation products is associated with insulin perturbations [38, 39]. In this study, the plasma antioxidant levels were verified using two independent parameters, TBARS and TAS. After 6 months of Cr or Cr + C + E supplementation, the mean plasma TAS levels had increased significantly (11 and 18%, respectively) (Table 4). It appears reasonable to suggest, therefore, that high-dosage supplementation with Cr in combination with vitamins C and E may be suitable for

treatment of severely hyperglycemic diabetes.

Vitamin E is considered the most important lipid-soluble exogenous antioxidant in humans. Vitamin C serves as an antioxidant, directly by scavenging aqueous peroxy radicals, and indirectly by regenerating reduced vitamin E [19]. Vitamins C and E defend against the damaging effects of high oxidative stress owing to nonenzymatic and autoxidative glycosylation, and metabolic stress in persons with diabetes [20]. Chromium, like vitamin E, has been shown to protect rats from oxidative damage related to carbon tetrachloride exposure [14], and also decreases lipid peroxidations in isolated rat hepatocytes [40]. Hepatic and renal TBARS were also reduced in hypertensive rats receiving Cr as polynicotinate [40].

Most Cr nutrition studies have focused on the role of Cr for prevention of insulin resistance. In Vladeva's study [41], they mentioned that insulin resistance index decreased after a two month application of Cr 30 mg daily in type 2 DM patients. So they demonstrated that Cr included early in the complex therapy of diabetes is beneficial in the reduction of the degree of insulin resistance. In Martin's study [42], they found that insulin sensitivity increased after a six month application of Cr picolinate 1000 μg daily in type 2 DM patients. They supposed that Cr supplementation in subjects with type 2 DM improved insulin sensitivity and glucose control. In our study, the results show a markedly decrease of blood insulin and insulin resistance index after a six month supplementation of Cr and Cr combined with vitamin C and E groups, these results are similar to those mentioned above. The mechanism of Cr for decreasing the insulin resistance is analyzed in the light of an improved first phase of secretion of insulin or facilitated post-receptor insulin sensibility as a way of potentiating the insulin action [41]. In addition, in Duman's study [2], they demonstrated that chromium through enhance action of insulin at adipocytes by increasing intracellular triglyceride synthesis and decreasing extracellular lipid. Thus, it decreases tendency to extracellular lipid peroxidation, and results in increasing tendency to plasma TAS. So we suppose that the effect of antioxidant of Cr could be an important factor for insulin resistance improvement. In some studies, vitamin C supplementation was provided by systemic infusion in the dose range of 500–2000 mg, and it was observed that this vitamin enhanced glucose disposal by enhancing insulin sensitivity [43]. In another *in vitro* study indicated that vitamin E may improve insulin action and insulin secretion by protecting peripheral tissues and β -cells from free radical-mediated damage [44]. However, in our study, we also found the TBARS value significantly decreased in Cr + C + E group compared to that in Cr alone group, but we did not observe markedly diminished for the insulin resistance index in Cr + C + E group compared to Cr group. Thus, we think the relationship between antioxidant and insulin resistance

needs further study.

In conclusion, Cr alone and combining Cr with vitamins C and E supplementation elicited lower levels of plasma TBARS, blood glucose, HbA1c and insulin resistance index; higher levels of TAS as well in subjects with type 2 diabetes mellitus (HbA1C>8.5%) in this study. TBARS value significantly decreased in Cr + C + E group compared to that in Cr alone group. However, further investigation is needed to confirm these encouraging results.

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