RESEARCH ARTICLE

Effect of chromium dinicocysteinate supplementation on circulating levels of insulin, TNF-α, oxidative stress, and insulin resistance in type 2 diabetic subjects: Randomized, double-blind, placebo-controlled study

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Scope: Chromium and cysteine supplementation have been shown to improve glucose metabolism in animal studies. This study examined the hypothesis that chromium dinicocysteinate (CDNC), a complex of chromium and L-cysteine, is beneficial in lowering oxidative stress, vascular inflammation, and glycemia in type 2 diabetic subjects.

Methods and results: Type 2 diabetic subjects enrolled in this study were given placebo for 1 month for stabilization and then randomized into one of three groups: placebo (P), chromium picolinate (CP), or CDNC, after which they received daily oral supplementation for 3 months. Of the 100 patients enrolled in the study, 74 patients completed it. There were 25 patients in the P supplemented group, 25 in the CP supplemented and 24 in the CDNC supplemented group who completed the study. Blood markers of glycemia, vascular inflammation, HOMA insulin resistance, and oxidative stress were determined at randomization and after 3 months of supplementation with P, CP, or CDNC. There was a significant decrease at 3 months in insulin resistance ($p = 0.02$) and in the levels of protein oxidation ($p = 0.02$) and TNF-α ($p = 0.01$) in the CDNC supplemented cohort compared to baseline. However, there was no statistically significant change in these markers in the CP supplemented group compared to baseline. Insulin levels significantly decreased ($p = 0.01$) for subjects receiving CDNC but not CP. There was no significant impact of supplementation on HbA_{1c} or glucose levels in either of the groups.

Conclusion: CDNC supplementation lowers insulin resistance by reducing blood levels of TNF-α, insulin, and oxidative stress in type 2 diabetic subjects. Therefore, CDNC supplementation has potential as an adjunct therapy for individuals with type 2 diabetes.

Keywords:
Chromium / Diabetes / Insulin resistance / L-cysteine / Oxidative stress

1 Introduction

Diabetes now affects 366 million people worldwide and is responsible for one death every 7 s, or about 4.6 million deaths each year. According to the American Diabetes Association, 23.5 million or 10.7% of the US population aged 20 years and older have diabetes. Intensive blood glucose control dramatically reduces the devastating complications that result from poorly controlled diabetes. However, for many, achievement of tight glucose control is difficult with

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Abbreviations: ALT, alanine aminotransferase; **AP**, alkaline phosphatase; **AST**, aspartate aminotransferase; **BUN**, blood urea nitrogen; **CDNC**, chromium dinicocysteinate; **CP**, chromium picolinate; **CRP**, c-reactive protein; **MCP-1**, monocyte chemotactic protein-1; **GLUT**, glucose transporter; **HbA1c**, Hemoglobin A1c; **HOMA-IR**, homeostasis model assessment-estimated insulin resistance; **ICAM-1**, intercellular adhesion molecule 1; **IRS-2**, insulin receptor substrate 2; **LC**, L-cysteine; **NAC**, N-acetylcysteine; **NF-kB**, nuclear factor-kappaB; **TNF-α**, tumor necrosis factor-alpha; **ZDF**, Zucker diabetic fatty rats

current regimens. Chromium supplementation in the form of commercially available chromium dinicocysteinate (CDNC) or chromium picolinate (CP) is widely used by the diabetic patient population.

Trivalent chromium is an essential nutrient and has been shown to lower oxidative stress and improve glucose and lipid metabolism [1–5]. Subclinical chromium deficiency may contribute to insulin resistance and cardiovascular disease, particularly in aging and diabetic populations [6]. It has been proposed that chromium supplementation increases the amount of a chromium-containing oligopeptide present in the insulin-sensitive cells that bind to the insulin receptor, markedly increasing the activity of insulin-stimulated tyrosine kinase and phosphorylation of insulin receptor substrate-1 and glucose transporter GLUT4 [7].

Diabetes is associated with elevated levels of oxidative stress, which potentially impairs cellular glucose metabolism via a variety of mechanisms, including redox imbalance and insulin resistance [8–12]. Recent studies report lower blood levels of L-cysteine and altered cysteine homeostasis in diabetic patients [13, 14]. Along with a host of proteins, L-cysteine is a precursor of glutathione, which is considered essential for the reduction of cellular oxidative stress [15]. Dietary supplementation with N-acetylcysteine (NAC) or whey protein and α-lactoalbumin (cysteine-rich proteins) lowered the oxidative stress and insulin resistance induced by sucrose or fructose in rats and streptozotocin-treated diabetic mice [16–19]. Oral supplementation with L-cysteine lowered oxidative stress, vascular inflammation, and glycemia markers in ZDF rats, an animal model of type 2 diabetes [20]. Recent studies report that L-cysteine supplementation lowered oxidative stress markers in type 2 diabetic subjects and normal subjects [13, 21].

Previous animal studies reported the results of a head-to-head comparison between three chromium complexes in order to determine if any one of these complexes demonstrated a superior ability to modulate risk factors linked with diabetes [22]. One of these, CDNC, a complex of trivalent chromium with L-cysteine and niacin, proved to be the most efficacious in decreasing fasting glucose levels, glycated hemoglobin levels, insulin levels, and vascular inflammation in Zucker diabetic fatty rats as assessed by CRP, MCP-1, ICAM-1, and oxidative stress levels. We, therefore, undertook this pilot study to determine whether CDNC is superior to CP at lowering oxidative stress and insulin resistance in type 2 diabetic subjects. This study examined the effect of daily supplementation for 3 months on oxidative stress, insulin resistance, markers of vascular inflammation, and glycemia in type 2 adult diabetic patients.

2 Research design and methods

2.1 Patient enrollment

Informed written consent was obtained from all patients according to the protocol approved by the Louisiana State University Health Sciences Center Institutional Review Board (IRB). All patients included in this study were adults with type 2 diabetes. One hundred diabetic subjects were enrolled in this study. Fasting blood was collected at the screening visit and all subjects were given placebo supplementation for 1 month. After the 1-month placebo run-in period, subjects were randomized into three groups. Patients in one group continued taking placebo and those in the other two groups were given either chromium dinicocysteinate (orally, 400 μg Cr^{3+}/day) or CP (orally, 400 μg Cr^{3+}/day) supplementation

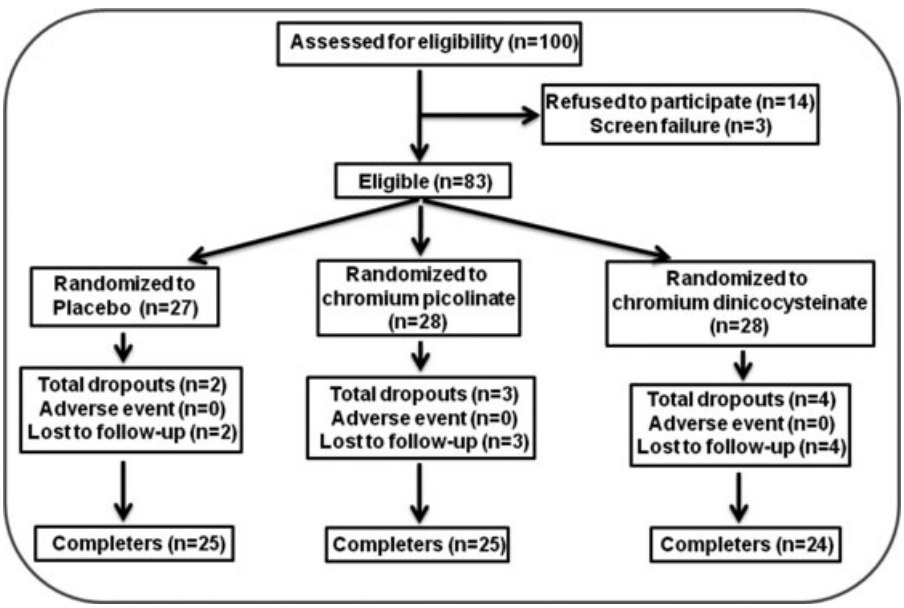

Figure 1. Enrollment, screen failure, randomization, and completion flow chart for all type 2 diabetic subjects.

for up to 3 months while receiving the usual standard care for diabetes. Medication usage was tracked throughout the duration of the study. Both subjects and investigators were blinded to the supplement (placebo or either formulation of Cr^{3+}) each subject received. Figure 1 gives flow chart for number of patients that were enrolled, screen failure, randomized, and those completed the study in each supplementation group. Of the 100 subjects screened, 74 subjects completed the study. Among those who discontinued, three were screen failures, 14 refused to enter the study, and nine were lost to follow-up.

2.2 Inclusion/exclusion criteria

Adult type 2 diabetic subjects ages 30–55 years were included in the study. Table 1 outlines the specific inclusion and exclusion criteria for patient enrollment in this study. Patients were excluded if they had any history of cardiovascular disease, sickle cell disease, treatment with insulin, or metabolic disorders, including: uncontrolled hypertension, hypothyroidism, or hyperthyroidism. Patients were excluded at baseline or at any visit if they showed signs of significant hepatic dysfunction, defined as any underlying chronic liver disease or liver function tests greater than 1.5 times the upper limit of normal or renal dysfunction, defined as a serum creatinine greater than 1.5 mg/dL. Women with a positive pregnancy test or those nursing infants were also excluded. Women who were sexually active were not included unless they agreed to use an effective form of contraceptive. All participants agreed not to take any supplemental vitamins or herbal products and those who started taking any supplements during the study period were withdrawn from the trial.

2.3 Chromium or placebo supplementation

Diabetic patients who agreed to participate in this study were asked to come to the clinic after overnight fasting. Initially all of the study subjects were given a placebo for 1 month run-in period prior to randomization. The outline of each clinic visit and clinical trial design is provided in Table 2. The blood levels determined at the randomization visit after the run-in period were considered baseline values. During the run-in period and before randomization, any subject identified to have an exclusion criterion was excluded from the study. The remaining subjects were randomized to receive supplementation with a placebo, chromium dinicocysteinate (400 μg Cr^{3+}/day), or CP (400 μg Cr^{3+}/day) for up to 3 months while receiving the usual standard care for diabetes. Both subjects and investigators were blinded to the medication (placebo or either formulation of chromium) each subject received. Pregnancy tests and renal and liver function tests were done at −1 (screening visit), 0 (baseline or randomization visit), and at the 1-, 2-, and 3-month supplementation visits to monitor for any sign of toxicity. All data presented here are from randomization (baseline) and after 3 months of supplementation. The placebo run-in period was designed to stabilize patients and prevent any effect due solely to inclusion in the study.

Table 1. Inclusion and exclusion criteria for the random trial

Inclusion criteria
Adults ages 30–55 years.
Participants must understand the risks and benefits of the protocol and sign an informed consent form provided
Willingness to participate in four additional clinic visits (Baseline, 1, 2, and 3 months)
Willingness to complete standard health history questionnaire before and during the study
Women with a negative pregnancy test
Exclusion criteria
History of cardiovascular disease, sickle cell disease, insulin intake, and metabolic disorders including hypertension, hypothyroid, and hyperthyroid
Any sign of hepatic and renal dysfunction as assessed from blood chemistry tests
Presently taking weight loss or other dietary supplements
Women with a positive pregnancy test or nursing females

2.4 Adverse effects (AE)

The nurse kept track of all the symptoms reported by the patient at each visit. In some instances, these symptoms were similar to preexisting conditions that were recorded in the patient charts. Other times, some symptoms were clearly unrelated to treatment (sports injuries, tooth extractions, decreased sex drive). To be in compliance with safety monitoring, all these symptoms regardless of relevance to the study were recorded. All of these were compiled into a single spreadsheet and summary is provided in Table 7. Overall, there do not appear to be any differences from one group to another group.

2.5 Blood collection

Blood was drawn from patients after an overnight fast (8 h). Following the blood collection at each visit, serum tubes for chemistry profile, EDTA tubes for HbA_{1C} and complete blood counts were promptly delivered to the LSUHSC clinical laboratories. Additional tubes of EDTA-blood were brought to research laboratory. Clear plasma was separated via centrifugation at 3000 rpm (1500 × *g*) for 15 min. All plasma samples were stored at −80°C for analyses of the biochemical parameters.

2.6 TNF-α, IL-6, IL-8, ICAM-1, insulin, oxidative stress (protein oxidation), and insulin resistance assays

TNF-α, IL-6, IL-8, ICAM-1, and insulin levels in the plasma were determined by the sandwich ELISA method using commercially available kits from Fisher Thermo Scientific Co.

Table 2. Trial design and time line of the clinical study

Screening	Trial period			
Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
• Subject enrollment • Supplementation with placebo • Prerandomization placebo-run-in period	• Randomization • Supplementation • Data collection[a)]	Data collection	Data collection	Data collection
Placebo-run-in 1 month	0 (Baseline)	1 month	2 months	3 months

a) Overnight fasting (12 h) blood samples, anthropomorphic data, record of daily insulin use, and blood glucose levels were collected at each visit.

(Rockford, IL). All appropriate controls and standards as specified by the manufacturer's kit were used. In the cytokine assay, control samples were analyzed each time to check the variation from plate to plate on different days of analysis. The protein oxidation was assessed by determining protein carbonyl level in the plasma using an ELISA kit from ENZO life sciences International Inc. (Plymouth Meeting, PA). Insulin resistance was calculated from blood glucose and insulin levels using HOMA insulin resistance method as described previously [12].

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise mentioned. Chromium dinicocysteinate was furnished by InterHealth Nutraceuticals, Inc. (Benicia, CA). CP was obtained from Nutrition-21 (Purchase, NY). Data were analyzed statistically using the Wilcoxon signed rank test to determine significant changes after 3-month supplementation for each treatment group and the Kruskal–Wallis test to compare the three treatment groups on changes. SAS version 9.2 (Cary, NC) was used for statistical computing. A p value of less than 0.05 for a statistical test was considered significant.

3 Results

Table 3 gives the ages, weights, BMI, glucose, HbA1c, and number of subjects in the placebo, CP, and CDNC supplemented groups. Table 4 also shows the glucose, glycated hemoglobin, and insulin levels of patients at baseline and after 3 months of supplementation. The ages, weights, BMI, glycemia levels of patients were similar in all three groups (Table 3). There were no differences in the glucose or HbA_{1c} levels after supplementation versus baseline in any of the groups (Table 4). However, there was a significant reduction in the insulin level after supplementation with CDNC versus before supplementation (Table 4). There was no significant difference in insulin levels after supplementation in the placebo or CP groups.

Figure 2 illustrates the effect of placebo, CP, or CDNC supplementation on insulin resistance in type 2 diabetic subjects. There was a significant decrease in insulin resistance after supplementation with CDNC compared with baseline levels. However, there was no significant difference in insulin resistance after placebo or CP supplementation. This suggests that CDNC supplementation can lower insulin resistance levels as determined by HOMA-IR in type 2 diabetic subjects. When subjects were divided into male and female groups; similar significant reduction in HOMA-IR was observed after CDNC supplementation in female group ($p = 0.035$) but not in male group ($p = 0.19$), which is likely due to number of male (n = 8) participants were lower than female (n = 16) participants. The differences were not significant when data was compared between CDNC versus CP or placebo-supplementation groups irrespective of when data was analyzed with all, male or female participants.

Table 5 shows no difference in levels of IL-6, IL-8, or ICAM-1 in any of the groups before versus after supplementation with placebo, CP, or CDNC in type 2 diabetic subjects. However, there was a significant decrease in levels of TNF-α (Fig. 3) and oxidative stress as determined by protein carbonyl (Fig. 4) in diabetic patients supplemented with CDNC for 3 months compared to baseline. Levels of oxidative stress or TNF-α did not change after placebo or CP supplementation in diabetic patients. This suggests that CDNC supplementation can lower oxidative stress and TNF-α levels in type 2

Table 3. Demographic and baseline characteristics of the trial subjects

	Placebo (n = 25)	Chromium picolinate (n = 25)	Chromium dinicocysteinate (n = 24)
Age (years)	48.64 ± 1.99	51.12 ± 2.03	48.79 ± 1.82
Gender: male/female (percentage)	2/23 (8.70%)	7/18 (38.89%)	8/16 (50%)
Weight (kg)	100.47 ± 5.52	103.94 ± 6.39	104.31 ± 6.93
BMI	38.00 ± 1.71	35.44 ± 2.06	36.85 ± 2.20
Fasting glucose (mg/dL)	142.4 ± 12.32	128.88 ± 8.78	131.95 ± 9.61
HbA_{1C} (percentage)	7.84 ± 0.34	7.54 ± 0.32	7.65 ± 0.35

Where applicable, values are expressed as Mean ± SEM. No significant difference was observed among the groups.

Table 4. Effect of chromium supplementation (daily for 3 months at 400 μg Cr/day) on various blood biomarkers in type 2 diabetic subjects

	Placebo (n = 25)			Chromium picolinate (n = 25)			Chromium dinicocysteinate (n = 24)		
	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value
Glucose (mg/dL)	142.4 ± 12.3	135.9 ± 13.3	NS	128.9 ± 8.8	137.2 ± 12.0	NS	131.9 ± 9.6	129.2 ± 12.6	NS
HbA_{1C} (percentage)	7.8 ± 0.3	7.8 ± 0.4	NS	7.5 ± 0.3	7.4 ± 0.3	NS	7.7 ± 0.4	7.5 ± 0.4	NS
Insulin (μU/mL)	30.6 ± 5.9	32.4 ± 5.2	NS	32.7 ± 5.6	25.3 ± 3.2	NS	34.7 ± 5.6	24.3 ± 3.9	0.01

Values are Mean ± SEM. $P < 0.05$ is considered statistically significant.

diabetic subjects. When participants were divided into male and female groups; similar significant reduction in TNF-α was observed after CDNC supplementation in female group ($p = 0.035$) and in male group ($p = 0.01$), for protein carbonyl differences were significant in female group ($p = 0.005$) but not for male group. The differences in TNF-α or protein carbonyl were not significant when data was compared between CDNC versus CP or placebo supplementation groups irrespective of when data was analyzed with all, male or female participants.

There were no differences in the liver function tests (AST, AP, ALT) or the renal function tests (BUN/creatinine ratio, serum creatinine), nor in the complete blood counts of patients after supplementation compared to baseline in any of the groups (Table 6). This suggests that daily supplementation with either CP or CDNC was not toxic in type 2 diabetic subjects. No differences were observed among the three cohorts with regards to medications used to control diabetes. Similarly, there was no adverse event reported related with supplementation in either of the groups (Table 7). No change in either body weight or BMI was detected using the DEXA assessment of body composition in either group (data not shown here).

4 Discussion

Uncontrolled hyperglycemia is known to increase reactive oxygen species (ROS) generation and oxidative stress in diabetic rats and diabetic patients [10, 11, 23, 24]. Oxidative stress and TNF-α levels are elevated in the blood of many diabetics and are positively associated with an increase in vascular inflammation and insulin resistance [10, 11, 25]. Insulin resistance and vascular inflammation are major risk factors that contribute to development of cardiovascular disease in diabetic patients.

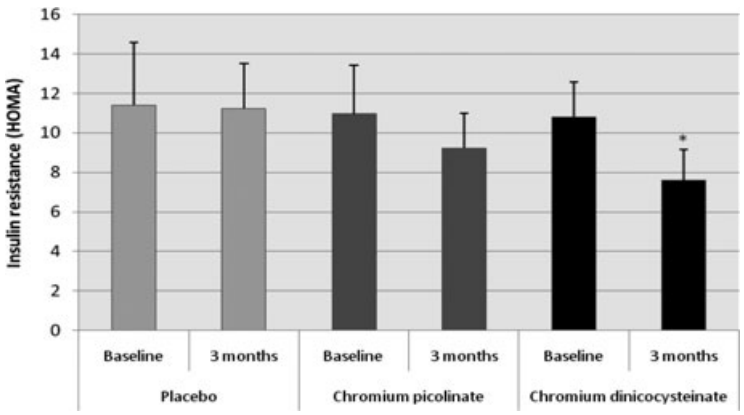

Figure 2. Effect of chromium dinicocysteinate, CP, and placebo supplementation (daily for 3 months, 400 μg Cr/day) on insulin resistance levels in type 2 diabetic subjects. Values are Mean ± SE. $^{*}p < 0.02$ compare with baseline values.

Table 5. Effect of chromium supplementation (daily for 3 months at 400 μg Cr/day) on various blood biomarkers in type 2 diabetic subjects

	Placebo (n = 25)			Chromium picolinate (n = 25)			Chromium dinicocysteinate (n = 24)		
	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value
IL-6 (pg/mL)	7.2 ± 0.6	7.4 ± 0.9	NS	8.1 ± 0.7	8.9 ± 0.6	NS	6.9 ± 0.5	7.1 ± 0.6	NS
IL-8 (pg/mL)	2.3 ± 0.3	2.8 ± 0.5	NS	2.9 ± 0.3	2.5 ± 0.4	NS	2.9 ± 0.7	3.3 ± 0.7	NS
ICAM-1 (ng/mL)	75.1 ± 10.1	80.8 ± 9.6	NS	107.8 ± 18.5	87.3 ± 13.4	NS	101.3 ± 14.6	91.8 ± 12.2	NS

Values are Mean ± SEM. No significant difference was observed among the treatment groups.

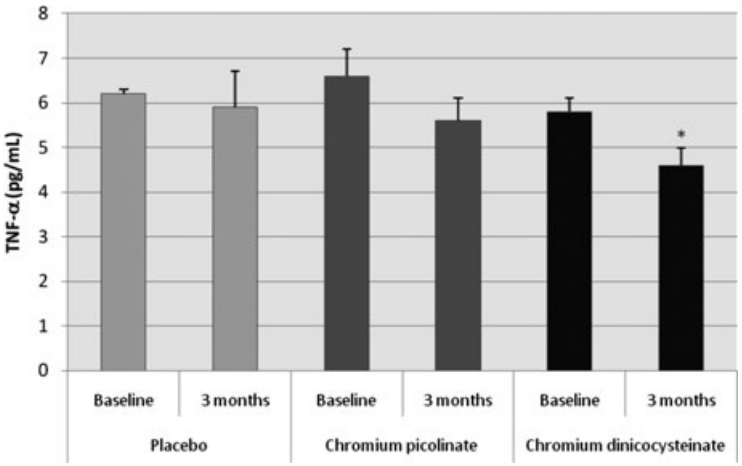

Figure 3. Effect of chromium dinicocysteinate, CP and placebo supplementation (daily for 3 months, 400 μg Cr/day) on TNF-α levels in type 2 diabetic subjects. Values are Mean ± SE. $^{*}p < 0.01$ compared with baseline values.

This study demonstrates a significantly lower protein oxidation level in diabetic patients supplemented with CDNC but not in those supplemented with placebo or CP. Oxidative stress is a known activator of NFkB, which undergoes nuclear translocation and serine phosphorylation at residue 276 of its p65 subunit. It then associates with surrounding chromatin components and binds with DNA, which promotes the transcription of pro-inflammatory cytokine TNF-α, which mediates the insulin resistance cascade. This study demonstrates that supplementation with CDNC significantly reduced circulating levels of TNF-α, insulin, and HOMA insulin resistance over a 3-month period in subjects already on diabetic medications. Conversely, supplementation with placebo or CP did not show any effect on TNF-α, protein oxidation, HOMA

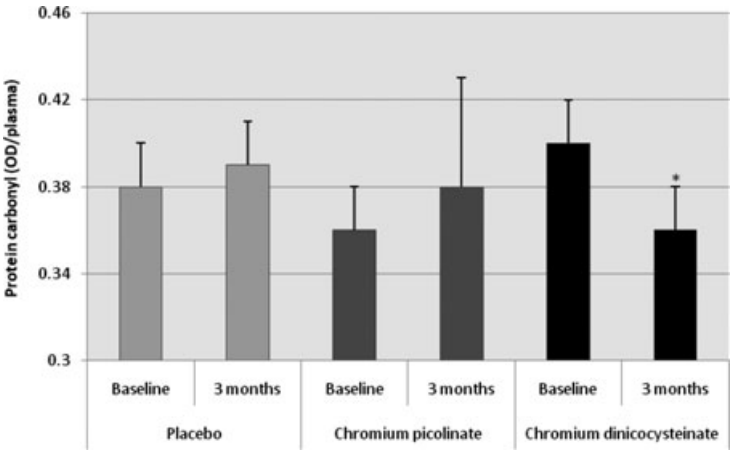

Figure 4. Effect of chromium dinicocysteinate, CP, and placebo supplementation (daily for 3 months, 400 μg Cr/day) on protein carbonyl in type 2 diabetic subjects. Values are Mean ± SE. $^{*}p < 0.02$ compared with baseline values.

Table 6. Effect of chromium supplementation (daily for 3 months at 400 μg Cr/day) on various blood biomarkers in type 2 diabetic subjects

	Placebo (n = 25)			Chromium picolinate (n = 25)			Chromium dinicocysteinate (n = 24)		
	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value	Baseline	3 Months	*p*-value
AST (U/L)	19.2 ± 2.7	19.4 ± 1.8	NS	20.1 ± 1.5	19.4 ± 1.6	0.54	19.33 ± 1.45	19.67 ± 2.29	NS
ALT (U/L)	30.0 ± 3.6	27.9 ± 2.4	NS	27.3 ± 1.9	28.9 ± 1.9	NS	26.4 ± 1.6	28.9 ± 2.3	NS
AP (U/L)	78.0 ± 3.8	75.9 ± 3.3	NS	76.4 ± 4.8	73.2 ± 3.8	NS	80.5 ± 5.3	80.7 ± 6.9	NS
Creatinine (mg/dL)	0.86 ± 0.20	0.85 ± 0.05	NS	0.90 ± 0.05	0.91 ± 0.05	NS	0.85 ± 0.04	0.82 ± 0.04	NS
BUN/creatinine (ratio)	17.6 ± 1.2	18.22 ± 1.45	NS	16.9 ± 1.4	17.8 ± 1.4	NS	16.1 ± 1.0	15.6 ± 1.2	NS
RBC (M/μL)	4.3 ± 0.4	4.4 ± 0.5	NS	4.4 ± 0.1	4.3 ± 0.1	NS	4.6 ± 0.5	4.6 ± 0.1	NS
Hemoglobin (g/dL)	12.4 ± 0.3	12.4 ± 0.3	NS	12.6 ± 0.3	12.5 ± 0.2	NS	13.2 ± 0.2	12.9 ± 0.3	NS
Hematocrit (percentage)	37.1 ± 0.7	37.3 ± 0.7	NS	37.6 ± 0.7	37.2 ± 0.7	NS	39.3 ± 0.6	38.6 ± 0.7	NS

Values are Mean ± SEM. No significant difference was observed among the treatment groups.

Table 7. Summary of occurrence of adverse events (AEs).

	Number of AEs	Severity			Subjects reporting AEs	Treatment related
		Mild	Moderate	Severe		
Placebo	45	35	9	1	19	None
Chromium picolinate	44	40	4	0	17	None
Chromium dinicocysteinate	28	24	2	2	14	None

insulin resistance, or glycemia. Lack of effect of supplementation of CDNC or CP on changes in HbA_{1c} may likely be due to the short duration of this clinical trial. Similarly, we did not observe a significant impact on blood glucose levels. It is unlikely that the effects of CDNC on circulating insulin, TNF-α, oxidative stress, and insulin resistance reported herein are dependent on any chromium deficiency in the diabetic patients because these effects were seen only in CDNC supplemented group but not in CP supplemented group. It seems that the chromium dinicocysteinate adduct may preferentially activate the insulin receptor transduction system [22]. Additional studies are required to address this issue and understand the underlying mechanisms involved.

Oxidative stress plays a key role in the regulatory pathway that progresses from hyperglycemia to monocyte and endothelial cell activation in the enhanced vascular inflammation of diabetes [10, 11, 25]. The effect of CDNC on TNF-α inhibition may be mediated in part by reduction in the oxidative stress pathways due to an enhanced effect of chromium facilitated by L-cysteine [15, 22, 26–28], as L-cysteine (LC) supplementation inhibits NFkB activation and improves insulin signaling [20]. When combined with chromium, it produced better effects, which could explain the greater efficacy of CDNC over P or CP. This study indicates that CDNC supplementation is more effective in lowering circulating levels of TNF-α, protein oxidation, and insulin resistance compared with P or CP supplementation in type 2 diabetic subjects.

Normal glucose homeostasis is maintained by a delicate balance between insulin secretion by the pancreatic β-cells and insulin sensitivity of the peripheral tissues (muscle, liver, and adipose tissue). Visceral adiposity is considered a risk factor for insulin resistance metabolic syndrome [29] and type 2 diabetes in adults [30], as well as in first-degree relatives of subjects with type 2 diabetes with normal glucose levels [31]. TNF-α is expressed in adipose tissues, with visceral fat responsible for more TNF-α production than subcutaneous fat. Obese mice lacking either TNF-α or its receptor show protection against developing insulin resistance. TNF-α inhibits tyrosine kinase phosphorylation of the insulin receptor, resulting in defects in insulin signaling and ultimately leading to insulin resistance and impaired glucose transport [32, 33]. The mechanism involves Ser phosphorylation of IRS-2 mediated by TNF-α activation of MAP kinases [34]. TNF-α may play a crucial role in the systemic insulin resistance of type 2 diabetes [35, 36]. The association of endothelial dysfunction with insulin resistance in the absence of overt diabetes or metabolic syndrome provides evidence that atherosclerosis may actually begin earlier in the pathogenesis of insulin resistance, ultimately resulting in a progression of metabolic syndrome to prediabetes and then to type 2 diabetes [37, 38].

When people are insulin resistant, their muscle, fat, and liver cells do not respond properly to insulin. As a result, the body requires more insulin to maintain normoglycemia. Initially, the pancreas increases insulin production to counterbalance the insulin resistance and maintain normal glucose levels. However, in many individuals the pancreas eventually fails to keep up with the body's demand for insulin leading to hyperglycemia and setting the stage for the development of diabetes. Insulin resistance increases the chances of developing both type 2 diabetes and cardiovascular disease. Physical activity and weight loss can help people with insulin resistance or prediabetes delay or prevent developing type 2 diabetes. However, maintaining a healthy lifestyle is difficult for many individuals to sustain and many ultimately develop diabetes. In addition to the role that physical activity and weight loss, CDNC supplementation appears to be helpful in alleviating insulin resistance. As stated above, our ability to increase insulin sensitivity may help to manage disease progression by restoring glucose homeostasis at a lower level of insulin. Such an approach could spare the pancreas. This cascade may take time to be fully implemented biochemically, thereby explaining why we saw a trend toward lower glucose levels in the CDNC supplemented group that had not yet reached statistical significance. Further long-term studies are required to better determine if this trend reaches statistical significance.

In conclusion, CDNC supplementation has the potential to lower blood levels of oxidative stress, TNF-α, and insulin resistance in type 2 diabetic subjects. CDNC is a complex of L-cysteine and chromium dinicotinate. L-cysteine derivatives such as N-acetyl cysteine are known to scavenge oxygen radicals. Results of this study indicate that the presence of the cysteinate molecule in CDNC helps provide better protection against the oxidative stress and the activation of signal transduction pathways associated with the insulin resistance and vascular inflammation of type 2 diabetes. Whether or not CDNC supplementation can similarly prevent insulin resistance and vascular inflammation and thereby, delay or prevent the onset of diabetes in the prediabetic population is not known and will be interesting to investigate. If it works as well as this research hints that it may, then chromium dinicocysteinate supplementation could be used as an adjunct therapy

for those with established diabetes. Furthermore, it might delay or prevent the development of diabetes in subjects with insulin resistance or prediabetes.

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The authors have declared no conflict of interest.

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