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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1233 – 1240
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat

Kazim Sahin[a,*], Muhittin Onderci[b], Mehmet Tuzcu[c], Bilal Ustundag[d], Gurkan Cikim[e], İbrahim H. Ozercan[f], Vidyasagar Sriramoju[g], Vijaya Juturu[h], James R. Komorowski[h]

[a]*Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, 23119 Elazig, Turkey*
[b]*Department of Biochemistry, Veterinary Control and Research Institute, 23100 Elazig, Turkey*
[c]*Department of Biology, Faculty of Science, Firat University, 23100 Elazig, Turkey*
[d]*Department of Biochemistry, School of Medicine, Firat University, 23100 Elazig, Turkey*
[e]*Department of Biochemistry, Sarahatun Hospital, 23100 Elazig, Turkey*
[f]*Department of Pathology, School of Medicine, Firat University, 23100 Elazig, Turkey*
[g]*Institute for Ultrafast Spectroscopy and Lasers, The City College of New York, NY 10031 USA*
[h]*Technical Services and Scientific Affairs, Nutrition 21 Inc, Purchase, NY 10577, USA*

Received 5 January 2007; accepted 12 April 2007

Abstract

Chromium supplements are widely used as an alternative remedy for type 2 diabetes mellitus (T2DM). In vitro study findings show that chromium picolinate (CrPic) may improve insulin sensitivity by enhancing intracellular insulin receptor. In this study, we evaluated the metabolic effects of CrPic in a rat model of T2DM. Male Sprague-Dawley rats (n = 45, 8 weeks old) were divided into 3 groups. The controls (group I) received a standard diet (12% of calories as fat); group II received a high-fat diet (HFD; 40% of calories as fat) for 2 weeks and then were intraperitoneally injected with streptozotocin (STZ, 40 mg/kg; HFD/STZ) on day 14; group III rats were given group II diets with the addition of 80 μg CrPic per kilogram body weight per day. The addition of CrPic in the group III treatment lowered glucose by an average of 63% ($P < .001$), total cholesterol by 9.7% ($P < .001$), and triglycerides by 6.6% ($P < .001$) compared with group II treatment. Compared with group II, CrPic treatment also lowered free fatty acid levels by 24% ($P < .001$), blood urea by 33% ($P < .05$), and creatinine level by 25% ($P < .01$), and reduced the severity of glomerular sclerosis ($P < .0001$). Histopathologic findings suggest that the CrPic-treated group had normal renal tubular appearance compared with the HFD/STZ-treated group. Normal appearance of hepatocytes was observed in the CrPic-treated group. These results showed that CrPic has marked beneficial effects against microvascular complications. In conclusion, HFD/STZ rats provide a novel animal model for T2DM. Further treatment with CrPic for 10 weeks significantly ameliorated changes in metabolic risk factors including favorable changes in histopathology of the liver, kidney, and pancreas, suggesting its potential role in the management of diabetes.

1. Introduction

Diabetes mellitus is a heterogeneous disease characterized by microvascular pathology leading to long-term complications clinically manifested principally in the kidney and retina. It is a common metabolic disorder characterized by relative or absolute lack of insulin. The insulin-sensitizing action of chromium picolinate (CrPic) has been indicated as the mechanism of its antidiabetic activity in experimental models of type 1 and type 2 diabetes mellitus (T2DM). Chromium is required for optimal insulin activity and normal carbohydrate and lipid metabolism [1]. Human studies suggest that CrPic increases insulin sensitivity, decreases insulin levels, and improves glucose disposal in obese populations with T2DM. CrPic is a widely used dietary supplement in the United States. A few studies have shown that CrPic lowers blood glucose without increasing insulin secretion, and CrPic has been considered as an insulin sensitizer [1,2]. In fact, CrPic showed multiple beneficial

Presented at the 66th American Diabetes Association Scientific Sessions, June 9 to 13, 2006, Washington, DC.
* Corresponding author. Tel.: +90 424 237 00 00x3938.
E-mail address: nsahinkm@yahoo.com (K. Sahin).

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doi:10.1016/j.metabol.2007.04.021

Table 1
Composition of diets (in grams per kilogram diet)

Ingredients	Normal diet	High fat diet
Casein	200.0	200.0
Starch	615.0	150.0
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 cloride	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*-α-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; calcium 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.

effects in T2DM, including attenuating body weight gain [3], improving lipid profiles [1-6], and enhancing endothelial function [4]. Chromium picolinate improves insulin sensitivity and may reduce other associated complications [7]. The mechanisms of CrPic action have remained obscure, despite multiple pathways of action being proposed, including a decrease in hepatic glucose production, an increase in peripheral glucose disposal, and a reduction of intestinal glucose absorption [8,9]. It has been documented that CrPic activated 5′-adenosine monophosphate (AMP)–activated protein kinase (AMPK). Thus, AMPK seems to be a major signal for the action of CrPic to suppress lipogenesis and induce fatty acid oxidation [9]. It has been demonstrated that CrPic administration increases glucose uptake in the skeletal muscle [8]. Chromium supplementation of obese, insulin-resistant rats may also improve insulin action by enhancing intracellular signaling [8,9]. The translocation of glucose transporter 4 is mediated through insulin-independent phosphorylation and activation of AMPK [9]. In addition, recent studies [10,11] reported chromium action was absent in methyl-β-cyclodextrin–pretreated cells already displaying reduced plasma membrane cholesterol and increased glucose transporter 4 translocation, and these findings at the cell level are consistent with in vivo observations of improved glucose tolerance and decreased circulating cholesterol levels after chromium supplementation and serotonergic pathway involvement [12]. Early reports suggested that chromium enhances insulin binding, insulin receptor number, insulin internalization, and beta-cell sensitivity [13]. Chromium enhances tyrosine phosphorylation of the insulin receptor in response to low doses of insulin, but this does not change insulin binding or inhibit the tyrosine phosphatase (PTP1B) that dephosphorylates the receptor [11]; more recent work showed that chromium enhances glucose uptake [14]. Pattar et al [15] found the effect of CrPic on proteins involved in cholesterol homeostasis revealed that the activity of sterol regulatory element–binding protein (SREBP), a membrane-bound transcription factor ultimately responsible for controlling cellular cholesterol balance, was up-regulated by CrPic. In addition, adenosine triphosphate–binding cassette transporter A1 (ABCA1) was decreased, consistent with SREBP transcriptional repression of the *ABCA1* gene. Chromium treatment decreased plasma membrane cholesterol. These cellular responses suggest a significant effect of chromium on cholesterol homeostasis [16].

Reed et al [17] suggested that fat-fed/streptozotocin (STZ)-treated rats is a novel animal model for T2DM and is suitable for the testing of antidiabetic compounds. Therefore, the study is designed to evaluate the effects of CrPic supplementation on the metabolic risk factors and to determine the effects of chromium supplementation on the histopathologic status of tissues in diabetic rats.

2. Materials and methods

2.1. Animals

Forty-five (8 weeks old) Sprague-Dawley rats weighing between 200 and 250 g were purchased from Firat University Laboratory Animal Research Center (Elazig, Turkey). These animals were reared at the temperature of 22 ± 2°C, humidity of 55% ± 5%, and a 12/12-hour light/dark cycle. The experiment was conducted under the protocol approved by the Firat University. All procedures involving rats were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the university.

Rats consumed a standard diet and tap water ad libitum. The rats were weighed every week and at the end of the study. Blood sample was collected from the tail vein of each rat for the measurement of biochemical efficacy and safety markers.

2.2. Materials

Chromium picolinate (Nutrition 21, Purchase, NY) was dissolved in water and administered at a concentration of 80 μg/kg per day in the drinking water for 10 weeks to get 8 μg chromium per day, which is an equivalent dose of 560 μg chromium for a 70-kg adult human. STZ was obtained from Sigma (St. Louis, MO). To induce experimental diabetes, STZ was dissolved in citrate buffer (pH 4.5) and injected once intraperitoneally (IP) at a dose of 40 mg/kg to the remainder of the animals. A control group was given citrate buffer via IP injection.

Ingredients and chemical composition of the basal diet are shown in Table 1. The diets were stored in a cold chamber at 4°C. Animals were fed either a regular diet consisting of 12% fat (as a percentage of total kilocalories) and 26% protein or a high-fat diet (HFD) consisting of 40% fat and 18% protein.

2.3. Experimental design and induction of T2DM

The fat-fed, STZ-treated rat model provides a novel animal model for T2DM that simulates the human syndrome and is suitable for the testing of antidiabetic compounds [17]. This study was conducted to evaluate the effects of CrPic on metabolic risk factors in an animal model of T2DM.

The animals were divided into 3 groups: group I (control group) rats were fed standard diet (12% of calories as fat); group II (HFD/STZ group) rats were fed HFD (40% of calories as fat) for 2 weeks and then injected with STZ (40 mg/kg IP); group III (HFD/STZ+CrPic) rats were fed HFD (40% of calories as fat) for 2 weeks and then injected with STZ (40 mg/kg IP) and CrPic was added to the drinking water at a concentration of 80 μg/kg body weight per day for 10 weeks.

Before STZ injection, glucose concentrations of study rats and controls were measured and compared. After the injection of STZ, animals that exhibited fasting glucose levels greater than 140 mg/dL were considered as neonatal STZ diabetic resembling T2DM in humans. Plasma insulin concentrations in response to oral glucose (2 g/kg) were determined.

2.4. Analysis of metabolic markers

In all groups, tail vein blood was collected for the analysis of metabolic markers. Blood samples were centrifuged at 3000*g* for 10 minutes and sera were collected. Estimation of insulin sensitivity made from oral glucose tolerance test data was performed using the composite insulin sensitivity index (CISI) proposed by Matsuda and De Fronzo [18]. Calculation of the index was made according to the following equation:

$$\mathrm{CISI} = \frac{10\,000}{\sqrt{(\mathrm{FSG} \times \mathrm{FSI})(\mathrm{MG} \times \mathrm{MI})}}$$

where FSG and FSI are fasting serum glucose and insulin concentrations, respectively, and MG and MI are the mean glucose and insulin concentrations, respectively, over the course of the oral glucose tolerance test.

Plasma glucose concentrations were measured by using ACCU-Chek Active (Roche Diagnostics, Basel, Switzerland). Serum insulin levels were measured with the Rat Insulin Kit (Linco Research, St Charles, MO) by enzyme-linked immunosorbent assay (ELISA) (El_x-800, BioTek Instruments, Winooski, VT). Serum concentrations of cholesterol and triglycerides (TGs) were measured by diagnostic kits (Sigma Diagnostics, St Louis, MO). Free fatty acid (FFA) levels were measured by a diagnostic kit (Boehringer Mannheim, Mannheim, Germany). Serum urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were measured with a biochemical analyzer (Olympus AU-660, Tokyo, Japan). Lipid peroxidation was assessed as thiobarbituric acid–reactive substance concentrations in serum and liver samples by the method of Mihara and Uchiyama [19]. Values are reported as the concentration of malondialdehyde (MDA). Chemical analyses of the diet samples were performed using Association of Official Analytical Chemists procedures [20].

Table 2
Effects of CrPic and biotin supplementation on biochemical parameters in diabetic rats

Metabolic markers	Control	HFD/STZ	HFD/STZ+CrPic
Body weight (g)	320 (3.5)[a]	215 (3.4)[b]	240 (3.4)[c]
Glucose (mg/dL)	103 (2.2)[a]	469 (7.9)[b]	287 (2.7)[c]
Insulin (μU/mL)	48.6 (0.21)[a]	23.2 (0.29)[b]	26.0 (0.26)[c]
CISI	2.65 (0.03)[a]	0.87 (0.04)[b]	1.23 (0.04)[c]
TC (mg/dL)	95 (0.68)[a]	250 (0.94)[b]	228 (0.84)[c]
TG (mg/dL)	135 (7)[a]	390 (7.8)[b]	366 (5.8)[c]
FFA (mmol/L)	1.6 (0.24)[a]	4.1 (0.21)[b]	3.3 (0.25)[c]

Data are expressed as mean (SD). Superscripts denote significant differences ($P < .05$) between groups.

Table 3
Effects of CrPic supplementation on safety markers in diabetic rats

	Control	HFD/STZ	HFD/STZ+CrPic
MDA (μg/g)			
Serum	0.92 (0.02)[a]	3.82 (0.02)[b]	2.78 (0.019)[c]
Liver	6.1 (0.2)[a]	11.2 (0.22)[b]	9.5 (0.21)[c]
Urea (mg/dL)	34 (2.02)[a]	65 (2.12)[b]	49 (1.9)[c]
Creatinine (mg/dL)	0.41 (0.02)[a]	0.70 (0.03)[b]	0.56 (0.03)[c]
AST (U/L)	115 (6.9)[a]	241 (6.6)[b]	189 (9.0)[c]
ALT (U/L)	80 (3.4)[a]	150 (4.2)[b]	13 (3.6)[c]

Data are expressed as mean (SD). Superscripts denote significant differences ($P < .05$) between groups.

2.5. Histopathology

After laparatomy, tissues (liver, kidney, and pancreas) of each rat were examined grossly. The tissues were removed for histologic study, washed with normal saline, and immersion-fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin for histologic examination according to standard procedures [21].

2.6. Statistical methods

All results were presented as means ± SD for the number of animals indicated. Sample size was calculated based on a power of 85% and a P value of .05. Given that assumption, a sample size of 10 per treatment was calculated. The data were analyzed using the GLM procedure of SAS software (1999; Cary, NC). Least squares treatments were compared if a significant F statistic (5% level of P) was detected by analysis of variance. Treatments were also compared using Student unpaired t test for comparison of individual treatment. $P < .05$ was considered statistically significant.

3. Results

Animals were fed the test diets for 2 weeks before the STZ injection and the effects of the CrPic on metabolic and safety markers are briefly summarized in Tables 2 and 3.

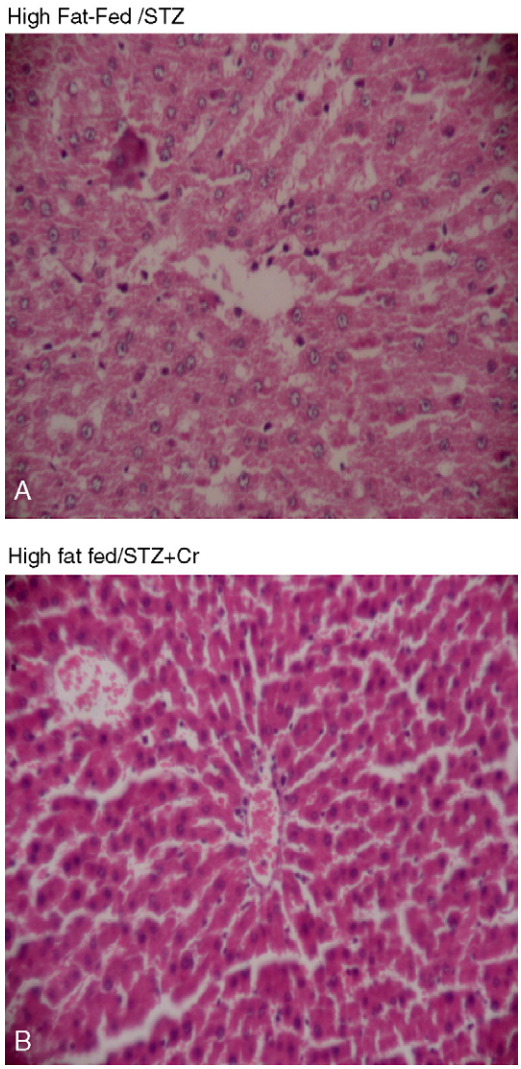

Fig. 1. Liver morphology: HFD/STZ vs HFD/STZ+CrPic.

3.1. Body weight and metabolic markers

Body weight was significantly decreased in group II (HFD/STZ, $P < .001$) rats compared with group I (control) and group III (HFD/STZ+CrPic rats). Compared with group II, group III rats had significantly reduced glucose concentrations and a significantly increased insulin concentration ($P < .001$). Total cholesterol (TC), TGs, and FFAs were also significantly decreased in group III ($P < .001$). Compared with group II, the addition of CrPic in the group III treatment lowered glucose by an average of 63% ($P < .001$), TC by 9.7% ($P < .001$), and TGs by 6.6% ($P < .001$). Compared with group II, CrPic treatment also lowered FFAs by 24% ($P < .001$).

3.2. Safety markers

In comparison to group II (HFD/STZ) rats, a significant decrease in blood urea, creatinine, AST, MDA in serum and liver, and ALT were observed in group III ($P < .01$) rats.

3.3. Histopathology

Chromium picolinate was effective in preventing/reverting diabetes condition. CrPic treatment induced functional and histologic improvements in kidneys, livers, and pancreata of HFD/STZ rats.

3.4. Liver morphology

Control rat livers had normal histology with normal hepatocellular architecture with normal central vein (Fig 1). Hepatocytes have pink eosinophilic cytoplasm without any inclusions and with mostly central single nuclei. These cells have well-defined cell borders, are polygonal, and are arranged in sheets. Liver sinusoids were not dilated. There were no areas of hemorrhage or fibrosis. In HFD/STZ rats, hepatocytes had vacuolated eosinophilic cytoplasm with ground-glass appearance and fatty inclusions.

The nuclei were enlarged, displaced, and vacuolated. There were several pycnotic nuclei suggesting significant hepatocellular degeneration. The cells were irregular in size, shape, and orientation. Moderate macrovesicular fatty degeneration of liver with dilated sinusoids was observed.

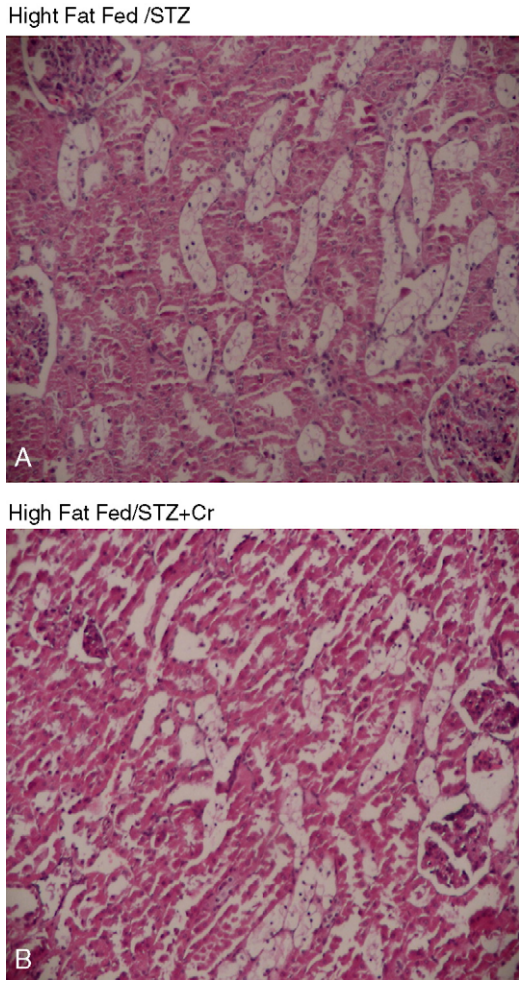

Fig. 2. Kidney morphology: HFD/STZ vs HFD/STZ+CrPic.

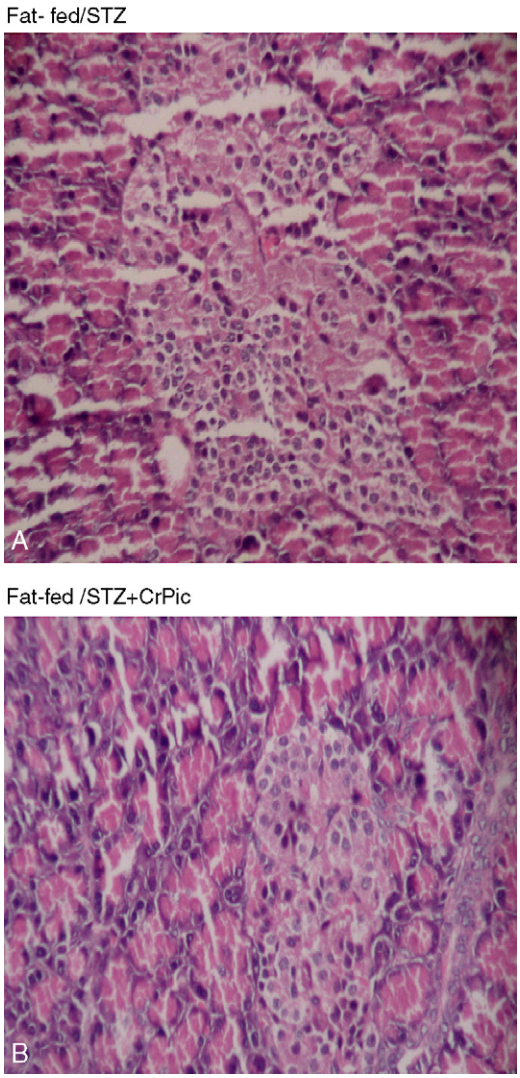

Fig. 3. Pancreas morphology: HFD/STZ vs HFD/STZ+CrPic.

The incidence and intensity of changes in group III (HFD/STZ+CrPic) rats was much lower compared with group II (HFD/STZ) rats. In group III rats, dilated vessels and sinusoids with no significant hepatocellular degeneration were observed. Hepatocytes had pink eosinophilic cytoplasm without any inclusions and with mostly central single nuclei showing lobules with significantly dilated central vein and hepatoportal vein. These cells, with well-defined cell borders, were polygonal and arranged in sheets.

3.5. Kidney morphology

In group I rats, normal renal tubular architecture and normal glomeruli were observed. In group II rats, renal parenchyma showing significant vacuolar degeneration of renal tubular cells with displaced pycnotic nuclei was observed (Fig 2). Renal glomeruli showing mesangial hyperplasia and eosinophilic fibrinous deposits were observed. Diffuse infiltration of monomorphonuclear cells in renal parenchyma and the glomeruli was observed. There was tubulointerstitial nephropathy with significant mesangial proliferation in the glomeruli. In group III rats, renal glomeruli showing normal histology and tubulointerstitial nephropathy with no major glomerular pathology were observed.

3.6. Pancreas morphology

Group I rats had normal exocrine pancreatic acinar architecture and pancreatic islets showing predominantly insulin-producing beta cells with granular basophilic cytoplasm and few eosinophilic glucagon-producing alpha cells (Fig 3). In group II rats, pancreatic islets showed a relatively decreased population of insulin-producing beta cells with granular basophilic cytoplasm and several eosinophilic glucagon-producing alpha cells. There were areas of eosinophilic amorphous deposits within islets, suggesting cellular necrosis.

Degenerative changes and islet cell necrosis in endocrine pancreas was observed. In group III rats, normal exocrine pancreatic acinar architecture and pancreatic islets showing predominantly insulin-producing beta cells with basophilic cytoplasm and few eosinophilic glucagon-producing alpha cells were observed. There is no evidence of necrosis or cellular degeneration within islets. Normal pancreatic islet histology was observed.

4. Discussion

High-fat diets induce insulin resistance in rodents [22-24]. Insulin resistance and hyperinsulinemia have been shown to predict T2DM. In the current study, group II rats had hyperglycemia but lower insulin concentrations. The increased magnitude was not due to a greater decline in beta cell function [17]. The results in Table 2 indicate that serum glucose concentrations decreased significantly in group III rats. In T2DM clinical trials, CrPic significantly reduced glucose concentrations [1,2]. Some of the anti-hyperglycemic agents commonly used to treat T2DM had no significant effects, or increased lipids and were dose dependent [25,26]. However, in this study, CrPic treatment reduced TC, TGs, and FFAs. Similar observations were reported in human T2DM clinical trials [1,2,5,6]. Consistent with findings of other researchers [8,9,27-31], the effect of CrPic administration produced a simple additive response, reducing serum and improving insulin sensitivity (as calculated by CISI) further, while exhibiting lipid-lowering effects. In this study, CrPic-treated rats (group III) reduced glucose concentrations and improved insulin sensitivity by increasing insulin levels. It should also be noted that HFD/STZ rats were hypercholesterolemic and hypertriglyceridemic, the characteristic abnormality of lipoprotein metabolism in patients with T2DM. This study provides an opportunity to study the effect of hyperglycemia on beta cells that are still capable of secreting substantial amounts of insulin. This model is interesting for the study of the pathophysiologic changes in HFD/STZ rats treated with

CrPic. In group II rats, the sinusoids are significantly dilated, hyperemic, and lined with several Kupffer cells, and in liver lobule, significantly altered hepatocellular architecture with moderate to severe degenerative changes was observed. No significant hepatocellular degeneration was observed in CrPic-treated HFD/STZ rats. Pancreatic islets showed predominantly insulin-producing beta cells with basophilic cytoplasm and few eosinophilic glucagon-producing alpha cells [32]. There is no evidence of necrosis or cellular degeneration within islets in CrPic-treated rats. In HFD/STZ rats, pancreatic islets showed relatively decreased population of insulin-producing beta cells with granular basophilic cytoplasm and several eosinophilic glucagon-producing alpha cells. There were areas of eosinophilic amorphous deposits within islets, suggesting cellular necrosis. The morphologic changes in HFD/STZ rats indicated an impaired renal function. Lack of changes in the renal morphology and decrease in oxidative stress, decrease in urea, creatinine, AST, and ALT levels associated with HFD/STZ rats treated with CrPic indicated lack of nephrotoxicity and hepatotoxicity effects of CrPic.

No toxic effects were observed in in vivo models at different doses [33,34] as well as in diabetic in vivo models [27,35,36]. In vitro mutagenicity is controversial based on reported studies [37-42]. The mutagenic effects may be due to different conditions, use of solvents, and exposure of cells at higher doses beyond physiologic conditions [37-43], which do not occur in in vivo models and in humans. Delayed pupation and decreased pupal viability were observed in a *Drosophila* study [43]. In the same study, CrPic increased lethal mutations and dominant female sterility. It is not possible to extrapolate such data to in vivo exposure in mammals and to quantitatively relate this finding in insects to the magnitude of the human risk of similar damage, if any. The difficulty in extrapolating from an insect model to man precludes a firm conclusion about these results, but the lack of genotoxic activity in the rodent assays noted in other studies [27,35,44] argues against such activity in mammalian germ cells because somatic cell mutagenicity is usually seen concurrently with germinal cell damage. This does not appear to be the case, at least at levels used for dietary supplementation, because $Cr(Pic)_3$ has been effectively used as a dietary supplement to increase the fertility of swine, as reviewed in an Institute of Medicine monograph [44].

The Institute of Medicine [44] reviewed more than 15 human clinical trials and observed no toxic effects with CrPic. Other government regulatory agencies reviewed the safety of CrPic and observed no safety concerns [45,46]. Mita et al [27] reported that renal chromium content and the recovery of renal chromium concentration after chromium supplementation were significantly lower in diabetic mice than in nondiabetic mice ($P < .001$). These observations suggest that CrPic supplementation improves insulin sensitivity and improves renal function by recovering renal chromium concentration.

Chromium can modulate the activity of insulin by increasing the insulin-sensitive cell receptors or binding activity and enhancing intracellular insulin signaling activity [6,9]. Insulin can act as a stimulator in anabolism and as an inhibitor in catabolism, subsequently increasing the blood glucose uptake and utilization by cells [47]. Insulin depresses lipolysis in adipocytes and inhibits gluconeogenesis [48]. The insulinotropic effect of stress-related hormones such as epinephrine are depressed, which consequently inhibits lipid mobilization [49]. The hypoglycemic effect of trivalent chromium was reported under insulin-deficient conditions [35,50-52] and exhibited significant antidiabetic potential in STZ-induced diabetes in rats. The current study was designed to develop insulin resistance and the transition of insulin resistance to the development of hyperglycemia that occurs in T2DM.

Fat-fed/STZ rats (group II) had significant increases in MDA, creatinine, blood urea, AST, and ALT. CrPic-treated fat-fed /STZ rats had significant decreases in all tested safety markers ($P < .01$). These markers are sensitive measures for oxidative stress metabolite, liver, and kidney functions. The test results indicate the safety of CrPic as a antihyperglycemic agent and in improving insulin health. Chromium supplementation reduced serum concentration of MDA in stressed hens [53,54]. Preuss et al [55] reported a decrease in hepatic thiobarbituric acid–reactive substance formation by supplementation of CrPic and chromium nicotinate in rats. Decrease in MDA levels could be related to inhibition of epinephrine because of the insulinotropic effect of chromium. Changes in biochemical values detected in the present study could be due to the stimulatory effect of CrPic on insulin activity. Similarly, Shinde and Goyal [35] reported significant decrease in creatinine, urea, AST, and ALT levels in diabetic rats treated with CrPic.

In conclusion, results from this study suggest that CrPic has significant effects on glucose and lipid profiles and improving insulin sensitivity in HFD/STZ rats. No hepatotoxic and nephrotoxic effects were observed in CrPic-treated rats. Thus, CrPic supplementation improves insulin sensitivity and pathophysiologic features of an insulin-resistant rat model.

Acknowledgment

Funding and product supplies for this study were provided by Nutrition 21 Inc, Purchase, NY.

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