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Effect of chromium supplementation on the diabetes induced-oxidative stress in liver and brain of adult rats

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Abstract This study was designed to investigate the susceptibility of liver and brain tissues, as insulin-independent tissues, of normal adult male rats to the oxidative challenge of subchronic supplementation with chromium picolinate (CrPic) at low (human equivalent) and high doses (2.90 and 13.20 μg Cr kg^{-1} day^{-1}, respectively). Also, the modulative effect of CrPic administration on the enhanced oxidative stress in the liver and brain tissues of alloxan-diabetic rats was studied. Fasting serum glucose level was not modified in normal rats but significantly reduced in diabetic rats that had received CrPic supplement. A mild oxidative stress was observed in the liver and brain of CrPic-supplemented normal rats confirmed by the dose-dependent reductions in the levels of hepatic and cerebral free fatty acids, superoxide dismutase and glutathione peroxidase activities, and in contrast increased tissue malondialdehyde concentration. On the other hand, hepatic and cerebral catalase activity was reduced in the high dose group only. CrPic supplementation did not act as a peroxisome proliferator confirmed by the significant reductions in liver and brain peroxisomal palmitoyl CoA oxidase activity. The non significant alterations in liver protein/DNA and RNA/DNA ratios indicate that CrPic did not affect protein synthesis per cell, and that mild elevations in hepatic total protein and RNA concentrations might be due to block or decrease in the export rate of synthesized proteins from the liver to the plasma. In diabetic rats, elevated levels of hepatic and cerebral free fatty acids and malondialdehyde, and in contrast the overwhelmed antioxidant enzymes, were significantly modulated in the low dose group and near-normalized in the high dose group. The significant increases observed in liver total protein and RNA concentrations, as well as protein/DNA and RNA/DNA ratios in diabetic rats supplemented with the high dose of Cr, compared to untreated diabetics, may be related to the improvement in the glycemic status of the diabetic animals rather than the direct effect of CrPic on protein anabolism.

Keywords Chromium picolinate · Alloxan-diabetes · Liver · Brain · Glucose · Palmitoyl CoA oxidase · Free fatty acids · Malondialdehyde · Antioxidant enzymes · Protein · Nucleic acids

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Introduction

Diabetes mellitus is a major endocrine disorder and a growing health problem in the world, especially in the

Middle East countries due to the prevalent positive consanguinity. Under conditions of elevated metabolism, such as diabetes mellitus many tissue specific cells are continuously subject to insult from reactive oxygen species (ROS). Overproduction of ROS or a failure in intracellular defenses against ROS will result in the pathogenesis of secondary diabetic complications like cardiovascular, renal and ocular, in addition to cerebrovascular like reduction in cerebral blood flow (ischemia), disruption of the blood brain barrier and cerebral oedema (Garg et al. 1996; Warner et al. 2004; Fridlyand and Philipson 2005).

Recent evidence reported that hyperglycemia also causes endoplasmic reticulum (ER) oxidative stress in the β cells, which attempt to compensate for insulin resistance by increased insulin synthesis and secretion. As a consequence, the ER is overwhelmed and an overall increase in protein misfolding occurs (Nakatami et al. 2005). When the production of misfolded proteins exceeds degradation, the proteins often aggregate leading to intracellular accumulation (Bukau et al. 2006). Protein aggregating diseases include Huntington's and Alzheimer's diseases (Gow and Sharma 2003).

Free radicals (superoxide anion; $^{\bullet}O_2^-$, hydroxyl radical; OH and peroxynitrite; $ONOO^-$) and singlet oxygen can inactivate proteins, induce breaks in DNA, degrade sugars, oxidize lipoproteins and initiate lipid peroxidation in cell membranes by attacking polyunsaturated fatty acids leading to loss of energy metabolism, cell signaling, transport and other major functions (Warner et al. 2004). Cells require antioxidant systems to neutralize ROS. For example, superoxide anions ($^{\bullet}O_2^-$) are enzymatically converted to hydrogen peroxide (H_2O_2) by superoxide dismutase, which is present in 3 forms: cytosolic (CuZnSOD), mitochondrial (MnSOD) and extracellular SOD. Decreased expression of MnSOD leads to decreased mitochondrial glutathione (GSH) and increased oxidative stress (Williams et al. 1998). Hydrogen peroxide can then be rapidly removed by the enzyme glutathione peroxidase (GPx), which is present in the cytosol and mitochondria. It also acts as a peroxynitrite reductase (Sies et al. 1997). A further antioxidant enzyme, catalase (CAT), found exclusively in peroxisomes, catalyses the dismutation of hydrogen peroxide generated by oxidases involved in β-oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism into water and oxygen (Turrens 2003; Newsholme et al. 2007).

Chromium (Cr), a group VIb transition element, exists in several valence states, the most prevalent oxidation states being hexavalent Cr (which is associated with industrial exposure and toxicity) and trivalent Cr (which is stable and the biologically active form). Cr supplements are available as trivalent Cr in the chloride ($CrCl_3$) or picolinate (CrPic) salt forms. The first suggestion that Cr participates in carbohydrate metabolism in animals was reported by Schwarz and Mertz (1957, 1959). Glucose tolerance factor (GTF), which was shown to contain Cr, was deficient in animals with impaired glucose tolerance.

Considerable controversy exists regarding the effectiveness of dietary Cr supplementation to improve carbohydrate metabolism in patients with type II diabetes mellitus. The controversy stems from the lack of definitive randomized trials, of gold standard techniques to assess glucose metabolism, the use of different doses and formulations and the study of heterogeneous populations (Cefalu and Hu 2004). However, more studies support the concept that dietary Cr supplementation yields more consistent clinical effects on carbohydrate metabolism, particularly when consumed at higher doses ($\geq$200 μg/day; Anderson et al. 1997; Cefalu et al. 1999).

Animal studies have shown that a deficiency in dietary Cr can result in an inability to remove glucose efficiently from the bloodstream (Striffler et al. 1999). Evidence also suggests that diet-induced insulin resistance in experimental animals can be improved by Cr supplementation (Striffler et al. 1998). In humans, there also seems to be an association between insulin resistance and Cr status (Fulop et al. 1987). Earlier reports suggested that Cr enhanced insulin sensitivity by direct interaction (Mertz 1969), increasing insulin receptors number (Anderson et al. 1987), membrane fluidity and the rate of insulin internalization (Evans and Bowman 1992). In addition, Cr was reported to modulate the activity of phosphotyrosine phosphatase, the enzyme that cleaves phosphate from the insulin receptor (Davis et al. 1996), and that Cr enhanced skeletal muscle glucose transporter-4 (GLUT-4) translocation and insulin sensitivity in a rat model of obesity and insulin resistance (Cefalu et al. 2002) and also recruits intracellular localization of GLUT-4 to the cytoplasmic side of the plasma membrane of murine adipocytes (Chen et al. 2006).

Peroxisomes are inducible cytoplasmic organelles present in all mammalian cells except red blood cells

(Stott 1988). They are involved in lipid degradation and biosynthesis. One of the most characteristic features of peroxisomes is the remarkable proliferation in response to xenobiotic agents, so-called peroxisome proliferators (Reddy et al. 1980). Peroxisome proliferation causes the induction of peroxisomal enzymes, one of which is palmitoyl CoA oxidase (a rate limiting enzyme of β-fatty acid oxidation) that oxidizes esters of medium, long and very long chain fatty acids and causes a lesser induction of peroxisomal catalase that degrades H_2O_2. The inducible palmitoyl CoA oxidase is well characterized and implicated in the oxidative DNA damage resulting from exposure to peroxisome proliferators (Chu et al. 1995).

The main objective of this study was to investigate the effect of subchronic Cr supplementation in alleviating the diabetes-induced oxidative stress in the liver and brain of adult rats, as examples of insulin-independent tissues. The aim of this study was also extended to assess the susceptibility of liver and brain tissues to the oxidative challenge of Cr and its role in peroxisomes proliferation.

Materials and methods

Animals

A total of 42 adult male Sprague-Dawley albino rats weighing 180–220 g were used in this study and obtained from the breeding unit of Egyptian Company for Biological Products and Vaccines (Cairo). The animals were maintained on a commercial pellet diet and tap water ad libitum.

Preparation and administration of chromium picolinate

Pure chromium (III) picolinate ($C_{18}H_{12}N_3O_6Cr$, MW418.3) was provided from ICN Biomedical Company (Germany). The human therapeutic dose of Cr is 200–600 μg/day (equivalent to 1.60–4.80 mg chromium picolinate/day), assuming an average human body mass of 75 kg. This is equivalent to 1.30–3.90 μg Cr day^{-1} 0.5 kg rat (Hepburn and Vincent 2002). In the current study Cr was daily administered to rats by intragastric tubing at low or human equivalent dose (0.023 mg/Kg b.w. or 2.90 μg Cr kg^{-1} day^{-1}) and high dose (0.1 mg/Kg b.w. or 13.20 μg Cr kg^{-1} day^{-1}). Accurately weighed 3.28 and 15.30 mg of chromium picolinate were individually dissolved in 850 ml of distilled water and the solutions were stored at 4°C during treatment.

Animal protocol

Rats were fasted for 12 h before being i.p injected with a single dose of freshly prepared alloxan monohydrate solution (MW 160.1, Sigma Co., USA) at a dose of 120 mg/Kg b.w. (Sheweita et al. 2002). After a period of 3 days for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (serum glucose level of 213.86 ± 10.82, range of 198–230 mg/dl) were used for the experiment. Fasting serum glucose level in normal rats averaged 90.14 ± 5.49 (83–98 mg/dl).

Rats were randomly allocated to two equal groups, which were normal and diabetic rats. Each group was then subdivided equally into three treatment groups (each contained 7 rats). In the first group normal rats were left intact (normal controls, NC) or supplemented daily with chromium (III) picolinate at concentration 0.023 mg/Kg b.w. (Low dose, NLD) or 0.1 mg/Kg b.w. (High dose, NHD). Likely, diabetic rats in the second group were left untreated (Diabetics, D) or supplemented daily with low dose (DLD) or high dose (DHD) of chromium (III) picolinate, respectively. The animals in all groups were weighed weekly throughout the treatment period (4 weeks) and the percentage of change in the body weight gain at each weekpoint was calculated then compared to its respective initial body weight (before starting Cr supplementation).

At the end of the treatment period (4 weeks) individual blood samples were taken from the retro-orbital venous plexus under light ether anaesthesia after a fast of 12 h. Serum was collected by centrifuging blood samples at 4,000 rpm for 5 min. Animals were dissected and livers and brains were immediately excised, rinsed thoroughly from blood in physiological saline, blotted dry and stored individually in plastic vials containing a small amount of ice cold saline at −70°C until analysis.

Preparation of tissue homogenate fractions

The livers and brains were accurately weighed to prepare 5% tissue homogenates in 50 mM Tris–HCl/

0.25 M sucrose buffer (pH 7.2–7.5). The whole tissue homogenate was used for determination of total free fatty acids (FFA), malondialdehyde, total soluble protein, RNA and DNA concentrations. An aliquot of the whole tissue homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the cytosolic supernatant was used for determination of the antioxidant enzymes. Palmitoyl Co-A oxidase activity was determined in the light mitochondrial (peroxisome-enriched) fraction prepared by centrifugation of the whole tissue homogenate at 107,000*g* for 30 min at 4°C.

Biochemical assays

Fasting serum glucose level was determined colorimetrically (Trinder 1969). using a commercial assay kit (Biodiagnostic, Egypt). Malondialdehyde (MDA) concentration was assessed as thiobarbituric reactive substances (TBRS; Esterbauer and Cheesman 1990) and calculated using an extinction coefficient of 1.53×10^5 $M^{-1}cm^{-1}$(Wills 1969). Total FFA extracted in chloroform/methanol mixture (2:1 v/v) were assayed as previously described (Duncombe 1963). Total protein was estimated in the whole tissue homogenate, cytosolic and light mitochondrial fractions by the dye binding method of Bradford (1976). Total RNA and DNA were extracted from the whole tissue homogenate in 5% perchloric acid according to the method of Schneider and coworkers (1950) then determined quantitatively by the colorimetric orcinol and diphenylamine methods, respectively (Dische 1957; Dische and Schwartz 1954).

Enzyme assays

Palmitoyl CoA oxidase activity (PCOX, EC 1.3.3.6) was assayed by measuring the acyl CoA dependent H_2O_2 production according to method of Hryb and Hogg (1979) modified by Von Veldhoven et al. (1991). The reaction was started by adding 100 µl of tissue homogenate, which was preincubated on ice with 50 µl of FAD/NaN_3 solution (50 µm/25 mM) for 5–10 min., to the reaction mixture (1 ml) prewarmed at 37°C and contained: 25 mM *p*-hydroxybenzoic acid, 1 mM 4-aminoantipyrine, 200 µg/ml BSA, 0.05% (v/v) triton X-100, 500U/ml horseradish peroxidase and 50 µM palmitoyl CoA in 50 mM potassium phosphate buffer (pH7.6). The increase in absorbance was read after 1, 2 and 3 min at 500 nm. PCOX activity was calculated using an extinction coefficient of 6.390 $M^{-1}cm^{-1}$ (Chu et al. 1995). One unit of the enzyme was defined as the amount which catalyzes the formation of 1 µmol of product/min. Superoxide dismutase activity (SOD, EC 1.15.1.1) assay is based on pyrogallol, which auto-oxidizes rapidly in an aqueous solution to produce a yellow colour in the presence of superoxide anions that can be read at 420 nm (Roth and Gilbert 1984). The SOD inhibits the auto-oxidation of pyrogallol by catalyzing the breakdown of superoxide anions. The enzyme activity was expressed as 50% inhibitory units per mg protein. Glutathione peroxidase activity (GPx, EC 1.11.1.9) was determined according to the method of Ursini et al. (1985) using a commercial assay kit (IBL, Germany). Catalase activity (CAT, EC 1.11.1.6) was assayed colorimetrically and expressed as µmole H_2O_2 consumed min^{-1} mg^{-1} protein (Sinha 1972). The reaction mixture (1.5 ml) contained 1.0 ml 0.01 M phosphate buffer (pH7.0), 0.4 ml of 2 M H_2O_2 and 0.1 ml of tissue supernatant. The reaction was stopped by the addition of 2 ml dichromate/acetic acid reagent (5% potassium dichromate and glacial acetic acid, 1:3 v/v).

Statistical analysis

All the grouped data were statistically evaluated with SPSS/11 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test; *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for seven animals in each group.

Results

Effect of Cr on body weight

The effect of subchronic Cr supplementation on the percentage of body weight gain of normal and diabetic animals was presented in Figs. 1 and 2. In normal-supplemented groups, a gradual increase in the percentage of body weight gain was recorded in rats received low (Gr.II) and high doses (Gr.III) of Cr throughout the experimental period. In comparison to normal controls (Gr.I) at the 4th weekpoint,

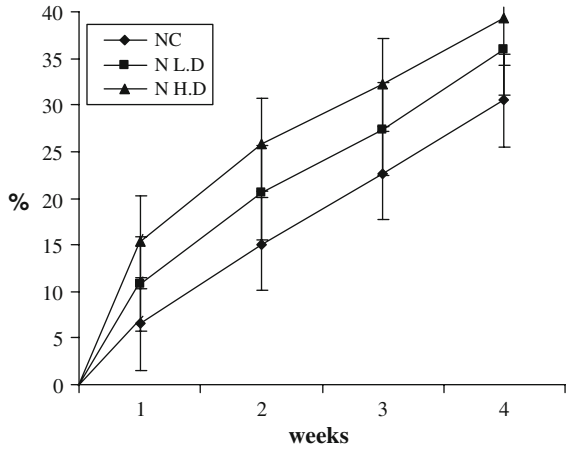

Fig. 1 Changes in the body weight gain (%) of normal rats supplemented with low or high doses of trivalent chromium throughout the experimental period, compared to their initial body weights

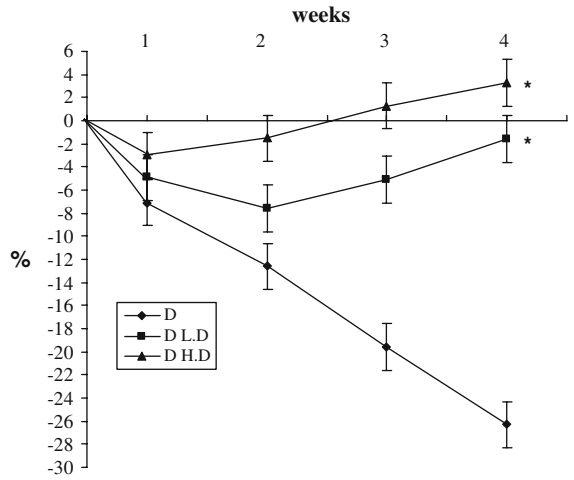

Fig. 2 Changes in the body weight gain (%) of diabetic rats supplemented with low or high doses of trivalent chromium throughout the experimental period, compared to their initial body weight. * Significantly different from its respective control at $P < 0.05$

non significant change in the percentage of body weight gain was recorded in both groups (Fig. 1). The untreated diabetic rats (Gr.IV) showed a persistent reduction in the percentage of body weight gain, which reached 26% by the end of the experiment, compared to the initial body weight. Diabetic animals supplemented with the low dose of Cr (Gr.V) showed a mild reduction in the percentage of body weight gain, which reached a maximum value of 8% at the 2nd weekpoint and a minimum of 2% by the end of the experiment (4 weeks). On the other hand, diabetic animals supplemented with the high dose of Cr (Gr.VI) showed a mild reduction (1.8%) in the percentage of body weight at the 2nd weekpoint, which was modified at the 4th weekpoint to a slight increase of 3.4%. In comparison to diabetic rats at the 4th weekpoint, significant increases in the percentage of body weight gain was recorded in the low and high dose groups (Fig. 2).

Effect of Cr on fasting blood glucose

Trivalent Cr supplementation of normal rats at low and high concentrations for 4 weeks produced non significant decrease in the fasting serum glucose level (7 and 9%, respectively), compared to normal controls (Fig. 3). Alloxan-diabetes caused a significant elevation (137%) in the fasting serum glucose level, compared to normal controls. Supplementation

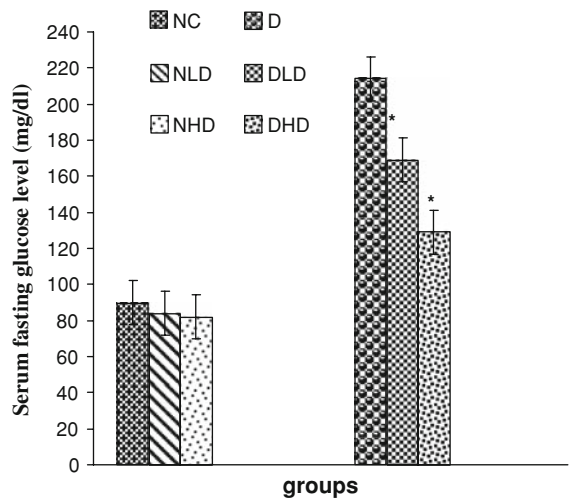

Fig. 3 Fasting serum glucose level in normal and diabetic rats supplemented with chromium picolinate at low or high doses. Each value represents mean ± SD. * Significantly different from its respective control at $P < 0.05$

of diabetic rats with Cr at low and high levels significantly reduced the fasting serum glucose level by 21 and 40%, respectively, compared to diabetic controls.

Effect of Cr on liver and cerebral peroxisomal fatty acyl CoA oxidase activity, FFA and malondialdehyde levels

Low dose of Cr caused significant reductions in hepatic and cerebral fatty acyl CoA oxidase activity (16 and 22%) and total FFA concentration (14 and 12%, respectively), compared to normal controls (Table 1). In contrast, administration of low and high doses of Cr to normal rats increased significantly malondialdehyde concentration in the liver (34 and 64%) and brain (27 and 49%, respectively), compared to normal controls. Alloxan-diabetes caused significant elevations in hepatic and cerebral peroxisomal fatty acyl CoA oxidase (23 and 22%), total FFA (48 and 41%) and malondialdehyde (22 and 26%) concentrations, compared to normal controls. Oral administration of diabetic rats with low dose of Cr gave rise to slight but significant decreases in hepatic and cerebral peroxisomal fatty acyl CoA oxidase (11%), total FFA (16 and 17%) and malondialdehyde (14%) concentrations, which were augmented at high dose level (27, 21 and 34, 25 and 21, 23%, respectively), compared to diabetic controls.

Effect of Cr on liver and cerebral antioxidant enzymes

The changes in hepatic and cerebral SOD, GPx and CAT activities in normal and diabetic rats supplemented with low and high doses of Cr were shown in Table 2. Significant reductions in liver and brain SOD (38 and 35%) and GPx (8 and 17%, respectively) activities were recorded in normal rats in response to supplementation with low dose of Cr, which were more pronounced at high dose level (58, 54 and 15, 49%, respectively), compared to normal controls. Significant reductions in liver and brain CAT activity were recorded only in the high dose group (23 and 22%, respectively). In diabetic rats, significant reductions in hepatic and cerebral SOD (56 and 39%), GPx (16 and 23%) and CAT (34 and 17%) activities were observed, compared to normal controls. Oral administration of diabetic rats with Cr at low and high doses significantly increased the hepatic and cerebral antioxidant defense enzymes activity. However, a more pronounced increase in hepatic SOD (112%) and GPx (16%) activities was recorded in the high dose group, except for CAT,

Table 1 Statistical significance of liver and brain palmitoyl CoA oxidase activity, free fatty acids and malondialdehyde levels in normal and diabetic rats supplemented with chromium

Group	PCOX (nmol min^{-1} mg^{-1} protein)		FFA (mg/100 g tissue)		MDA (nmol/g tissue)	
	Liver	Brain	Liver	Brain	Liver	Brain
Gr.I NC						
Mean ± SD	4.26 ± 0.52	6.88 ± 0.83	710 ± 31.41	790 ± 20.51	5.70 ± 0.76	6.25 ± 0.74
Gr.II NLD						
Mean ± SD	$3.56 \pm 0.38^{+}$	$5.38 \pm 0.73^{+}$	$608 \pm 25.37^{+}$	$695 \pm 25.12^{+}$	$7.65 \pm 0.57^{+}$	$7.93 \pm 0.89^{+}$
Change %	−16	−22	−14	−12	34	27
Gr.III NHD						
Mean ± SD	$3.15 \pm 0.41^{+}$	$4.52 \pm 0.65^{+}$	$498 \pm 26.22^{+}$	$565 \pm 26.61^{+}$	$9.33 \pm 1.21^{+}$	$9.93 \pm 1.04^{+}$
Change %	−26	−34	−30	−28	64	49
Gr.IV D						
Mean ± SD	5.23 ± 0.84^{1}	8.39 ± 0.75^{1}	$1{,}050 \pm 28.49^{1}$	$1{,}110 \pm 24.71^{1}$	6.96 ± 0.49^{1}	7.85 ± 1.03^{1}
Gr.V DLD						
Mean ± SD	4.65 ± 0.68^{a}	$7.50 \pm 0.56^{*a}$	$885 \pm 30.35^{*a}$	$926 \pm 29.41^{*a}$	$6.02 \pm 0.67^{*a}$	$6.72 \pm 0.78^{*a}$
Change %	−11	−11	−16	−17	−14	−14
Gr.VI DHD						
Mean ± SD	$3.83 \pm 0.45^{*b}$	$6.65 \pm 0.48^{*b}$	$695 \pm 35.43^{*b}$	$830 \pm 44.23^{*b}$	$5.52 \pm 0.86^{*b}$	$6.02 \pm 0.55^{*b}$
Change %	−27	−21	−34	−25	−21	−23

Notes: $^{+}$ $P < 0.05$ for Gr.II and Gr.III vs Gr.I, ^{1} $P < 0.05$ for Gr.IV vs Gr.I, * $P < 0.05$ for Gr.V and Gr.VI vs Gr.IV, ^{a} $P < 0.05$ for Gr.V vs Gr.II, ^{b} $P < 0.05$ for Gr.VI vs Gr.III

Table 2 Statistical significance of liver and brain antioxidant enzymes activity in normal and diabetic rats supplemented with chromium

Group	SOD (U/mg protein)		GPX (nmol min^{-1} mg^{-1} protein)		CAT (Kf/mg protein)	
	Liver	Brain	Liver	Brain	Liver	Brain
Gr.I NC						
Mean ± SD	6.84 ± 0.73	7.21 ± 0.62	231.57 ± 16.83	18.29 ± 3.4	0.56 ± 0.04	0.23 ± 0.03
Gr.II NLD						
Mean ± SD	4.23 ± 0.36^{+}	4.67 ± 0.3^{+}	213.71 ± 8.98^{+}	15.14 ± 2.8^{+}	0.48 ± 0.06	0.21 ± 0.06
Change %	−38	−35	−8	−17	−14	−13
Gr.III NHD						
Mean ± SD	2.87 ± 0.22^{+}	3.29 ± 0.36^{+}	197.86 ± 5.81^{+}	9.4 ± 1.70^{+}	0.43 ± 0.03^{+}	0.18 ± 0.04^{+}
Change %	−58	−54	−15	−49	−23	−22
Gr.IV D						
Mean ± SD	3.04 ± 0.40^{1}	4.4 ± 0.49^{1}	195.29 ± 6.97^{1}	14.14 ± 2.9^{1}	0.37 ± 0.03^{1}	0.19 ± 0.02^{1}
Gr.V DLD						
Mean ± SD	4.61 ± 0.57*	5.74 ± 0.52*^{a}	212.14 ± 11.63*	15.0 ± 3.16	0.42 ± 0.05^{a}	0.20 ± 0.03^{a}
Change %	52	30	9	6	14	5
Gr.VI DHD						
Mean ± SD	6.44 ± 0.59*^{b}	7.11 ± 0.63*^{b}	227.43 ± 10.03*^{b}	18.43 ± 2.70*^{b}	0.48 ± 0.07*^{b}	0.25 ± 0.04*^{b}
Change %	112	62	16	30	30	32

Notes: $^{+}$ $P < 0.05$ for Gr.II and Gr.III vs Gr.I, ^{1} $P < 0.05$ for Gr.IV vs Gr.I, * $P < 0.05$ for Gr.V and Gr.VI vs Gr.IV, ^{a} $P < 0.05$ for Gr.V vs Gr.II, ^{b} $P < 0.05$ for Gr.VI vs Gr.III

which was significantly increased (30%) in the high dose group only. As to brain, a more pronounced increase in SOD activity (62%) was recorded in the high dose group, while increased activities of GPx and CAT were reported in the high dose group only (30 and 32%, respectively).

Effect of Cr on liver and cerebral total protein, DNA and RNA levels

Oral administration of low dose of Cr to normal rats had non significant effect on hepatic and cerebral total protein, RNA and DNA concentrations, compared to normal controls (Table 3). However, the high dose of Cr produced mild but significant elevations in hepatic and cerebral total protein and RNA concentrations only (16, 10% and 19, 15%, respectively), while DNA concentration remains unchanged, compared to normal controls. Alloxan-diabetes caused slight but significant reductions in liver and cerebral total protein concentrations (12 and 15%, respectively), whereas hepatic and cerebral RNA and DNA concentrations remain unchanged, compared to normal controls. Supplementation of diabetic rats with low dose of Cr caused non significant changes in liver and brain RNA and DNA concentrations but increased significantly hepatic total protein by 9.10%. High dose treatment significantly increased hepatic and cerebral RNA concentrations (14 and 12%), whereas DNA concentration remains unchanged, compared to diabetic controls. Liver and brain total protein concentrations were significantly elevated in the high dose group (13 and 23%), compared to diabetic controls.

Effect of Cr on liver and cerebral protein/DNA and RNA/DNA ratios

Treatment of normal rats with low and high doses of Cr showed non significant changes in liver and brain protein/DNA and RNA/DNA ratios, compared to normal controls (Table 4). Diabetic rats showed a highly significant reduction in protein/DNA ratio (23%), whereas RNA/DNA remains unchanged, compared to normal controls. Oral treatment of diabetic rats with low dose of Cr had non significant effect on hepatic and cerebral protein/DNA or RNA/DNA ratio, compared to diabetic controls. However,

Table 3 Statistical significance of liver and brain total protein, RNA and DNA concentrations in normal and diabetic rats supplemented with chromium

Group	Protein (mg/g tissue)		RNA (mg/g tissue)		DNA (mg/g tissue)	
	Liver	Brain	Liver	Brain	Liver	Brain
Gr.I NC						
Mean ± SD	226.0 ± 12.40	79.20 ± 3.7	7.03 ± 0.30	5.30 ± 0.34	2.03 ± 0.13	3.33 ± 0.31
Gr.II NLD						
Mean ± SD	232.4 ± 8.20	82.50 ± 4.41	7.68 ± 0.35	5.34 ± 0.45	2.12 ± 0.23	3.46 ± 0.45
Change %	3	4	9	0.75	4	4
Gr.III NHD						
Mean ± SD	262.4 ± 7.50^{+}	87.50 ± 3.28^{+}	8.36 ± 0.42^{+}	6.11 ± 0.46^{+}	2.35 ± 0.17	3.44 ± 0.37
Change %	16	10	19	15	16	3
Gr.IV D						
Mean ± SD	198.71 ± 8.08^{1}	67.57 ± 2.91^{1}	7.23 ± 0.36	5.10 ± 0.47	2.30 ± 0.25^{1}	3.70 ± 0.31
Gr.V DLD						
Mean ± SD	216.80 ± 10*^{a}	71.0 ± 3.66^{a}	7.46 ± 0.44	5.40 ± 0.47	2.10 ± 0.25	3.50 ± 0.27
Change %	9	5	3	6	−9	−5
Gr.VI DHD						
Mean ± SD	223.6 ± 10.2*^{b}	83.36 ± 4.85*	8.26 ± 0.56*	5.70 ± 0.40*	2.06 ± 0.32	3.30 ± 0.35
Change %	13	23	14	12	−10	−11

Notes: $^{+}$ $P < 0.05$ for Gr.II and Gr.III vs Gr.I, ^{1} $P < 0.05$ for Gr.IV vs Gr.I, * $P < 0.05$ for Gr.V and Gr.VI vs Gr.IV, ^{a} $P < 0.05$ for Gr.V vs Gr.II, ^{b} $P < 0.05$ for Gr.VI vs Gr.III

high dose of Cr gave rise to slight but significant increases in liver and brain protein/DNA (26 and 41%) and RNA/DNA (27 and 21%) ratios, compared to diabetic controls.

Discussion

In the present study, oral administration of low (human equivalent) dose (2.90 μg Kg^{-1} day^{-1}) or high dose (13.20 μg Kg^{-1} day^{-1}) of Cr(III) to normal rats for four consecutive weeks did not produce any significant modification in the body weight of the animals by the end of the experimental period, compared to normal controls (Fig. 1). This indicates that Cr, at both doses, produced no toxic effect on the host or a favorable effect on the growth rate of animals. Sellei and associates (1970) reported that treatment with a therapeutic dose of any compound on the body weight loss must not exceed 10%. Also, Bissery and coworkers (1991) stated that a drug dosage that produces a loss in body weight ≥20% is considered as excessively toxic. On the other hand, treatment of diabetic rats with Cr showed a much less reduction in the body weight, compared to diabetic controls (Fig. 2).

Supplementation with Cr(III) picolinate at both doses for 4 weeks did not modify significantly the fasting serum glucose level in normal rats, however, it caused a dose-dependent reduction in hyperglycemia of diabetic rats, compared to diabetic controls (Fig. 3). These results revealed the anti-diabetic effect of Cr and that it acted differently according to the glycemic state of the host. In our opinion the antihyperglycemic effect of Cr could be attributed due to enhanced glucose uptake via increasing the number of and/or potentiating the translocation of glucose transporters. This hypothesis warranted verification. Our findings are in accordance with previous reports (Anderson 1993, 1998; Shinde and Goyal 2002, 2003). Recently, Cr(III) in the chloride or picolinate salt forms was reported to recruit mobilization of the glucose transporter (GLUT-4) to the cytosolic side of the plasma membrane of 3T3-L1 adipocytes concomitant with insulin-stimulated glucose transport (Sinha 1972). The authors attributed their findings to the effect of Cr treatment on reduction of plasma membrane cholesterol, which

Table 4 Statistical significance of liver and brain total protein/DNA and RNA/DNA ratios in normal and diabetic rats supplemented with chromium

Group	Protein/DNA (mg/mg)		RNA/DNA (mg/mg)	
	Liver	Brain	Liver	Brain
Gr.I NC				
Mean ± SD	111.80 ± 8.60	24.10 ± 2.30	3.46 ± 0.32	1.59 ± 0.18
Gr.II NLD				
Mean ± SD	110.48 ± 10.90	24.20 ± 3.50	3.66 ± 0.42	1.56 ± 0.16
Change %	−1	0.41	6	−2
Gr.III NHD				
Mean ± SD	111.49 ± 9.50	22.70 ± 1.60	3.56 ± 0.22	1.57 ± 0.18
Change %	−0.28	−6	3	−1
Gr.IV D				
Mean ± SD	86.40 ± 9.30[1]	18.40 ± 2.0[1]	3.14 ± 0.50	1.40 ± 0.11
Gr.V DLD				
Mean ± SD	103.24 ± 15.70	19.80 ± 1.90[a]	3.56 ± 0.44	1.49 ± 0.15
Change %	19	8	13	6
Gr.VI DHD				
Mean ± SD	108.54 ± 21.40*	25.90 ± 4.20*	4.0 ± 0.62*[b]	1.70 ± 0.25*
Change %	26	41	27	21

Notes: [+] $P < 0.05$ for Gr.II and Gr.III vs Gr.I, [1] $P < 0.05$ for Gr.IV vs Gr.I, * $P < 0.05$ for Gr.V and Gr.VI vs Gr.IV, [a] $P < 0.05$ for Gr.V vs Gr.II, [b] $P < 0.05$ for Gr.VI vs Gr.III

resulted in increased membrane fluidity and enhanced mobilization of glucose transporters. In contrast, the chromium-mobilized pool of transporters was not active in the absence of insulin.

Subchronic administration of low dose of Cr was found to exert a mild oxidative stress in the liver and brain tissues of normal rats, which was more notable at the high dose level. This effect was manifested by the dose-dependent reduction in hepatic and cerebral total FFA concentrations and elevated lipid peroxides concentration, which suggests enhanced fatty acids oxidation (Table 1). FFA metabolism has multiple adverse effects including inhibition of oxidative phosphorylation, oxidative conversion of free arachidonic acid via the cyclooxygenase pathway to eicosanoids (thromboxanes and prostaglandins), free radical generation and lipid peroxidation-mediated chain reactions and cytotoxicity from lipid peroxidation products, which may stimulate apoptosis (Warner et al. 2004). Recently, it has been reported (Strydom et al. 2006) that Cr plays a role in enhancement of glycolysis and Krebs cycle (through activation of pyruvate dehydrogenase complex, which converts pyruvate to acetyl CoA). The active glycolysis, Krebs cycle, electron transport and oxidative phosphorylation produce ROS, which may cause lipid peroxidation and oxidative DNA damage (Bagchi et al. 2002). Alloxan-diabetes showed an enhanced oxidative stress in the liver and brain tissues of rats. This was demonstrated by sharp elevations in peroxisomal fatty acyl CoA oxidase activity, total FFA and lipid peroxides concentrations in the liver and brain, compared to normal controls (Table 1). This reflects increased peroxidation of lipids in plasma membranes in diabetes mellitus, which was reported as an index of oxidative insult (Kesavulu et al. 2001). Our findings are in line with previous reports (Bagchi et al. 2002; Mahboob et al. 2002; Ramalingam and Leelavinothan 2005; Refaie et al. 2005). Multiple comparison analysis revealed that the significant reduction in palmitoyl CoA oxidase activity in the liver and brain tissues in the normal-treated groups and diabetes-treated groups, compared to their respective controls, was mainly due to supplemental Cr, whereas the significant elevation in its activity in the diabetes-treated groups, compared to their respective normal-treated groups, was due to diabetes mellitus. This led to the notion that Cr

does not act as a peroxisome proliferater and that it might potentiate β-oxidation of FFA in the liver and brain by fatty acyl CoA oxidase of mitochondrial rather than peroxisomal origin. Also, the implication that Cr could enhance the oxidation of LDL might be considered.

Oral administration of low and high doses of Cr to normal animals caused significant reductions in hepatic and cerebral SOD and GPx activities, with the exception of CAT activity, which was significantly reduced in the high dose group only, in comparison to normal controls (Table 2). These findings suggested the dose-dependent mild oxidative potential of Cr supplement. The insignificant change in the levels of liver and cerebral CAT activity and reduced fatty acyl CoA oxidase activity confirmed that Cr at low dose did not act as a peroxisome proliferator and that Cr could modulate the imbalance between the generation of ROS and the scavenging enzyme activity. The significant reductions in the liver and cerebral SOD, GPx and CAT activities in diabetic rats could simply be interpreted due to the overproduction of ROS that overwhelmed the activity of these enzymes. This clearly shows that brain and liver are among the body organs affected by the oxidative stress of diabetes mellitus, and that some tissues are affected by free radicals more than others. It seems probably that some tissues in response to diabetes and/or oxidative stress may overexpress the genes for the antioxidant enzymes. Numerous studies affirmed enhanced peroxidative status due to lowered antioxidant enzymes in diabetes mellitus (Wohaieb and Godin 1987; Saxena et al. 1993; Giugliano et al. 1998). Oral treatment of the diabetic group with low dose of Cr increased significantly the SOD and GPx activities in the liver and brain tissues, compared to diabetic controls, with a more pronounced effect in the high dose group attaining normal values at the end of the experiment. The reversion in the activity of the two enzymes may account for the reduced production of ROS demonstrated by decreased MDA concentration. Supplementation of diabetic rats with high dose of Cr showed a significant increase in the liver and brain CAT activity, whereas the low dose did not cause any significant change in the enzyme activity, compared to diabetic controls (Table 2). Hence, it could be concluded that Cr supplement stimulates the antioxidant defense system of the body, which prevents the secondary diabetic complications initiated mainly due to lipid peroxidation and free radicals production, resulting in significant modulation in lipid and carbohydrate metabolism.

In the present study the effect of Cr supplementation on total protein, RNA and DNA concentrations in the liver and brain of normal and diabetic rats was assessed. Also, protein/DNA and RNA/DNA ratios were reported, the former ratio gives a reflection of protein synthesis per cell. Treatment of normal rats with low dose of CrPic showed non significant effect on the hepatic and cerebral total protein, DNA and RNA concentrations, whereas the high dose of Cr caused mild but significant elevations in hepatic and cerebral total protein and RNA concentrations, compared to normal controls (Table 3). The non significant alterations in liver protein/DNA and RNA/DNA ratios (Table 4) indicate that Cr does not affect protein synthesis, but may block or decrease the rate of export of synthesized proteins from the liver to the plasma. Our results are in harmony with previously reported findings (Bernao and Meseguer 2004) that although high dose of CrPic (500 μg) increases muscle mass (given the increase in gastrocnemius muscle weight and carcass weight), the differences were not significant. It was suggested that CrPic might stimulate protein anabolism, with Cr improving the efficacy of insulin, and thus facilitating the entry of amino acids into the muscle cells, where they are assembled into muscle protein.

The significant reduction in liver total protein concentration in untreated diabetic rats could be explained in terms of increased secretion of exported proteins other than albumin (Table 3). In harmony with our suggestion, Anjuman et al. (2005) affirmed significant elevations in α_1-and α_2-globulins in the serum of diabetic patients. As well, Jefferson et al. (1983), who previously studied liver protein synthesis in spontaneously diabetic BB/W rats, reported that diabetes-induced changes in protein synthesis are due to diabetes alone and not alloxan, demonstrated by the greatly reduced capacity of the liver to synthesize and secrete plasma proteins, particularly albumin, in addition to a moderate decline in the rate of synthesis of nonexported proteins. Two mechanisms account for these alterations; first the fall in ribosomal RNA content and the decrease in the relative abundance of albumin mRNA of the total mRNA. The significant increases reported in liver total protein and RNA concentrations, as well as protein/DNA and RNA/

DNA ratios in diabetic rats given high dose of Cr are more probably due to the improvement in the glycemic status of the diabetic animals rather than the direct effect of CrPic on protein anabolism. This suggestion is confirmed by the intergroup comparison using Duncan's multiple range test between high dose-diabetic group and high dose-normal group (Tables 3, 4).

In conclusion, supplementation with Cr(III) has no favorable effect on the body weight of normal rats, while it significantly increases the body weight of alloxan-diabetic rats. Fasting serum glucose level in normal rats is not affected by treatment with CrPic, but significantly reduced in diabetic rats, which indicates that the mode of action of Cr depends on the glucose tolerance in the host. Cr does not act as peroxisome proliferator and the reduced hepatic and brain total FFA concentrations and in contrast elevated MDA concentration following administration of Cr suggest enhanced mitochondrial β-oxidation of FFA. This effect may contribute indirectly to minimizing the esterified cholesterol, which is responsible for the formation of atherosclerotic plaque. Oral supplementation of normal rats with low dose of Cr caused a mild oxidative stress in the liver and brain tissues of normal rats, which was augmented in the high dose. The efficacy of Cr in restoring the balance between the generation of ROS and the activity of scavenging antioxidant enzymes might help in preventing/reverting long term secondary diabetic complications. Also, results obtained from this study recommend the beneficial use of CrPic by diabetic patients as it improves the impaired glucose tolerance and stimulates the antioxidant defence system, which prevents oxidative tissue DNA damage. However, the use of Cr as a supplement by normal subjects is not effective as it showed non significant effect on the body mass, besides the mild oxidative stress induced by the long term treatment in 2 important body organs as liver and brain.

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