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Effects of Dietary Chromium-Methionin Supplementation on Blood Metabolites and Insulin Resistance Index in Fructose-Fed Diabetic Rats

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Abstract: The aim of this study was to evaluate the effect of chromium-methionin supplementation in high dosage on blood parameters and Insulin Resistance (IR) index in fructose induced insulin resistant rats. At first stage of the experiment, ten wistar rats were assigned as the healthy control group and sixteen rats were given Fructose (Fr) for 5 weeks in order to inducing insulin resistance. In the 2nd stage the insulin resistant rats were divided into Cr-fed group that additionally fed 1000 μg day^{-1} chromium as Chromium-L-Mmethionin (Cr-Met) and insulin resistant rats with no chromium supplementation for 6 weeks. In the first stage, IR was significantly induced ($p<0.05$) in rats receiving Fr. Plasma Fasting glucose, insulin, IR index and triglyceride contents were significantly ($p<0.05$) increased in Fr-fed group in comparison with the control. According to the results of second stage, IR index significantly ($p<0.05$) decreased after Cr-Met supplementation. The fasting insulin, glucose and triglycerides concentrations in Cr-Met treated group were lower ($p<0.05$) than the Fr-fed insulin resistant group. The results of this study indicated that chromium supplementation as Cr-Met could be effective for lowering IR index, fasting insulin, glucose and triglyceride in insulin resistant rats.

Key words: Chromium-methionin, insulin resistance, fructose, rat, fasting insulin, triglycericles

INTRODUCTION

It has been predicted that in year 2030, the number of people with diabetes mellitus and metabolic syndrome will reach to 336 millions (Wild *et al*., 2004). This fact has encouraged many researchers to study on the effects of Anti-diabetic agents. It has been noted that trivalent Cromium (Cr) has anti diabetic and Cholesterol (Chrl) lowering characteristics (Althuis *et al*., 2002). Mertz *et al*. (1961) showed that trivalent Cr potentiates the insulin actions through increasing glucose uptake by epididymal fat cells of Sprague-Dawley rats.

Yamamoto *et al*. (1984) revealed the importance of one Low Molecular Weight Cr binding protein (LMWCr) or Chromodulin in insulin internalization and glucose regulation. Titration of Cr free Chromodulin (apo-chromodulin) with Cr determined that four equivalent of Cr ions required for maximum activity of Chromodulin (Davis and Vincent, 1997a; Hatfield *et al*., 2006). additionally, it was observed that type II diabetes Mellitus accompanied with increasing urinary Cr excretion and reducing the plasma Cr concentration (Anderson *et al*., 1991; Morris *et al*., 1999).

High urinary excretion of Cr in diabetic subjects is two fold more than healthy ones which is an indicator of relationship between Cr and insulin pathway (Morris *et al*., 1993). Recently, UV-Vis spectroscopy and fluorescence studies have determined the interrelationship between Cr (III) and B21site of Glutamine in insulin (Sreekanth *et al*., 2008).

For evaluating the effects of Cr compounds, different organic and inorganic forms of Cr such as $CrCl_3$ (Anderson *et al*., 1983), Cr Nitrate, Cr acetate, Nicotinate), niacin bound Cr (Crawford *et al*., 1999), Cr Picolinate (Gosh *et al*., 2002; Martin *et al*., 2006), Cr Sulfate (Chung *et al*., 1983), Cr Alum (Mertz *et al*., 1961), Cr Propionate (Sumner *et al*., 2007) and Cr nano composite (Wang *et al*., 2007) has been supplemented to animal

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diets. More studies have demonstrated that organic compounds could be more efficient for lowering plasma insulin and glucose in insulin resistant subjects (Vincent, 2004).

Some long term studies demonstrated that Cr Picolinate, the most popular organic dietary supplement of Cr, may cause deleterious effects on DNA structure (Hepburn *et al*., 2003). Chelating Cr with amino acids could be a suitable substitute for Cr Picolinate. Amino acid binding Cr is a novel Cr compound which is absorbed by amino acids mechanism in animal gut. Accordingly, these compounds such as Chromium-Methionin (Cr-Met) and chromium phenylalanine would be more efficient in insulin signaling pathways and glucose metabolisms (Smith *et al*., 2005). A reported recommendation for maximum tolerable concentration of dietary Cr soluble compounds is 100 mg kg^{-1} diet for laboratory animal's (National Research Council, 2005). Approximately, in all studies that observed positive effects of Cr, elemental Cr was fed between 1-1000 μg day^{-1} for organic and inorganic supplements (Balk *et al*., 2007). There is no sufficient information about appropriate dose of Cr-Met supplementation and effects of it's over dose feeding in laboratory animals with insulin resistance. Also the beneficial influences of Cr nutrition on type 2 diabetes mellitus require more studies (Heronenberg *et al*., 2008). The aim of this study was to evaluate the effects of high concentration Cr-Met supplementation on blood parameters and insulin resistance index in Fr induced insulin resistant rat as a model for nutritional metabolic syndrome.

MATERIALS AND METHODS

Animals and treatments: About 26 male Albino Wistar rats with mean body weight of 225±25 g were provided by Iranian pastor institute and housed individually in standard cages in an air conditioned room with temperature of 22±2°C humidity of 45-50% and 12 h photo period (Wang *et al*., 2006). Institutional Animal Care and Use Committee of Mashhad University of Medical Science approved all procedure-involving animals.

All rats were nourished with 15 g standard rat chow (Javaneh Khorasan, Mashhad, IR) based on recommendations of National Research Council (1995). At first stage of experiment after 2 weeks adaptation in an steady condition, ten animal randomly (Snedecor and Cochran, 1991) assigned to healthy control group (Ctrl) and sixteen ones received Fructose (Fr) (Merck Chemicals, ART: 105321) by 10% W/V in drinking water for 5 weeks in order to inducing insulin resistance (Fr-fed) (Juan *et al*., 2001).

In second stage of experiment the insulin resistant rats were randomly divided in to two groups. Eight animals were additionally fed 50 ppm elemental Cr supplement as Cr-L- methionin (Zinpro Inc, Eden Prairie, MN, USA, Cr-Met: a source which supplied 1000 ppm of Cr from a compound containing 3 molecules methionin and 1 atom Cr) in the diet, approximately equal to 1000 μg day^{-1} Cr (Cr group) and the others (Fr group) remained in the previous feeding manner for 6 weeks base on Vincent recommendations (Vincent, 2004). The healthy Ctrl group was nourished as well as at first stage of experiment. The Cr content of Ctrl diet was negligible (<0.1 μg g^{-1}).

Sampling and assays: Animals were blood sampled via tail snip procedure (Clodfelder and Vincent, 2005) prior to Cr administration in order to test for IR inducing and at the end of experiment for determination of blood plasma parameters including fasting Glucose, Triglyceride (TG), Chrl and insulin contents following overnight fasting. All blood samples were collected in Eppendorf tubes and centrifuged in 1000×g for 10 min.

Extracted plasma was stored at -20°C prior to analysis. The fasting plasma Glucose, TG and Chrl were analyzed using Auto analyzer system (BT-3000 plus, Biotechnica, Rome, Italy) and diagnostic kits (Pars Azmoon, Karaj, Iran) in Abu Ali Sina Research Center (Mashhad, Iran).

The plasma fasting insulin content were analyzed with immunoradiometric assay kit (Biosource INS-IRMA kit, Biosource, Europe, SA, Nivelles, Belgium) using a gamma counter system (Isodata 20/20 gamma counter, Isodata Inc, NJ, USA) in the Special Laboratory of Imam Reza Hospital (Mashhad, Iran). Dietary Cr validation was done using a flame atomic absorption spectrophotometer (Varian Specter AA 50B, Varian Ltd, Pty, Australia) (Jorhem, 2000).

Insulin resistance Index: The Insulin resistance Index was calculated based on Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula (Wallace *et al*., 2004) using HOMA-IR calculator software of Oxford University as follows:

$$\text{HomA-IR} = \text{fasting insulin}/22.5\times e^{-\ln(\text{fasting glucose})}$$

Statistical analysis: Data were analyzed using general linear model of SAS (1996) as completely randomized design with analysis of covariance. Duncan's multiple comparison was used for grouping the variables among treatments. All values are presented as mean±SD and the $p<0.05$ are considered as significant statistically.

RESULTS AND DISCUSSION

The effect of Fr on blood parameters are shown in Table 1. In the first stage of the study, IR was significantly ($p<0.05$) induced in rats receiving Fr. Plasma Fasting glucose, insulin, IR index (HOMA-IR) and TG contents were increased significantly ($p<0.05$) in Fr-fed group in comparison with the Ctrl. These changes could be the result of the Fr metabolite effects on insulin signaling in adipose tissues.

In first stage of this experiment the results of blood parameter analysis and HOMA-IR index values demonstrated that plasma fasting glucose, TG and insulin were significantly ($p<0.05$) increased in response to administration of Fr in drinking water compared with Ctrl group. HOMA-IR index in Fr fed group was increasingly ($p<0.01$) higher than Ctrl group. Zhao *et al.* (2003) reported significant increase in HOMA-IR index in response to Fr nutrition in rats. Considering that HOMA-IR index can be a useful tool in the detection of insulin resistance cases is used to judge (Albareda *et al.*, 2000). This results indicated the partially development of insulin resistance and diminished insulin sensitivity of tissues (Elliott *et al.*, 2002). The results about Fr-fed group were similar to findings of Juan *et al.* (2001) and Jalal *et al.* (2007) studies. Continues Fr feeding have been shown to induce insulin resistance, weight gain, hypertension and hyperglycemia in rats. Fr has the potential to induce fatty acid de novo synthesis that may result in insulin resistance emersion in hepatic cells and adipose tissues (Le and Tappy, 2006). The existence of Fr specific transporters in brush border and basolateral membrane of epithelial cells in jejunum may cause to high Fr conveyance in to the portal and consequently immoderate uptake in hepatic cells. In an analogous studies some key factors mentioned as insulin resistance developing agents related to Fr metabolism such as over loading of adipose tissues through unregulated synthesis of TG and glucose precursor with unlimited rate from Fr has been noted (Hansen *et al.*, 2006).

This may be as a signal for releasing of free fatty acids from adipose tissues into the blood stream. Also, the hydrolysis of TG by lipoprotein lipase in the blood accompanied with releasing of very low density lipoprotein from liver elucidated the Non Esterified Fatty Acids (NEFA) in blood plasma. These events may cause to ectopic storing of NEFA in skeletal muscles that could interfere with insulin signaling and developing of insulin resistance in response to NEFA influx in to the blood Lionetti *et al.* (2009), Fried *et al.* (1998) and Hotamisligil *et al.* (1996) were mentioned that the over loaded adipose tissue can secret the inflammatory cytokines such as inreleukine-6 and tumor necrosis factor-α. These cytokines can greatly increase the insulin resistance that our results were supported these mechanisms. In the second stage of experiment each group (Ctrl, Fr, Cr) were analyzed for plasma parameters and insulin resistance index (HOMA-IR) determination (Table 2). The plasma glucose was significantly ($p<0.05$) diminished after Cr-Met supplementation in Cr group compared with Fr group whereas was similar to the ctrl group.

Table 1: Blood serum content of the rats in first period of experiment

		Treatments	
Variables	Unit	Ctrl (n = 10)	Fr-fed (n = 16)
Glucose	mmol L^{-1}	10.02±0.1200	10.480±0.9000*
Insulin	mU L^{-1}	3.89±0.0500	5.090±0.0400*
HOMA-IR	-	1.81±0.0300	2.270±0.0300**
TG	mmol L^{-1}	1.459±0.011	1.711±0.0030*
Chrl	mmol L^{-1}	1.53±0.0700	1.790±0.0900^{NS}

$^{*}p<0.05$; $^{**}p<0.01$ ^{NS}:$p>0.05$

Table 2: Comparison of blood plasma parameters after Cr-Met administration

		Treatments		
Variables	Unit	Ctrl (n = 10)	Fr-fed (n = 8)	Cr-Met+Fr-fed (n = 8)
Glucose	mmol L^{-1}	10.46±0.16^{a}	11.56±0.16^{b}	10.91±0.16^{a}
Insulin	mU L^{-1}	4.22±0.35^{a}	6.10±0.27^{b}	5.32±0.21^{a}
HOMA-IR	-	1.68±0.04^{a}	3.38±0.11^{b}	2.78±0.09^{c}
TG	mmol L^{-1}	1.74±0.11^{a}	2.42±0.09^{b}	2.17±0.07^{c}
Chrl	mmol $L^{-}1$	1.65±0.05^{a}	2.01±0.05^{a}	1.86±0.04^{a}

The significant differences between treatments are shown with small alphabetic characters ($p<0.05$)

There was also significant difference between Fr group and ctrl in plasma glucose content ($p<0.01$) probably due to the possible mechanisms that mentioned above. This effect of Cr may be because of amplification in insulin signaling and consequently increase in glucose uptake by insulin sensitive adipose tissues (Yang *et al.*, 2006).

Fasting plasma insulin of Cr group was significantly ($p<0.01$) lower than the Fr group and was similar to ctrl. Generally, the insulin releasing from pancreas is increased in response to influx of glucose in blood stream due to glucose regulation. Regarding to the quality that Cr participates in insulin action a secondary synchronized pathway suggested along insulin cascade. Increasing of insulin secretion in to the blood acts as a signal for potentiating transformation of transferrin receptors form intra cellular vesicles to the surface of plasma lemma in insulin sensitive tissues (Kandror, 1999).

This leads to transferrin as a carrier of Cr) entry into the cytoplasm through endocytose. According to the acidic condition of the cell contents, Cr molecules release in cellular environment (Kozlovsky *et al.*, 1986) where apo-chromodulin bind with four Cr ions with intense

affinity to form chromodulin. Chromodulin is a low molecular weight Cr binding protein which has potential to activate the insulin receptors more and more via binding with active site of receptor and consequently make conformational changes (Davis and Vincent, 1997b; Vincent, 2000).

Therefore, reduction in plasma glucose and insulin is related to Cr effects via chromodulin as a cofactor for insulin receptor activators such as kinase enzymes. Regarding to higher urinary excretion of Cr in insulin resistant subjects (Morris *et al*., 1999) in response to insulin fluctuations (Anderson *et al*., 1982) and development of insulin resistance due to Cr deficiency, two possible mode of action for Cr has been suggested (Vincent, 2000; Sreekanth *et al*., 2008). Firstly, chromodulin acts as cofactor for tyrosine kinase in Insulin Receptor Substrate-1 (IRS-1) and stimulates the phosphorylation of tyrosine as well as amplifying the insulin signaling (Hotamisligil *et al*., 1996). Furthermore, chromodulin could be an activator for phosphatidylinositol-3 (PI-3) kinase in muscular cells that cause to more and more glucose uptake and consequently suppression insulin secretion from pancreas (Heronenberg *et al*., 2008).

The fasting plasma TG in Cr group was significantly ($p<0.05$) lower than Fr group. Cr effect on TG level in plasma may be related to amplifying insulin signals that cause to diminish in lipids catabolism. Jeejeebhoy (1999) reported that Cr deficiency due to long term parentral nutrition lead to high plasma glucose, insulin and TG contents due to insulin resistance development that supports the results. Fasting plasma Chrl in Cr group compared with Fr group has an insignificant descending trend. along with insulin signal amplification, it is expected that the Chrl uptake from adipose tissues is declined but all significant effects of Cr on the Chrl level were observed in long term studies (Anderson *et al*., 1997; Sun *et al*., 2002) statistically lower HOMA-IR index in Cr group in response to high dose Cr-Met supplementation ($p<0.05$) was similar to results of Rabinovitz *et al*. (2004) and Kuryl *et al*. (2006) that also HOMA-IR index were reduced noticeably. We didn't observe any symptoms of acute toxicosis such as diarrhea, lacrimation, weight losing, hypoactivity and mydriasis (National Research Council, 2005) along the experiment.

CONCLUSION

The results indicate that Cr-Met as an organic compound could be an insulin amplification agent that also may be an effective booster for insulin sensitivity regarding to the mechanisms that discussed previously.

Further studies to determine histopathology changes and the possibility of chronic toxicity in long-term supplementation of high dose trivalent Cr as organic compounded along with beneficial influences on insulin sensitivity are required and planned.

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