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Chromium Picolinate Modulates Serotonergic Properties and Carbohydrate Metabolism in a Rat Model of Diabetes

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Abstract Chromium picolinate (CrPic) has shown both antidepressant and antidiabetic properties. In this study, the effects of CrPic on serotonergic properties and carbohydrate metabolism in diabetic rats were evaluated. Sixty male Sprague–Dawley rats were divided into four groups. (1) The control group received only standard diet (8 % fat). (2) The CrPic group was fed standard diet and CrPic (80 μg CrPic per kilogram body mass (b.m.)/day), for 10 weeks (microgram/kilogram b.m./day). (3) The HFD/STZ group fed a high-fat diet (HFD, 40 % fat) for 2 weeks and then received streptozotocin (STZ, 40 mg/kg, i.p.) (i.v.) HFD-STZ–CrPic group treated as the previous group and then were administered CrPic. CrPic administration to HFD/STZ-treated rats increased brain chromium levels and improved all measurements of carbohydrate metabolism and serotonergic properties ($P<0.001$). CrPic also significantly increased levels of insulin, tryptophan, and serotonin ($P<0.001$) in the serum and brain, and decreased cortisol levels in the serum ($P<0.01$). Except chromium levels, no significant effect of CrPic supplementation was detected on the overall measured parameters in the control group. CrPic administration was well tolerated without any adverse events. The results support the use of CrPic supplementation which improves serotonergic properties of brain in diabetes.

Keywords Chromium · Glucose · Insulin · Cortisol · Serotonin · Tryptophan

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Introduction

The role of brain insulin action in insulin resistance and in the pathogenesis and treatment of diabetes is of interest whether neuronal insulin signaling is required for glucose lowering during the treatment of diabetes. A cluster of metabolic and vascular risk factors are associated with an increased risk of diabetes. Chronic hyperglycemia and hypoglycemia may both adversely affect the brain through different mechanisms leading to different cerebral deficits [1]. Diabetes mellitus (DM) is associated with modest impairments in cognition, increased risk of dementia, and neurophysiological and structural changes in the brain. There is an increasing body of evidence to support a relationship between type 2 diabetes and dementia in aging [2, 3].

Chromium (Cr), an essential trace element, has been used in the form of chromium picolinate (CrPic) to reduce elevated blood glucose levels in people with diabetes [4–6] and has been reported to improve symptoms of depression [7] and reducing cortisol levels in animal models [8]. It was proposed that chromium-bound chromodulin participates as part of an insulin signal amplification system as the complex binds to insulin-activated insulin receptors and results in stimulating its tyrosine kinase activity [9]. Recent studies have shown the antidepressant action of CrPic in a modified

forced swimming test affecting only swimming, suggesting involvement in serotonergic pathways [10, 11]. The addition of supranutritional amounts of Cr to the diet can lead to a pharmacological effect, as reflected by increased insulin sensitivity [12].

Several studies have evaluated CrPic effect on the central nervous system and cognition in diabetic humans and animals [13, 14]. Recent studies suggest that CrPic may have antidepressant properties, perhaps through increasing the peripheral availability of tryptophan for brain serotonin (5-HT) synthesis and decreasing the cortisol response to challenge with 5-hydroxytryptophan (5-HTP) [8]. Reed et al. [15] reported that the transition from insulin resistance to hyperglycemia in this model would be analogous to the decline in compensatory hyperinsulinemia and development of hyperglycemia that occurs in human type 2 diabetes. Therefore, this study was designed to evaluate the effects of CrPic supplementation on serotonergic properties and carbohydrate metabolism in diabetic rats.

Materials and Methods

Animals, Diets, and Experimental Design

Sixty male Sprague–Dawley rats (8 weeks old) weighing 200–250 g were purchased from Firat University Laboratory Animal Research Center (Elazig, Turkey). These animals were reared at 22±2°C°C, 55±5 % humidity, under a 12/12-h light/dark cycle. Diet and water were provided ad libitum. Body mass was measured every week throughout the study. Blood samples were collected from the tail vein for measurements of biochemical efficacy and safety markers. All procedures were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the University's local Institutional Animal Care and Use Committee.

A rat model of type 2 diabetes created by feeding a high-fat diet (HFD) and streptozotocin (STZ) treatment developed by Reed et al. [15] provides a novel animal model for type 2 diabetes that is applicable to the human syndrome making it suitable for testing antidiabetic compounds. In using such a model, the increased hyperglycemia after STZ injection in high fat-fed rats was not due to a greater decline in B cell function [15]. Animals were fed either a basal diet or an HFD to contain either 8 % or 40 % fat, respectively (Table 1). The batches of the diets were stored at 4 °C. Diabetes was induced by administering STZ (Sigma Chemical Co., St. Louis, MO, USA). A single STZ (40 mg/kg, i.p.) was dissolved in citrate buffer (pH 4.5) and injected once intraperitoneally (i.p.). The control group was given citrate buffer via i.p. injection. CrPic (CrPic; Chromax® chromium picolinate, Nutrition 21, Inc., Purchase, NY, USA) was dissolved in water and administered (80 μg/kg body mass (b.m.)/day) ad libitum in the drinking water for 10 weeks. The dose provided 8 μg of elemental Cr/day (equivalent to 560 μg of elemental Cr for a 70 kg adult human).

Table 1 Composition of diets (gram per kilogram diet)

Ingredients	Normal diet	High-fat diet
Casein	200.0	200.0
Starch	615.0	145.0
Sucrose	–	150.0
Corn oil	80.0	–
Beef tallow	–	400.0
Cellulose	50.0	50.0
Vitamin–mineral premix[a]	50.0	50.0
DL-methionine	3.0	3.0
Choline chloride	2.0	2.0

[a] The vitamin–mineral premix provides the following (per kilogram): all-*trans*-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*-a-tocopherol acetate, 12.5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B_6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B_{12}, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg. manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg

In total, 60 rats were divided into four treatment groups as (1) control group: rats were fed standard diet (8 % fat), (2) Cr-Pic group: rats were fed standard diet and CrPic was included into water at a concentration of 80 μg/kg b.m./day for 10 weeks. (3) HFD/STZ group: rats were fed HFD (40 % fat) for 2 weeks and then injected with STZ (40 mg/kg i.p.). (4) Group HFD/STZ+CrPic: rats were fed HFD for 2 weeks and then injected with streptozotocin, and CrPic was included into water at a concentration of 80 μg/kg b.m./day for 10 weeks.

Before STZ injection, the glucose concentrations of rats were measured and compared to the control. After the injection of STZ, the animals with fasting glucose >140 mg/dl were considered neonatal-STZ (nSTZ) diabetic that resembles type 2 diabetes in humans.

Analysis of Metabolic Markers

In all groups, tail vein blood was collected to analyze the biological markers. Blood samples were centrifuged at 3,000×*g* for 10 min, and sera were collected. Estimation of insulin sensitivity made from oral glucose tolerance test (OGTT) data was performed using the composite insulin sensitivity index (CISI) proposed by Matsuda and DeFronzo [16]. Blood glucose concentrations were measured by using ACCU-

Chek Active (Roche Diagnostics). Serum insulin levels were measured with the Rat Insulin Kit (Linco Research Inc., St. Charles, MO, USA) by ELISA (El_x-800; BioTek Instruments Inc., VT, USA). The homeostasis model assessment of insulin resistance (HOMA-IR), the CISI and the glucose to insulin ratio (GIR) were calculated. Insulin resistance is a strong predictor of the development of type 2 diabetes mellitus. The fasting glucose/insulin ratio (FGIR) was found to be a highly sensitive and specific measure of insulin sensitivity [17]. FGIR was calculated as fasting blood glucose (G0) divided by fasting serum insulin (I0) levels [18]. The CISI was calculated according to the following formula [16]. $\mathrm{CISI} = 10,000/\text{square root of } [(\mathrm{G0})\,(\mathrm{I0})\,(\text{mean serum insulin during OGTT})\,(\text{mean blood glucose during OGTT})]$

HOMA-IR A computer-solved model has been used to predict the homeostatic concentrations which arise from varying degrees beta cell deficiency and insulin resistance. A comparison of a patient's fasting values with the model's predictions allows a quantitative assessment of the contributions of insulin resistance and deficient beta cell function to the fasting hyperglycemia (HOMA). HOMA-IR was calculated as proposed by Matthews et al. [19] as follows:

$$\mathrm{HOMA-IR} = \text{insulin (milli-international units per liter)} \times \text{glucose (millimole per liter)}/22.5.$$

The HOMA approach has been widely used in clinical research to assess insulin sensitivity.

Brain Assays

Brain samples were rapidly removed and dissected in ice, wrapped in foil, and immediately frozen and stored at −80 °C until serotonin concentration and binding assay could be performed. Brain samples were homogenized in 0.1 N HCl (100 μl/mg of tissue) and centrifuged for 10 min at 1,500×*g* at 4 °C. From this extracts, serotonin levels were measured using enzyme-linked immunosorbent assay kits (IBL Immuno-Biological Laboratories, Inc., Hamburg, and Germany) according to the manufacturer's protocol. Brain insulin levels were measured as mentioned previously by Havrankova et al. [20]. Serum and brain tryptophan was measured using high-performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan). Separation was obtained with a reverse-phase column and a mobile phase (flow rate, 1 mL/min) composed of 5 % acetonitrile, 100 mm phosphate buffer pH 3.6, and 1 mm ethylenediaminetetraacetic acid. Detection was obtained with a Shimadzu model LC 120 (HPLC; Shimadzu, Tokyo, Japan). Excitation and emission wavelengths were 313 and 420 nm, respectively. Serum cortisol levels were determined using a commercially available ELISA kit (Cayman chemical, MI, USA). Serum melatonin levels were determined with a commercially supplied ELISA kit (IBL, Hamburg, Germany) using kit procedure.

For Cr content analysis, brain samples were digested with a mixture of concentrated HNO_3 (65 % Merck, Suprapur) and H_2O_2 (30 % Merck, Suprapur) in a Microwave Digestion System (Berghoff, Germany). Prior to decomposition, the samples with added reagents were allowed to digest at room temperature in loosely capped Teflon beakers for at least 10 min, in order to remove the excess of nitrous oxide vapors. The microwave digestion proceeded during 30 min. A Perkin Elmer AAnalyst 800 Atomic Absorption spectrometer with AA WinLab software (PerkinElmer Corp., Norwalk, CT), version 4.1 SP, was used for the determination of brain Cr concentration. The analysis of Cr was performed using graphite furnace atomic absorption spectrophotometer (PerkinElmer, Analyst 800, with Zeeman background correction).

Statistical Analyses

Sample size was calculated based on a power of 85 % and a *P* value of 0.05. Given that assumption, a sample size of ten per treatment was calculated. The data were analyzed using the GLM procedure of the SAS software [21]. Least square treatments were compared if a significant F statistic (5 % level of *P*) was detected by analysis of variance. Treatments were also compared using student's unpaired *t* test for comparison of individual treatment. A $P<0.05$ was considered to be statistically significant.

Results

Body Mass and Metabolic Profile

As shown in Table 2, HFD and STZ decreased body mass ($P<0.001$). Rats fed standard diet had greater body mass than rats fed HFD ($P<0.001$). Body mass increased with CrPic supplementation in treatment group ($P<0.001$). Table 2 also summarizes the effects of HFD/STZ administration and CrPic supplementation on metabolic profile.

HFD/STZ-treated rats had greater blood glucose concentration and lower serum and brain insulin concentration, insulin resistance (HOMA-IR) and the ratio of GIR values than rats fed standard diet ($P<0.0001$). However, insulin sensitivity (CISI) values were lower in HFD/STZ-treated rats ($P<0.0001$). Blood glucose concentration, HOMA-IR, and GIR values decreased, whereas insulin concentration and CISI values increased with CrPic supplementation in diabetic rats ($P<0.001$). Compared to the HFD/STZ group,

Table 2 Effects of HFD/STZ administration and CrPic supplementation on body mass, glucose, and insulin concentrations in rats

Metabolic markers	Groups				SEM
	Control	CrPic	HFD/STZ	HFD/STZ+CrPic	
Body mass, g	320a	318a	215c	240b	3.43
Glucose, mg/dl	103c	102c	469a	287b	4.26
Insulin, mg/dl	48.6a	49.8a	23.8c	26.0b	0.25
Cortisol, mg/dl	8.2c	7.8c	13.4a	11.5b	0.35
HOMA-IR	12.4c	12.1c	26.9a	18.3b	0.49
CISI, mg/dl	2.68a	2.61a	0.83c	1.28b	0.08
Glucose/insulin ratio	2.12c	2.05c	20.22a	11.04b	0.27
Brain insulin, ng/g	27.4b	31.8a	23.2c	25.9b	0.73

Letters a–c indicate that means in the same line without a common letter differs significantly ($P<0.05$)

SEM standard error mean

CrPic decreased insulin resistance (HOMA-IR) and reduced blood glucose levels, whereas CrPic significantly CISI, reduced blood glucose, and reduced insulin resistance ($P<0.001$). Serum cortisol concentration was higher in HFD+STZ group than control ($P<0.01$; Table 2).

Serotonergic Properties

Serotonergic properties parameters in response to HFD/STZ and supplemental CrPic are shown in Table 3. HFD/STZ treatment reduced serotonergic properties and brain chromium levels ($P<0.001$). CrPic administration in HFD/STZ-treated rats improved all measurements of serotonergic properties ($P<0.001$) and increased chromium levels (28.9 vs 5.3 ng/g; $P<0.001$). HFD/STZ-treated rats had lower tryptophan (22.5 vs. 12.3 μg/dl for serum; 8.1 vs 5.2 μg/dl for brain; $P<0.01$ for both), serotonin (225 vs. 165 μg/dl for serum; 752 vs 450 μg/dl for brain; $P<0.01$ for both) and serum melatonin (32.0 vs 12.5 μg/dl; $P<0.01$) concentrations than rats fed standard diet. CrPic significantly increased levels of serotonin and tryptophan in serum and brain, and levels of melatonin in serum ($P<0.01$). The mean values were 17.6, 28.9, 5.3, and 22.6 ng/g for brain chromium in control, CrPic, HFD+STZ, HFD+STZ+CrPic groups, respectively ($P<0.01$; Table 3).

Discussion

High-fat diets induce insulin resistance in rodents [15, 22, 23]. CrPic supplementation improves insulin action by enhancing intracellular signaling [24] and helps in translocation of glucose transporter 4 (GLUT-4). GLUT-4 is mediated through insulin-independent phosphorylation and activation of AMP-activated protein kinase [25]. Insulin resistance is a determinant of free fatty acids in the blood, which are in turn important in tryptophan metabolism and brain serotonin concentrations [26, 27]. In a large prospective study in which indicators of insulin sensitivity were associated with suicide risk [28, 29]. A higher basal glucose levels, greater cumulative glucose responses after the GTT, and larger cumulative insulin responses after the GTT was observed in depressed patients than control subjects [30].

Rats administrated with HFD/STZ had hyperglycemia but lower insulin concentrations [22, 23]. The increased magnitude was not due to a greater decline in β-cell function [15]. The metabolic effect in fat fed/STZ rats and patients with type 2 DM had similar changes. Neuroimaging techniques, such as, magnetic resonance imaging and computed tomography, have provided data on structural changes in the brain that may be more relevant to the general population of diabetic patients. In this study, a significant increase in blood glucose levels with

Table 3 Effects of HFD/STZ administration and CrPic supplementation on serotonergic properties and brain Cr levels in rats

Metabolic Markers	Groups				SEM
	Control	CrPic	HFD/STZ	HFD/STZ+CrPic	
Tryptophan					
Serum, μg/dl	22.1a	22.5a	12.3c	16.1b	0.27
Brain, μg/dl	7.5b	8.1a	5.2c	5.5c	0.48
Serotonin					
Serum, μg/dl	221.9a	225.0a	165.8c	174.9b	3.50
Brain, μg/dl	700a	752a	450c	520b	7.07
Serum melatonin, μg/dl	32.0a	31.2a	12.5c	16.3b	0.40
Brain chromium, ng/g	17.6c	28.9a	5.3d	22.6b	0.18

Letters a–d indicate that means in the same line without a common letter differs significantly ($P<0.05$)

SEM standard error mean

changes in serotonergic properties was observed. In animals fed with CrPic, there was an improvement in insulin sensitivity index and a decrease in insulin resistance and a decrease in cortisol levels and also changes in serotonin and tryptophan. The changes in brain chromium levels may be enhancing the favorable effects on serotonergic properties and improve brain insulin levels. There is a tendency for an increased occurrence of white matter hyperintensities on magnetic resonance imaging studies [31]. Davilla et al. [32] reported that a complementary role of insulin and insulin-like growth factor 1 (IGF-1) in the brain, unveiling the cellular and molecular pathways involved in brain insulin/IGF-I actions are helping to establish potentially new therapeutic targets and its exploitation may lead to new treatments for a wide array of brain diseases. Stewart and Liolitsa [33] concluded that there is a cross-sectional and prospective association between type 2 and cognitive impairment, probably both for memory and executive functions.

The limitations in this study, the morphological and histopathological changes before and after supplementation of CrPic in the brain were not studied. This is of interest; to see whether the changes in brain Cr and insulin levels may improve the changes occurred during insulin resistance and diabetes conditions. Studies are ongoing to study these effects and to see the improvements in cognitive function, and insulin sensitivity helps to improve the histochemical and morphological changes in the brain. The brain relies on the continuous nurture of glucose via the blood to maintain normal metabolic function [34]. The role of insulin in the regulation of brain glucose metabolism has been an area of controversy. It was observed that insulin receptors and insulin-sensitive GLUT4 [35] have been found at the blood–brain barrier, and insulin has been shown to be modulating the autonomic response to hypoglycemia and of feeding behavior [36]. Insulin crosses the blood–brain barrier via receptor-mediated transcytosis. A modest increase in insulin makes more glucose available to brain cells, resulting in improved memory. If insulin resistant and the level of insulin in the bloodstream remains high, the brain takes measures to slow the transport of insulin across the blood–brain barrier. That reduces the amount of insulin in the brain, making less glucose available to nourish brain cells. Recent evidence on brain insulin action suggests that brain insulin action is a determinant of insulin requirements among individuals with diabetes. Gelling et al. [37] found that insulin receptor substrate (IRS)-phosphatidylinositol 3-kinase (PI3K) signaling is reduced in the mediobasal hypothalamus of rats with STZ-DM that this reduction is readily reversed by insulin treatment and that infusion of an inhibitor of PI3K signaling into the third cerebral ventricle attenuates the glycemic response of diabetic animals to systemic insulin treatment. Earlier studies on chromium suggest that CrPic enhances PI3 kinase and helps to improve insulin sensitivity in insulin resistance and metabolic syndrome animal models; Wang et al. [38] observed an increase in IRS-1 phosphorylation and IRS-1–associated PI-3 kinase activity were observed in vivo in skeletal muscle after insulin stimulation in obese JCR:LA-cp rats (a model for insulin resistance) administered CrPic compared with obese controls. The enhanced signaling occurred without an increase in the content of proteins involved in the insulin signaling cascade, e.g., IRS, PI-3 kinase and Akt. However, the modulation of a protein tyrosine phosphatase, i.e., g protein tyrosine phosphatase (PTP1B), by chromium levels was suggested by the decreases in enzyme activity in obese rats administered CrPic.

The present study demonstrates for the first time that fat-induced insulin resistance significantly affects the serotonergic properties and fat-fed/STZ-induced rats further shows significant changes in cortisol, serotonin, and tryptophan levels in the serum and brain tissue. This may affect the neural insulin signaling pathway. It is very clear that CrPic significantly affects the neural insulin signaling pathway through its action through the insulin signaling transduction pathway. Wang et al. [25, 38] demonstrated that CrPic enhances protein kinase B (Akt) phosphorylation and PI3 kinase, and helps for translocation of GLUT4 transporters to enhance insulin function. A positive correlation of Cr levels and cognitive performance with a better glucose intake in central nervous system was reported [39]. Attenburrow et al. [8] observed that CrPic increased peripheral and central tryptophan availability and elevated brain 5-HT content in rats. CrPic appeared to reduce serum branched-chain amino acids and a consequential increase of the tryptophan to branched-chain amino acids (BCAAs) ratio [8]. Insulin may aid the transport of 5-HT precursor tryptophan (TRP) across the blood–brain barrier through its ability to increase muscle uptake of BCAAs. Earlier studies have reported that CrPic enhances BCAAs in skeletal muscle cells [40]. So a hypothesis may be a theory of transport of TRP across the blood–brain barrier through its ability to increase glucose uptake, and BCAAs in skeletal muscle cells may enhance insulin sensitivity and decreasing the brain insulin resistance. Liu et al. [41] conclude that an appropriate level of insulin plays an important role in maintaining the normal function of the blood–brain barrier through regulating the function and expression of P-glycoprotein in the diabetic and normal rats. In the present study, brain insulin levels were improved in the CrPic-treated group. Further long-term intervention studies are required to assess the physiological changes in the brain of insulin resistance and diabetes models and its association with insulin sensitivity.

In conclusion, HFD/STZ treatment lowered brain chromium levels and impaired glucose metabolism, insulin levels, and serotonergic properties. CrPic administration was effective in attenuating or restoring these negative effects. The

results from this study may provide a link between defects in glucose metabolism and serotonergic activity. In addition, CrPic administration may represent a viable nutritional treatment option for the management of depression and diabetes. Therapeutic strategies that target neuronal insulin signal transduction molecules may therefore prove beneficial in the management of diabetes in humans.

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Conflict of Interest Statement The study was funded by Nutrition 21, Inc., NY, USA. Nutrition 21 also supplied the chromium picolinate used in the study. James Komorowski is an employee of Nutrition 21, the distributors of chromium picolinate under a license from the USDA.

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