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Antioxidant Effects of Chromium Supplementation with Type 2 Diabetes Mellitus and Euglycemic Subjects

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To determine the effects of chromium (Cr) supplementations on oxidative stress of type 2 diabetes and euglycemic (EU) subjects, adult having HbA_{1C} values of <6.0% (EU), 6.8−8.5% (mildly hyperglycemic, MH), and >8.5% (severely hyperglycemic, SH) were supplemented for 6 months with 1000 μg/day of Cr (as Cr yeast) or with a placebo. In the beginning, the levels of the plasma Cr in the MH and SH groups were 25−30% lower than those of the EU subjects. The values of thiobarbituric acid reactive substances (TBARS) and total antioxidative status (TAS) of the MH and SH groups were significantly higher than those of the EU ones. Following supplementations, the levels of plasma TBARS in the Cr groups of MH and SH groups were significantly decreased (the inverse was found in the EU) and showed no significant changes in the placebo group. The levels of plasma TAS in the Cr groups of EU and MH were significantly decreased (the inverse was found in the SH) and showed no significant changes in the placebo group. No significant difference was found in the antioxidant enzyme (superoxide dismutase, glutathione peroxidase, catalase) activities during supplementations. These data suggest that Cr supplementation was an effective treatment strategy to minimize increased oxidative stress in type 2 diabetes mellitus patients whose HbA_{1C} level was >8.5%, and the Cr in EU groups might act as a prooxidant.

KEYWORDS: Antioxidants; chromium; diabetes; total antioxidative status (TAS); thiobarbituric acid reactive substances (TBARS)

INTRODUCTION

Diabetes is a disease believed to be associated with increased oxidative stress because increased blood concentrations of thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, have been reported (*1*). Oxygen-derived radicals and reactive oxygen species are known to attack cell membranes and result in the propagation of lipid peroxidations.

Oxidative damage due to free radicals is associated with vascular disease in people with types 1 and 2 diabetes mellitus (DM) (*2*). There are several potential resources of free radical production in diabetics including autoxidation of plasma glucose (*1*), activation of leucocytes, and increased transition metal bioavailability (*3*). The total antioxidant status (TAS) in type 1 or 2 DM was lower than that of age-matched controls, and this might be attributed to lower levels of vitamin C, vitamin E (*4*), or other factors including micronutrients (*5*−*8*) in blood.

Anderson et al. (*8*) reported the beneficial effects of supplemented Cr on plasma glucose and related variables in type 2 DM patients. Accompanying these data, there are also studies that suggest Cr also improves the cellular antioxidant capacity in rats (*9*−*11*). Therefore, a restored Cr status in people with type 2 DM may counteract the deleterious effects of oxidative stress and help prevent complications associated with diabetes (*8*, *12*, *13*).

In Taiwan, a 5-fold increased mortality exists from the incidence of complications from DM in the most recent 20 years. For type 2 DM, there are high incidences of oxidative complications such as retinopathies, glomerulopathies, and vascular complications. Therefore, this study was conducted in Taipei and investigated the effects of Cr supplementation on both mildly hyperglycemic (MH) and severely hyperglycemic (SH) diabetes variables associated with oxidative stresses. Healthy age- and gender-matched adults comprised the control group.

MATERIALS AND METHODS

Subjects. Volunteers were adult males and females under 56 years old who had been diagnosed with diabetes at least 5 years previously (a fasting glucose level >7.3 mmol/L and an HbA_{1C} level >6.8%). Key exclusion criteria included pregnant and lactating women, people receiving trace element supplements in the previous 3 months, people undergoing gastric or diuretic treatments, patients with acute renal failures (creatinine level <120 μmol/L), and patients who had recently

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Table 1. Baseline Characteristics of Subjects

	euglycemic (EU)		mildly hyperglycemic (MH)		severely hyperglycemic (SH)	
	placebo (11)[a]	Cr (12)	placebo (9)	Cr (11)	placebo (10)	Cr (11)
men/women (*n*)	5/10	3/9	6/3	8/3	5/5	4/7
age[b] (years)	46.7 ± 1.6	47.9 ± 2.1	50.8 ± 2.3	52.5 ± 2.0	50.5 ± 1.9	53.1 ± 2.0
body mass index[b] (BMI; kg/m^2)	23.9 ± 0.7	24.0 ± 0.9	26.8 ± 1.0	27.3 ± 0.7	27.8 ± 0.8	25.9 ± 0.7
fasting glucose[b] (mmol/L)	4.86 ± 0.09c	5.15 ± 0.17c	7.86 ± 0.47b	7.95 ± 0.47b	12.25 ± 0.93a	13.52 ± 0.51a
HbA_{1C}[b] (%)	5.0 ± 0.1c	5.1 ± 0.1c	7.4 ± 0.4b	7.7 ± 0.4b	9.1 ± 0.4a	10.3 ± 0.6a
insulin[b] (pmol/L)	80.36 ± 7.89b	81.79 ± 7.89b	116.23 ± 22.96a	96.86 ± 9.32a	118.38 ± 16.50a	97.58 ± 12.19a

[a] Numbers in parentheses denote the number of subjects per group. [b] Values are the mean ± SEM. Values with different letters in a given row significantly differ from one another at $P < 0.05$ as determined by Duncan's multiple-range test.

Table 2. Supplementation Effects on Blood and Urinary Chromium

	euglycemic (EU)		mildly hyperglycemic (MH)		severely hyperglycemic (SH)	
	placebo (15)[a]	Cr (12)	placebo (9)	Cr (11)	placebo (10)	Cr (11)
blood Cr (μg/dL)						
initial[b]	0.30 ± 0.05a	0.22 ± 0.04a	0.19 ± 0.04ab	0.20 ± 0.02ab	0.16 ± 0.02b	0.18 ± 0.07b
6 month[b]	0.24 ± 0.06b	0.59 ± 0.05*a	0.17 ± 0.01b	0.57 ± 0.07*a	0.15 ± 0.05b	0.57 ± 0.15*a
urinary Cr (ng/mg of creatinine)						
initial[b]	0.15 ± 0.04	0.19 ± 0.05	0.17 ± 0.03	0.18 ± 0.04	0.21 ± 0.05	0.17 ± 0.02
6 month[b]	0.14 ± 0.02c	1.15 ± 0.17*b	0.15 ± 0.04c	1.50 ± 0.30*a	0.25 ± 0.05c	1.34 ± 0.24*a

[a] Numbers in parentheses denote the number of subjects per group. [b] Values are the mean ± SEM. *, significant effects of supplementation at $P < 0.001$. Values with different letters in a given row significantly differ from one another at $P < 0.05$ as determined by Duncan's multiple-range test.

undergone surgery or had an acute infection. Patients were enrolled from Taipei Medical University Hospital. The study received the approval of the Human Studies Review Board of Taipei Medical University Hospital. Patients were informed of the purposes of the study, were free to ask questions throughout the study, and signed an informed consent form witnessed by one of the investigators. This study design was under double-blind and placebo control. Subjects, $n = 68$, were divided into three groups, euglycemia (EU, HbA_{1C} level of <6%), mild hyperglycemia (MH, HbA_{1C} level of 6.8−8.5%), and severely hyperglycemic (SH, HbA_{1C} level >8.5%). Each group was divided into two random subgroups, which were supplemented daily either with 1000 μg of Cr as Cr yeast chromium(III) or with a placebo; both were provided by Westar Nutrition Corp. (Costa Mesa, CA). The concentrations of fasting glucose of EU, MH, and SH subjects were 4.7−5.3, 7.3−8.4, and >8.5 mmol/L, respectively. The quantity of chromium was verified by chemical analysis. Each month volunteers who had received their daily doses for 1 month were asked to return the nonused supply to help measure their compliance. Subjects were also asked questions regarding any possible side effects and their degrees of compliance.

Analytical Methods. Blood samples were drawn after an overnight fast in the beginning of the study and after 6 months of daily supplementations. Blood was taken from the antecubital vein and collected in a Vacutainer trace element-free tube (Becton-Dickinson). Urine samples were collected in 100-mL polypropylene specimen containers (Falcon) and stored at −20 °C. All samples were run prior to the breaking of the code that was not available to the investigators until completion of all the samples. Blood and urinary Cr levels were determined with a Hitachi Z-5000 polarized Zeeman atomic absorption spectrophotometer, using standard electrothermal graphite furnace techniques (*14*). The in-house urinary sample (as a control check) was assayed at least twice a day for the accuracy of the urinary Cr analysis (*15*).

Plasma samples were stored at −70 °C prior to batch analyses. The autoanalyzer system (Hitachi-7170) and diagnostic kits (Shino) were used for plasma glucose concentrations. HbA_{1C} was assayed with the HbA_{1C} kit (Bayer). The insulin kit (DPC) was used for insulin analyses.

Plasma TBARS were determined for the assay of malondialdehyde and thiobarbituric acid complex (*16*) by fluorometry kit after extraction with *n*-butanol. TAS was determined for the assay of the rate of peroxidation through the loss of fluorescence of the protein *R*-phycoerythrin induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (*17*). The lag phase was compared to that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) used.

Catalase activity was measured spectrophotometrically (*18*) by the change in absorbance at 240 nm. Superoxide dismutase activity was determined according to the method of McCord and Fridovich (*19*). Glutathione peroxidase activity was measured according to the method of Lawrence and Burk (*20*). Activities were calculated as units per milligram of protein to normalize differences between control and diabetic blood. The Lowry method (*21*) was used for protein determinations.

Statistical Analysis. Statistical analyses of the data were performed using the analysis of variance. Individual mean comparisons were identified with Duncan's multiple-range tests (SAS, SAS Institute, Cary, NC). Values are expressed as means ± SEM. Group means were considered to be significantly different at $P < 0.05$.

RESULTS

Effects of Supplementation on Changes of Blood and Urinary Cr. In the beginning of the study (**Table 1**), the supplementation and placebo groups in each EU, MH, and SH group were similar on the basis of gender, age, body masss index (BMI), fasting glucose, HbA_{1C}, and insulin parameters. However, there were differences in concentrations of fasting glucose and HbA_{1C} values among EU, MH, and SH groups following 6 months of supplementation, with the SH group having the highest values. In the SH group, the fasting glucose decreased from 13.5 ± 0.5 to 12.9 ± 1.4 mmol/L following 6 months of Cr supplementation, and HbA_{1C} decreased from 10.3 ± 0.6 to 9.7 ± 0.6% (data not shown).

In the beginning, the levels of plasma Cr were similar and showed no significant differences between the supplementation and placebo subgroups, but showed the order of EU > MH > SH (**Table 2**). After 6 months of Cr supplementation, the levels of plasma Cr were significantly increased ($P < 0.001$) in the Cr supplementation groups compared to those of the placebo groups in all three groups (**Table 2**). The levels of plasma Cr in MH and SH subjects were 25−30% lower than that of EU subjects in the beginning (**Table 2**); however, there were no

Table 3. Effects of 6 Months of Chromium Supplementation on Plasma Thiobarbituric Acid Reactive Substances (TBARS) and Total Antioxidative Status (TAS)

	euglycemic (EU)		mildly hyperglycemic (MH)		severely hyperglycemic (SH)	
	placebo (15)[a]	Cr (12)	placebo (9)	Cr (11)	placebo (10)	Cr (11)
TBARS (μmol/L)						
initial[b]	3.01 ± 0.05c	2.89 ± 0.10c	4.14 ± 0.13b	4.00 ± 0.15b	5.43 ± 0.16a	5.41 ± 0.13a
6 month[b]	3.11 ± 0.09c	4.00 ± 0.09**b	4.22 ± 0.12b	3.71 ± 0.10**bc	5.58 ± 0.09a	4.43 ± 0.10**a
TAS (mmol/L)						
initial[b]	0.91 ± 0.05b	1.00 ± 0.04b	1.11 ± 0.04a	1.22 ± 0.04a	1.10 ± 0.04a	1.13 ± 0.06a
6 month[b]	0.96 ± 0.06b	0.85 ± 0.05*c	1.13 ± 0.08a	0.98 ± 0.06*b	1.11 ± 0.08b	1.26 ± 0.05*a

[a] Numbers in parentheses denote the number of subjects per group [b] Values are the mean ± SEM. *, significant effect of supplementation at $P < 0.01$; **, significant effect of supplementation at $P < 0.001$. Values with different letters in a given row significantly differ from one another at $P < 0.05$ as determined by Duncan's multiple-range test.

Table 4. Effects of 6 Months of Chromium Supplementation on Plasma Antioxidant Enzymes

	euglycemic (EU)		mildly hyperglycemic (MH)		severely hyperglycemic (SH)	
	placebo (15)[a]	Cr (12)	placebo (9)	Cr (11)	placebo (10)	Cr (11)
SOD (U/g of protein)						
initial[b]	866.9 ± 28.0	933.7 ± 22.9	959.0 ± 20.2	891.8 ± 22.0	889.3 ± 3 1.2	925.4 ± 23.7
6 month[b]	857.3 ± 83.1	961.5 ± 45.5	914.6 ± 28.6	852.0 ± 49.1	894.5 ± 48.7	914.0 ± 47.2
GPx (U/g of protein)						
initial[b]	8.09 ± 0.94	8.63 ± 0.62	7.17 ± 0.82	9.58 ± 0.53	9.57 ± 0.82	9.63 ± 0.56
6 month[b]	8.67 ± 0.98	8.29 ± 0.89	7.14 ± 0.90	9.99 ± 1.77	9.54 ± 1.25	9.25 ± 1.09
catalase (kU/g of protein)						
initial[b]	11.9 ± 0.5	11.6 ± 0.5	12.0 ± 0.4	12.3 ± 0.5	12.3 ± 0.8	12.1 ± 0.5
6 month[b]	11.5 ± 1.2	11.4 ± 0.6	12.6 ± 0.7	12.6 ± 0.8	12.4 ± 1.0	12.7 ± 1.0

[a] Numbers in parentheses denote the number of subjects per group. [b] Values are the mean ± SEM. SOD, superoxide dismutase; GPx, glutathione peroxidase.

significant differences among EU, MH, and SH after 6 months of supplementation. The levels of urinary Cr were similar in all groups in the beginning and increased 10-fold after Cr supplementation (**Table 2**).

Effects of Cr Supplementation on Blood TBARS and TAS. In the beginning, the mean levels of the TBARS were 2.89, 4.0, and 5.41 μmol/L, respectively, for the EU, MH, and SH groups. The levels of the TBARS in MH and SH subjects were significantly higher ($P < 0.05$) than those of EU subjects (**Table 3**). After 6 months of Cr supplementation, the mean levels of the TBARS were 4.0, 3.71, and 4.43 μmol/L, respectively, for the EU, MH, and SH groups. There were significant decreases ($P < 0.001$) of plasma TBARS in MH and SH subjects; however, inversely there was a significant increase ($P < 0.001$) in EU subjects. No significant changes of TBARS were found in any of the three placebo groups. The levels of plasma TBARS decreased 18.1 and 7.25%, respectively, in the SH group (5.41 ± 0.13 vs 4.43 ± 0.10 μmol/L) and MH group (4.00 ± 0.15 vs 3.71 ± 0.10 μmol/L). However, the levels of plasma TBARS increased 38.4% in the EU group (2.89 ± 0.10 vs 4.00 ± 0.09 μmol/L). In the beginning, the levels of plasma TAS were 1.0, 1.22, and 1.13 mmol/L, respectively, for the EU, MH, and SH groups. The levels of plasma TAS in the MH and SH groups were significantly higher ($P < 0.05$) than those in the EU group (**Table 3**). After 6 months of Cr supplementation, the mean levels of the TAS were 0.85, 0.98, and 1.26 mmol/L, respectively, for the EU, MH, and SH groups. There were significant increases ($P < 0.01$) of plasma TAS in the SH group; however, inversely there were significant decreases in both the EU and MH groups (**Table 3**). No significant changes of plasma TAS were found in the placebo group.

Effects of Cr Supplementation on Antioxidant Enzyme Activities. Antioxidant enzyme activities, such as superoxide dismutase, glutathione peroxidase, and catalase, were not changed before and after Cr supplementation (**Table 4**). However, catalase activities were higher in the MH and SH groups than in the EU group.

DISCUSSION

The present data demonstrated the significant effects both statistically and clinically of supplemental 1000 μg/day Cr on TBARS and TAS in people with type 2 DM. Three groups of EU, MH, and SH subjects had different results after 6 months of Cr supplementation. At the onset of this study, plasma TBARS were significantly higher in the MH and SH groups compared to that of EU group, which consisted of apparently healthy subjects. These differences of TBARS were 1.11 and 2.52 μmol/L (**Table 3**), which were similar to results of increased lipid peroxidation in DM (*13*, *22*, *23*). After 6 months of Cr supplementation, the mean levels of the TBARS were 4.0, 3.71, and 4.43 μmol/L, respectively, for the EU, MH, and SH groups. There were significant decreases ($P < 0.001$) of plasma TBARS in MH and SH subjects; however, inversely there was a significant increase ($P < 0.001$) in EU subjects. Anderson et al. (*13*) reported that 6 months of Cr supplementation (400 μg of chromium pidolate) in people with type 2 DM could significantly reduce plasma TBARS and not significantly change antioxidant enzymes (such as Cu/Zn superoxide dismutase and glutathione peroxidase). Our results (MH and SH groups) were similar to those of Anderson et al. (*13*). However, the present results also indicate that 6 months of Cr supplementation in the EU group might increase the end products of lipid peroxidation in plasma.

The lipid peroxides were proposed to be the end products of membrane damage, which were elevated with DM. These elevated levels of peroxides could result from the hyperglycemic state in relation to autoxidation of plasma glucose and other small autoxidizable molecules (*24*) and were associated with

poor metabolic controls of plasma glucose (*25*). In diabetes, the vulnerability to oxidative damage might be partly attributed to a lower antioxidative micronutrient status including trace elements. Impairments of Cr status (*7*, *26*) have been reported as aggravating factors in the progression of diabetes. The increased lipid peroxidation products were associated with insulin perturbations (*27*, *28*). The plasma antioxidant levels were verified by two independent parameters, TBARS and TAS. Therefore, this was the first report using TAS, which stated the total radical-trapping parameters, to determine the effect of Cr supplementation on EU, MH, and SH subjects. Even though plasma TBARS increased 38.4% in the EU group after 6 months of Cr supplementation (**Table 3**), a high dosage of Cr might not be suitable for EU subjects.

Chromium, like vitamin E, protected rats from oxidative damage related to carbon tetrachloride (*9*) and also decreased lipid peroxidations in isolated rat hepatocytes (*29*). In hypertensive rats receiving Cr as polynicotinate, hepatic and renal TBARS were also reduced (*29*). Most Cr nutrition studies were focused on the role of Cr in preventing insulin resistance; however, in the light of our results, interactions among insulin sensitizers and antioxidants should also be evaluated (*29*). The mechanism by which Cr acted as an antioxidant is still not totally understood. The amount of Cr used in this study might be adequate to result in an improved antioxidant status in SH subjects but might also be adequate to meet the requirements for measurable changes in the glucose/insulin system in SH subjects. Discrepancies in the response to Cr were dependent upon the forms of Cr (pidolate vs picolinate vs yeast) used and the duration of diabetes and the status of subjects, including their dietary habits. During this study, blood Cr increased 3-fold and urinary Cr losses were almost 10-fold in Cr-supplemented groups by the end of the study compared with those values at the onset of the study. We have not observed such changes in any of our studies involving nonsupplemented subjects. Urinary Cr losses were significantly higher in the MH and SH groups than in the EU group. The Cr intake of subjects appeared to increase during the study, and dietary Cr intake studies need to be completed to determine foods that are high in Cr for diabetes subjects. In this study, Cr elicited lower levels of plasma TBARS and higher levels of TAS in the $HbA_{1C} > 8.5\%$ type 2 diabetes mellitus (SH) group. Therefore, studies reporting effects of Cr on the glucose/insulin system may also be consistent with effects on free radical production. Further studies are needed to confirm these results.

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