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Rapid Letter

Trivalent Chromium Inhibits Protein Glycosylation and Lipid Peroxidation in High Glucose-Treated Erythrocytes

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ABSTRACT

Epidemiological studies have shown lower levels of chromium among men with diabetes and cardiovascular disease (CVD) compared with healthy control subjects. The mechanism by which chromium may decrease the incidence of CVD and insulin resistance is not known. Using erythrocytes as a model, this study demonstrates that chromium inhibits the glycosylation of proteins and oxidative stress, both risk factors in the development of CVD. Erythrocytes were treated with high levels of glucose (mimicking diabetes) in the presence or absence of chromium chloride in the medium at 37°C for 24 hours. Chromium supplementation prevented the increases in protein glycosylation and oxidative stress caused by the high levels of glucose in erythrocytes. This study demonstrates for the first time that chromium supplementation inhibits protein glycosylation in erythrocytes exposed to high glucose medium, which appears to be mediated by its antioxidative effect. This provides evidence for a novel mechanism by which chromium supplementation may decrease incidence of CVD in diabetic patients. *Antioxid. Redox Signal.* 8, 238–241.

INTRODUCTION

Chromium is an essential nutrient required for glucose and lipid metabolism (2, 29). Various studies have reported lower levels of chromium in the blood, lenses, and urine of diabetic patients compared with those of the normal population (8, 9, 21, 24). Clinical trials have demonstrated that supplementation with chromium chloride, chromium-niacinate, or chromium picolinate can lower blood glucose and triglyceride levels in diabetic patients and animals (1, 4, 5, 6, 20, 25, 28, 30, 32). Results from case control studies suggest an inverse association between chromium levels in toenails and the risk of myocardial infarction in the general population (12). Similarly, a recent report within the Health Professionals Follow-up Study has found lower levels of toenail chromium among men with diabetes and CVD compared with healthy control subjects (27). Chromium is a transition metal, and its trivalent state is the most prevalent form in organic complexes (31). Chromium supplements are widely consumed in the United States (19).

The molecular mechanism by which chromium supplementation may increase insulin sensitivity and reduce CVD is not known. Glycosylation of proteins, enzymes, and insulin can reduce insulin sensitivity, increase oxidative susceptibility, and is a risk factor in the development of CVD (3, 13, 19, 33, 34). However, no studies concerning the effect of chromium on the glycosylation of proteins in erythrocytes or any other cell types are available in the literature.

This study examined the hypothesis that chromium supplementation decreases protein glycosylation in an erythrocyte model. The results of this study demonstrate that chromium supplementation prevents increased hemoglobin glycosylation and decreases oxidative stress in erythrocytes exposed to high levels of glucose.

MATERIALS AND METHODS

Blood was collected into tubes containing ethylenediaminetetraacetate (EDTA) from normal human volunteers according to a protocol approved by the Institutional Human Review Board for the Protection of Human Subjects. EDTA-blood was centrifuged; the clear plasma and buffy coat layers

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were discarded. The red cell suspension was filtered through cotton wool to remove any leftover leukocytes. The cells were washed with cold 0.15 *M* sodium chloride solution three times after a one-to-ten dilution.

In vitro *treatment with glucose and chromium*

Washed RBC were suspended to 10% hematocrit in phosphate-buffered saline (15) containing 6 m*M* glucose. Aliquots of the cell suspension were placed in Erlenmeyer flasks, after which a freshly prepared stock solution of glucose or chromium chloride was added. Concentrations are expressed in terms of the total cell suspension. The contents of the flasks were incubated in a shaking water bath at 37°C for 24 hours. In certain experiments, the RBC suspension was preincubated with chromium for 30 minutes before addition of glucose to the suspension. Percent hemolysis was less than 1% in all incubations. Treated RBC were washed two times after a 1-to-10 dilution with 0.15 *M* NaCl before biochemical analyses. All incubations contained 10 µl of penicillin-streptomycin/ml of cell suspension to vitiate any microbial growth during the overnight incubations. The working solution of penicillin-streptomycin contained 300 mg penicillin G and 500 mg streptomycin in 10 ml buffer.

Membrane lipid peroxidation

Lipid peroxidation was assessed by measuring thiobarbituric-acid (TBA)-reactivity of malondialdehyde (MDA), an end product of fatty acid peroxidation (10). For this purpose, 0.2 ml cells were suspended in 0.8 ml phosphate-buffered saline and 0.025 ml butylated hydroxytoluene (88 mg/10 ml absolute alcohol). Thirty percent trichloroacetic acid (0.5 ml) was then added. The tubes were vortexed and allowed to stand on ice for at least 2 hours, then centrifuged at 2000 rpm for 15 minutes. For each sample, 1 ml supernatant was transferred to a new tube. To each of these was added 0.25 ml 1% TBA in 0.05 *N* NaOH. The tubes were then mixed and kept in a boiling water bath for 15 minutes. The concentration of the MDA-TBA complex was assessed using HPLC after its separation with ion exclusion and a reverse phase Shodex KC-811 column (Waters Corp., Milford, MA) with the UV/Vis detector set at 532 nm (10). The packed cell volume of washed RBC was determined using an Autocrit Centrifuge (Becton Dickinson Co., Sparks, MD).

Measurements of glycosylated hemoglobin (GHb)

The human erythrocyte is freely permeable to glucose, and within each erythrocyte, glycosylated hemoglobin is formed continuously from hemoglobin A at a rate dependent on the ambient glucose concentration (23). GHb values were measured by using Glyc-affinity columns and a kit (Iso-Lab Inc., Akron, OH) and expressed as a % of total hemoglobin.

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise mentioned. Data were analyzed statistically using unpaired Student's *t* tests between different groups using Sigma Plot statistical software (Jandel Scientific, San Rafael, CA). A *p* value of less than 0.05 was considered significant.

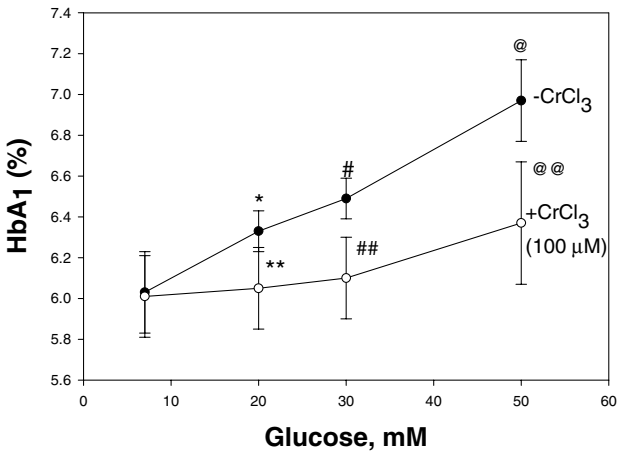

FIG. 1. Effect of different glucose concentrations on glycation of hemoglobin in the presence and absence of chromium chloride. Mean ± SE ($n = 5$). Differences between * vs. **, # vs. ##, and @ vs. @@ are significant ($p < 0.05$).

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of chromium on hemoglobin glycosylation. Increasing concentrations of glucose caused an increase in hemoglobin glycosylation. This increase in glycosylation was inhibited by supplementation with chromium. Figure 2 demonstrates the effect of different concentrations of chromium on the high glucose-induced glycosylation. This shows that the inhibitory effect of chromium can be seen at chromium concentrations as low as 0.5 µ*M*. Figure 3 shows that chromium supplementation prevented an increase in the lipid peroxidation caused by high glucose concentrations. The effect of chromium on inhibition of lipid peroxidation was seen even at 0.5 µ*M*. RBC exposed to normal glucose concentrations did not show any changes in glycosylated hemoglobin or lipid peroxidation values either with or without chromium.

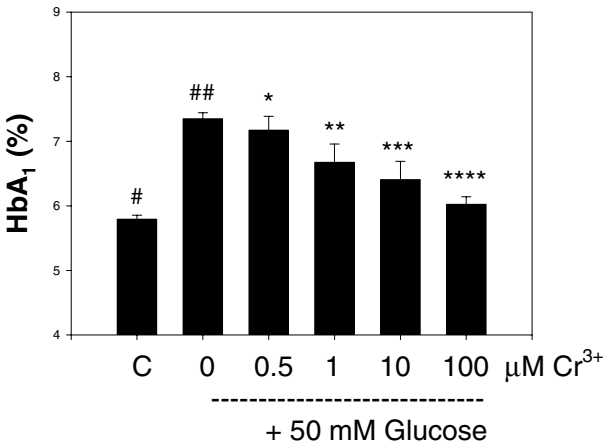

FIG. 2. Effect of different chromium concentrations on hemoglobin glycation in high glucose-treated erythrocytes. Mean ± SE ($n = 4$). Differences between # vs. ##, ## vs. **, ## vs. ***, and ## vs. **** are significant ($p < 0.05$).

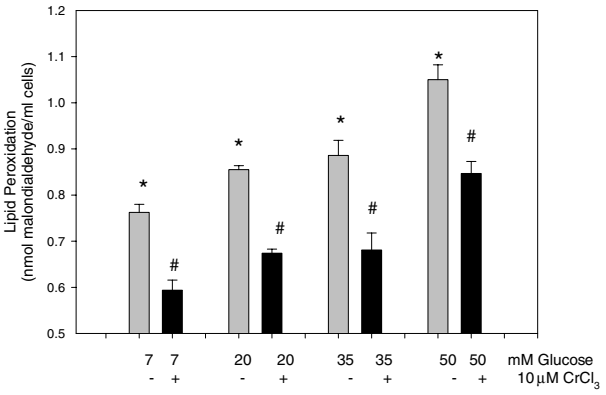

FIG. 3. Effect of chromium on lipid peroxidation in erythrocytes treated with different concentrations of glucose. Mean ± SE (n = 4). Differences between * vs. # are significant ($p < 0.05$).

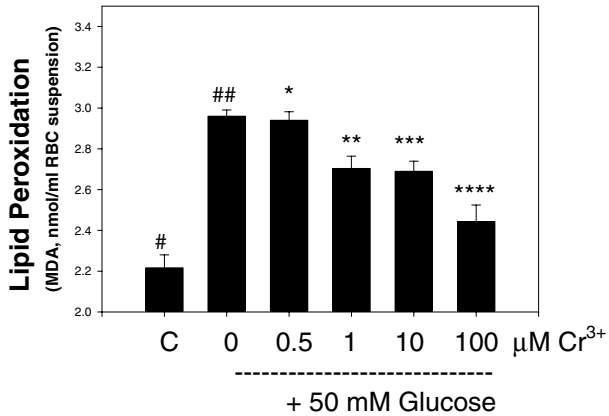

FIG. 4. Effect of different chromium concentrations on lipid peroxidation in high glucose-treated erythrocytes. Mean ± SE (n + 4). Differences between # vs. ##, ## vs. **, ## vs. ***, ## vs. **** are significant ($p < 0.05$).

Vascular inflammation and cardiovascular disease (CVD) are the leading causes of morbidity and mortality in the diabetic population and thus a major public health issue. The effects of Cr^{3+} on blood glucose homeostasis are accomplished by increased activation of insulin receptors through binding of chromium with glucose tolerance factor (31). A chromium-containing oligopeptide present in insulin-sensitive cells binds to the insulin receptor, markedly increasing the activity of the insulin-stimulated tyrosine kinase (31). Previous studies have demonstrated that Cr^{3+} supplementation inhibits the increase in TNF-α secretion levels caused by exposure to high levels of glucose in cultured U937 monocytes (14). The mechanisms by which chromium produces its effects on insulin sensitivity and CVD are not known.

Using RBC as a model, this study demonstrates that trivalent chromium can reduce glycosylation of proteins and lipid peroxidation in RBC exposed to high glucose. High glucose concentrations can result in increased oxidative stress from excessive oxygen radical production from the auto-oxidation of glucose, glycosylated proteins, or stimulation of cytochrome P450-like activity by the excessive NADPH produced by glucose metabolism (11, 16, 22, 26). The data on the inhibition of glycosylation by chromium is novel. A previous study has shown that oxidative stress by itself can increase glycosylation of proteins (17). This suggests that the inhibition of glycosylation may be mediated by an antioxidative effect of chromium. However, the precise mechanism by which chromium inhibits oxidative stress is not known. For instance, chromium may reduce oxidative stress by activation of glutathione reductase or some other antioxidative enzyme that detoxifies oxygen radicals, thereby reducing the oxidative stress caused by high glucose.

Lamson and Plaza (18) have written an excellent review on the safety and efficacy of chromium. Chromium levels of up to 0.6 μ*M* have been reported in the blood of normal subjects (7). Therefore, the chromium concentration of about 0.5–1 μ*M* used in these studies falls within a normal physiological range.

In conclusion, this study has demonstrated for the first time that chromium inhibits glycosylation of proteins in a high glucose-treated erythrocyte model. Both glycosylation of proteins and oxidative stress have been proposed to contribute to the pathogenesis of cellular dysfunction leading to the vascular complications of diabetes (11, 33). The evidence that chromium can prevent oxidative stress and protein glycosylation needs to be explored at the clinical level to see whether supplementation can lower levels of protein glycosylation and oxidative stress and thereby reduce the incidence of CVD in the diabetic patient population.

ABBREVIATIONS

Cr^{3+}, trivalent chromium; CVD, cardiovascular disease; RBC, red blood cells; GHb, glycosylated hemoglobin; TBA, thiobarbituric acid-reactivity; MDA, malondialdehyde; EDTA, ethylenediaminetetraacetate.

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