Effect of chromium citrate on the mechanism of glucose transport and insulin resistance in Buffalo rat liver cells

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Abstract:
OBJECTIVE: Our published literature indicated that chromium citrate could regulate the glycemic index in alloxaninduced diabetic mice. The present study investigated the mechanism of chromium citrate in insulin resistance (IR) buffalo rat liver (BRL) cells.

MATERIALS and METHODS: Chromium citrate was synthesized in our laboratory. BRL cells were purchased from the Chinese Academy of Sciences Cell Bank. The glucose transport and IR affected by chromium citrate in BRL cells were examined. The Thiazolyl Blue Tetrazolium Bromide (MTT) and glucose assay experiments were measured by microplate ELISA reader. The protein kinase B (Akt), glucose transporter-4 (Glut4), and phosphor-AMP-activated protein kinase (p1 levels were tested by Western blot, and the mRNA expression of glucose transport proteins (Akt2, Glut4, and AMPActivated protein kinase α2 (AMPKα2)) and insulin sensitivity proteins (insulin receptor substrate1 (IRS-1), phosphatidylinositol 3 kinase (PI3K), and peroxisome proliferator-activated receptor γ (PPARγ)) was measured by reverse transcription–polymerase chain reaction.

RESULTS: The results indicated that the glucose absorption level of chromium citrate groups was higher than model group significantly. It demonstrated that chromium citrate could significantly improve glucose absorption in IR BRL cells. The Akt, Glut4, and phosphor-AMPKβ1 levels in chromium citrate groups (at doses of 0.4, 0.2, and 0.1 µg Cr/mL) were markedly improved when compared with the other experiment groups, and chromium citrate could more effectively increase the Akt level than chromium trichloride. In addition, the mRNA expression of Akt2, Glut4, and AMPKα2 in chromium citrate groups was significantly improved when contrasted with model group.

CONCLUSION: The consequences illustrated that chromium citrate can affect the IR BRL cells’ ameliorating glucose transport and IR.

Keywords: Diabetes mellitus, glucose absorption, reverse transcription–polymerase chain reaction, Western blot

Introduction

Diabetes mellitus is a metabolic syndrome resulting from genetic and environmental factors.[1] The International Diabetes Federation had pointed out that the prevalence rate of diabetes mellitus has reached 8.3%. Diabetes mellitus will be more than 5.92 hundred million in 2035 and has been a serious public health problem.[3] The pathogenesis of diabetes mellitus is the absolute or relative absence of insulin in human beings.[4] Type 2 diabetes (T2D), which is one of the most serious diseases, accounts for more than 90% in diabetes cases and has been ranked as an intractable disease by the World Health Organization.
Previous studies have shown that hyperglycemia and insulin resistance (IR) are the main symptoms of T2D. Moreover, it is also associated with complications such as coronary heart disease, diabetic nephropathy, diabetic peripheral neuropathy, and bad cognitive ability. However, T2D and T2D complications could not be cured completely and could bring great trouble to the healthy body and wonderful life.

Currently, nutritional supplements such as chromium (III), magnesium, calcium, and multivitamin supplements had become an effective way to assist the treatment of T2D. Chromium (III), as a common beneficial micronutrient, can significantly improve glycometabolism and lipid metabolism in organisms. Romero and Moran had reported that lack of sufficient chromium could result in T2D, angiocardiopathy, and neurological disorders. Studies have also reported that appropriate chromium supplements could ameliorate glucose tolerance, IR, atherosclerosis, and immune function. At present, inorganic and organic chromium complexes are the main categories of chromium supplements in the market. Organic chromium complexes have acted as a functional food additive and a dietary supplement widely, for example, chromium yeast, chromium nicotinate, and chromium picolinate. Meanwhile, published literature indicated that chromium picolinate could retard the IR through regulating intracellular pathways of glucose metabolism. However, studies had proved that chromium picolinate could result in DNA damage and cytotoxicity. In addition, inorganic chromium complexes were not widespread used because of its poor absorption and high toxicity. In view of the above problems, characterizing a novel and fewer side effects, antihyperglycemic organic chromium complex is a considerable subject.

Chromium citrate is a novel organic chromium complex and is synthesized by citric acid and chromium trichloride hexahydrate in our laboratory. Li et al. had discussed that chromium citrate was capable of adjusting blood glucose level in alloxan-induced diabetic mice. Citrate acid, which has been used widely in food, beverage, and cosmetic production, is environment-friendly and low toxicity as an organic ligand. Our previous study showed that chromium citrate did not give rise to oxidative damage in DNA level under physiological status and did not generate any critical evidences or deaths in acute oral toxicity test. This study was to demonstrate the activity and mechanism of chromium citrate on improving glucose tolerance and IR in IR buffalo rat liver (BRL) cells. This study will furnish foundation for researching and developing a novel, nonhazardous organic chromium complex as a functional food ingredient and nutritional supplement.

Materials and Methods

Materials and chemicals
Chromium citrate was synthesized in our laboratory. Insulin (PubChem CID: 16131099) and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Group Company Limited (Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase, glucose transporter-4 (Glut4), phosphor-AMP-activated protein kinase β1 (p-AMPKβ1), and protein kinase B (Akt) primary antibodies and the corresponding second antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA, America). RNA extraction kit and RNA polymerase chain reaction (PCR) kit were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The other reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Cell culture
BRL cells were grown in dulbecco’s modified eagle medium (DMEM) supplemented with 10.0% fetal bovine serum and cultured in 5% CO₂ at 37°C in atmosphere of 95% air-5% CO₂.

Study design and models of insulin resistance
BRL cells were cultured in 96-well plates at the density of 4 × 10⁴ cells/mL for 24 h and then exposed to 1 µM insulin for another 4 h. 100-nM insulin was incubated for 30 min, and the cells were collected.

MTT assay
The IR BRL cells were cultured in 96-well plates at the density of 4 × 10⁴ cells/mL and exposed to different chromium compounds for 24 h. The supernatant was removed thoroughly, and 100-µL MTT (1 mg/mL) was immitted into 96-well plates. The BRL cells were incubated for another 4 h. 100 µL DMSO was added into each well for another 1-h incubation after removing the supernatant. The absorbance was detected by microplate ELISA reader at 570 nm.

Effects of chromium citrate on glucose absorption of cells
The IR BRL cells were cultured in 96-well plates at the density of 4 × 10⁴ cells/mL and incubated in different chromium compounds. The BRL cells were randomly allocated into planned groups [Table 1]. The supernatant was collected after incubating for 24 h, and the dextrose level was measured by a glucose assay kit.
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**Western blot analysis**
BRL cells (4 × 10^4 cells/mL) were plated in 6-well plates and incubated with different chromium compounds for 24 h. The cells were collected after removing the supernatant. The Akt, Glut4, and p-AMPKβ1 of BRL cells were extracted and measured. The expression of Akt, Glut4, and p-AMPKβ1 was analyzed by a method described in Cordero-Herrera et al. The equal amount of the protein samples was separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Moreover, the protein samples were transferred into polyvinylidene difluoride (PVDF) membrane, and the PVDF membrane was incubated with primary antibodies and the corresponding second antibodies after blocking with 5% nonfat milk for 1 h at room temperature. The blot was visualized using the enhanced chemiluminescence detection with chemiluminescence system, and the comparative degree of each blots was calculated by Quantity One software (Bio-Rad Laboratories, Hercules, California, USA).

**Reverse transcription–polymerase chain reaction analysis**
BRL cells were collected after the cells (4 × 10^4 cells/mL) were incubated in various concentrations of different chromium compounds for 24 h. The IR BRL cells’ RNA levels were analyzed as described by Mulik et al. The BRL cells’ total RNA was extracted by RNA extraction kit, and the RNA was reversed transcription as cRNA. The cRNA was measured by real-time polymerase chain reaction (PCR). The Cq of reverse transcription-PCR (RT-PCR) was calculated with 2^ΔΔCq. The primer sequences are expressed in Table 2.

**Statistical analysis**
The data analysis was performed using Statistical Product and Service Solutions 16.0 (SPSS 16.0, IBM, Armonk, New York, USA), and the data were presented as mean ± standard deviation. One-way analysis of variance was used to detect significant differences between the control and treatment groups. Tukey’s test was used for multiple comparisons. Values of P < 0.05 were considered to be statistically significant.

**Results**

**Insulin resistance model**
The dextrose levels in model and normal BRL cells were 22.85 ± 0.207 and 20.70 ± 0.180 mmol/L, respectively. The glucose level in model group was higher than that of normal group significantly.

**MTT assay**
The effect of chromium citrate on cell viability changes of IR BRL cells is shown in Figure 1. It could be concluded that chromium supplements cannot cause statistically significant among the groups. Moreover, the optical density value of model group has no significant change in this experiment.

**Effects of chromium citrate on glucose absorption in insulin resistance buffalo rat liver cells**
The ability of chromium citrate on improving glucose absorption in IR BRL cells is exhibited in Figure 2. The results indicated that chromium supplements could increase glucose absorption in IR BRL cells. The glucose levels of chromium picolinate, chromium trichloride, and chromium citrate (at doses of 0.4, 0.2, and 0.1 µg/mL) were significantly different from the control group.

**Table 1: The experiment design and the dose of chromium citrate, chromium picolinate, and chromium trichloride (n=5 for each group)**

| Group                          | Cells              | Addition            | Dose (Cr µg/mL DMEM) |
|--------------------------------|--------------------|---------------------|----------------------|
| Normal control group           | BRL cells          | -                   | -                    |
| Model group                    | Insulin resistance BRL cells | -                   | -                    |
| Chromium picolinate group      | Insulin resistance BRL cells | Chromium picolinate | 0.4                  |
| Chromium trichloride group     | Insulin resistance BRL cells | Chromium trichloride | 0.4                  |
| Chromium citrate high-dose group | Insulin resistance BRL cells | Chromium citrate | 0.4                  |
| Chromium citrate middle-dose group | Insulin resistance BRL cells | Chromium citrate | 0.2                  |
| Chromium citrate low-dose group | Insulin resistance BRL cells | Chromium citrate | 0.1                  |
| Chromium citrate control group | BRL cells          | Chromium citrate    | 0.4                  |

BRL=Buffalo rat liver, DMEM=Dulbecco’s modified eagle medium

**Table 2: The primer sequences of target genes in this study**

| Target genes | Primer sequences                                           |
|--------------|------------------------------------------------------------|
| Glut4        | 5'-GGGCTGTGAGTGAGTGCTTTC-3'                                 |
|              | 5'-CAGCGAGCAAGGCTGAGTCA-3'                                  |
| AMPKα2       | 5'-GGGTATCCTGATGCTCCCT-3'                                   |
|              | 5'-TGTCTTGTATGCTGCTCCCT-3'                                  |
| Akt2         | 5'-ATGATAGCTCCAGATGAG-3'                                    |
|              | 5'-TGATAATCAGGCTGCTGCT-3'                                   |
| Irs-1        | 5'-TGTGCCAAGGCAAGGAAAG-3'                                   |
|              | 5'-ACGTTTTCCAAGGAGGAA-3'                                    |
| PPARγ        | 5'-CTTACCGCGCTGATTTTTC-3'                                   |
|              | 5'-CAGGCTCTACCTACTTCTG-3'                                   |
| PI3K         | 5'-ACTGGAGGAAGACTTGAAG-3'                                   |
|              | 5'-CGTTTCCCAACCATCATTG-3'                                   |
| GAPDH        | 5'-TGGTGACGTCATGCTAC-3'                                     |
|              | 5'-CAGCAATCGAGGGCCTCT-3'                                    |

GAPDH=Glyceraldehyde-3-phosphate dehydrogenase, Irs-1=Insulin receptor substrate-1

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**Table 2: The experiment design and the dose of chromium citrate, chromium picolinate, and chromium trichloride (n=5 for each group)**

| Group                          | Cells              | Addition            | Dose (Cr µg/mL DMEM) |
|--------------------------------|--------------------|---------------------|----------------------|
| Normal control group           | BRL cells          | -                   | -                    |
| Model group                    | Insulin resistance BRL cells | -                   | -                    |
| Chromium picolinate group      | Insulin resistance BRL cells | Chromium picolinate | 0.4                  |
| Chromium trichloride group     | Insulin resistance BRL cells | Chromium trichloride | 0.4                  |
| Chromium citrate high-dose group | Insulin resistance BRL cells | Chromium citrate | 0.4                  |
| Chromium citrate middle-dose group | Insulin resistance BRL cells | Chromium citrate | 0.2                  |
| Chromium citrate low-dose group | Insulin resistance BRL cells | Chromium citrate | 0.1                  |
| Chromium citrate control group | BRL cells          | Chromium citrate    | 0.4                  |

BRL=Buffalo rat liver, DMEM=Dulbecco’s modified eagle medium

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**Table 2: The primer sequences of target genes in this study**

| Target genes | Primer sequences                                           |
|--------------|------------------------------------------------------------|
| Glut4        | 5'-GGGCTGTGAGTGAGTGCTTTC-3'                                 |
|              | 5'-CAGCGAGCAAGGCTGAGTCA-3'                                  |
| AMPKα2       | 5'-GGGTATCCTGATGCTCCCT-3'                                   |
|              | 5'-TGTCTTGTATGCTGCTCCCT-3'                                  |
| Akt2         | 5'-ATGATAGCTCCAGATGAG-3'                                    |
|              | 5'-TGATAATCAGGCTGCTGCT-3'                                   |
| Irs-1        | 5'-TGTGCCAAGGCAAGGAAAG-3'                                   |
|              | 5'-ACGTTTTCCAAGGAGGAA-3'                                    |
| PPARγ        | 5'-CTTACCGCGCTGATTTTTC-3'                                   |
|              | 5'-CAGGCTCTACCTACTTCTG-3'                                   |
| PI3K         | 5'-ACTGGAGGAAGACTTGAAG-3'                                   |
|              | 5'-CGTTTCCCAACCATCATTG-3'                                   |
| GAPDH        | 5'-TGGTGACGTCATGCTAC-3'                                     |
|              | 5'-CAGCAATCGAGGGCCTCT-3'                                    |

GAPDH=Glyceraldehyde-3-phosphate dehydrogenase, Irs-1=Insulin receptor substrate-1
Cr/mL DMEM) groups were higher than model group (22.85 ± 0.21 mmol/L) significantly. However, the glucose concentrations of chromium citrate and chromium citrate control groups expressed no obvious change among the chromium supplement groups.

**Effects of chromium citrate on glucose transport in insulin resistance buffalo rat liver cells**

Effects of chromium citrate on Akt, Glut4, and p-AMPKβ1 expression of IR BRL cells are shown in Figure 3. The Akt, Glut4, and p-AMPKβ1 levels in chromium citrate (at doses of 0.4, 0.2, and 0.1 µg Cr/mL DMEM) groups were obviously improved when contrast with the model group. Moreover, the Akt levels of chromium citrate (at doses of 0.4, 0.2, and 0.1 µg Cr/mL DMEM) groups still had markedly improved when compared with the chromium trichloride group. However, the Akt, Glut4, and p-AMPKβ1 levels of chromium citrate and chromium citrate control groups have no significant difference when contrasted with the chromium picolinate group.

**Effect of chromium citrate on mRNA expression of glucose transport proteins**

Effects of chromium citrate on mRNA of glucose transport protein expression (Akt2, Glut4, and AMPKα2) in IR BRL cells are expressed in Figure 4. Chromium supplement groups can increase the mRNA levels of Akt2, Glut4, and AMPKα2 in IR BRL cells. The mRNA levels of Akt2, Glut4, and AMPKα2 in chromium citrate (at doses of 0.4, 0.2, and 0.1 µg Cr/mL DMEM), chromium picolinate, and chromium trichloride groups were obviously improved than that of model group.

Moreover, the mRNA level of Glut4 in chromium citrate group (at a dose of 0.4 µg Cr/mL DMEM) had a marked increase trend when compared with the chromium trichloride group. However, the mRNA levels of Akt2, Glut4, and AMPKα2 in chromium citrate and chromium citrate control groups showed no notable variation contrasted with the chromium picolinate group. As a result, it can be verified that chromium citrate increased the mRNA levels of Glut4 more notable than that of chromium trichloride.

**Effect on chromium citrate on mRNA expression of insulin resistance proteins**

Effects of chromium citrate on mRNA of IR protein expression (insulin receptor substrate-1 [Irs-1], PPARγ, and PI3K) in IR BRL cells are exhibited in Figure 5. The mRNA levels of Irs-1, PPARγ, and PI3K were found to be significantly decreased in IR BRL cells when contrasted with normal BRL cells. The chromium supplements can improve the mRNA levels of Irs-1, PPARγ, and PI3K in IR BRL cells. The Irs-1, PPARγ, and PI3K mRNA levels in chromium supplement groups were notably improved than that of model group. However, the Irs-1, PPARγ, and PI3K mRNA levels in chromium citrate and chromium citrate control groups showed no statistical variations contrasted with the other chromium supplement groups.

**Discussion**

The modeling experiment results signified that the IR BRL cells were established successfully. Prior researches had illustrated that chromium complexes could affect

![Figure 1: Effects of chromium citrate on cell viability of buffalo rat liver rat liver cells with insulin resistance. Chromium picolinate and chromium trichloride were used as positive controls. Each value was presented as means ± standard deviation (n = 20).]({} 

| Group | OD Value |
|-------|---------|
| Normal control group | 0.6 |
| Model group | 0.7 |
| Chromium picolinate group | 0.8 |
| Chromium trichloride group | 0.9 |

- Significant different from normal group (P < 0.05)
- Significant different from model group (P < 0.05)
- Significant different from chromium picolinate group (P < 0.05)
- Significant different from chromium trichloride group (P < 0.05)

![Figure 2: The glucose absorption (mmol/L) changes of buffalo rat liver rat liver cells with insulin resistance were treated with chromium citrate. Chromium picolinate and chromium trichloride were used as positive controls. Each value was presented as means ± standard deviation (n = 20).]({} 

| Group | Glucose absorption mmol/L |
|-------|---------------------------|
| Normal control group | 1.0 |
| Model group | 1.2 |
| Chromium picolinate group | 1.4 |
| Chromium trichloride group | 1.6 |

- Significant different from normal group (P < 0.05)
- Significant different from model group (P < 0.05)
- Significant different from chromium picolinate group (P < 0.05)
- Significant different from chromium trichloride group (P < 0.05)
the cell viability and have toxicity for human beings in high concentration.\cite{23,24} However, the effects will be disappeared in low concentrations, which were consistent with this result.\cite{25} Therefore, chromium citrate has no obvious effect on cell viability of BRL cell, and the dosage used in this study (at doses of 0.4, 0.2, and 0.1 µg Cr/mL DMEM) could be investigated in the further study. The results of glucose absorption experiment confirmed that chromium citrate could improve glucose absorption in BRL cells with IR and has no obvious effect on glucose absorption of normal BRL cell.

The published research indicated that Akt may play an important role in insulin stimulation of glucose transport.\cite{26} Another essential function of Akt is the regulation of glycogen synthesis through phosphorylation and inactivation of Glycogen Synthase Kinase (GSK)-3α and GSK-3β.\cite{27} Glut4, which is a glucose transport protein, plays a principal role in maintaining balance between intracellular glucose and extracellular glucose.\cite{28} Adenosine 5’-monophosphate (AMP)-activated protein kinase (AMPK) could activate and accelerate Glut4 shift from cytoplasm to cytomembrane. The result of glucose transport experiment illustrated that chromium citrate could improve glucose transport by increasing Akt, Glut4, and p-AMPKβ1 expression in BRL cells with IR and has no obvious effect on glucose transport in normal BRL cell. In addition, the improving

\begin{figure}
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\caption{(a-c) Effect of chromium citrate on Akt (a), Glut4 (b), and phosphor-AMPKβ1 (c) expression in in buffalo rat liver rat liver cells with insulin resistance. Chromium picolinate and chromium trichloride were used as positive controls. Each value was presented as means ± standard deviation (n = 20). *Significant different from normal group (P < 0.05); †Significant different from model group (P < 0.05); ‡Significant different from chromium picolinate group (P < 0.05); §Significant different from chromium trichloride group (P < 0.05)}
\end{figure}
effect of chromium citrate was superior to that in chromium trichloride. Yang et al. had reported that Cr (pa)₃ could enhance glucose uptake in mouse 3T3-adipocytes stimulated by insulin, and they also found that Cr (pa)₃ enhanced Akt phosphorylation in insulin-stimulated condition in a time- and concentration-dependent manner.⁴⁹ Feng et al. had researched that chromium malate could remarkably promote the expression levels of Glut4, Akt, and AMPKβ1 phosphorylation in IR L6 cells. The related mRNA expression of chromium malate was also significantly promoted.⁵⁰

As shown in mRNA expression experiments, the results indicated that chromium citrate can improve glucose transport by increasing mRNA levels of glucose transport protein expression (Akt2, Glut4, and AMPKα2) in BRL cells with IR and has no obvious effect on mRNA of glucose transport in normal BRL cell. Irs-1 is one of the major substrates of the insulin receptor kinase and a potential mechanism for IR in the obese model.⁵¹ PPARγ, which is an important biomarker of signal pathway, can modulate the insulin sensitivity in human beings with IR. PI3K is a major protein in insulin signal transduction pathway and can improve glucose transport and IR in the liver.⁵² It also can be observed in “Effect on chromium citrate on mRNA expression of insulin resistance proteins” section that chromium citrate could improve IR by increasing mRNA of IR protein expression (Irs-1, PPARγ, and PI3K) in BRL cells with IR and has no obvious effect on mRNA of IR in normal BRL cell.

**Conclusion**

Chromium citrate can improve glucose transport and IR by upregulation of glucose transport proteins (Akt2, Glut4, and AMPKα2) and insulin sensitivity proteins (Irs-1, PI3K, and PPARγ) in IR BRL cells. The improving of chromium citrate on increasing the Akt levels and mRNA levels of Glut4 is better than that of chromium trichloride.
Chromium citrate can notably strengthen the glucose absorption in IR BRL cells. Therefore, it can be concluded that the therapeutic effects of chromium citrate on improving glucose transport and IR are better than that of chromium trichloride. Chromium citrate could be utilized as an antidiabetic dietary supplement. This study investigated that the effects of chromium citrate on improving glucose transport and IR were mainly based on Glut4 mechanism, the action mechanism should be studied more systematically and comprehensively in our further research, such as the effect of Glut2-mediated glucose-stimulated insulin release mechanism in diabetic milieu.

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Conflicts of interest
There are no conflicts of interest.

References
1. Liu J, Wang Y, Gong L, Sun C. Oxidation of glyceraldehyde-3-phosphate dehydrogenase decreases sperm motility in diabetes mellitus. Biochem Biophys Res Commun 2015;465:245-8.
2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014;103:137-49.
3. Kabadi UM. Starting insulin in type 2 diabetes: Overcoming barriers to insulin therapy. Int J Diabetes Dev Ctries 2008;28:65-8.
4. Chodick G, Porath A, Alapi H, Sella T, Flash S, Wood F, et al. The direct medical cost of cardiovascular diseases, hypertension, diabetes, cancer, pregnancy and female infertility in a large HMO in Israel. Health Policy 2010;95:271-6.
5. Kohli P, Greenland P. Role of the metabolic syndrome in risk assessment for coronary heart disease. JAMA 2006;295:819-21.
6. Zia A, Bhatti A, Jalil F, Xingbin W, Peter J, Kiani K, et al. Prevalence of type 2 diabetes-associated complications in Pakistan. Int J Diabetes Dev Ctries 2015;36:1-10.
7. Groop L, Storm P, Rosengren A. Can genetics improve precision of therapy in diabetes? Trends Endocrinol Metab 2014;25:440-3.
8. Bhattacharyya S, Pal D, Ghosal S, Biswas T, Polley G, Pandit S, et al. Effects of adjunct therapy of a proprietary herbo-chromium
supplement in type 2 diabetes: A randomized clinical trial. Int J Diabetes Dev C 2010;30:153-61.
9. Wells IC, Claassen JP, Anderson RJ. A test for adequacy of chromium nutrition in humans-relation to type 2 diabetes mellitus. Biochem Biophys Res Commun 2003;303:825-7.
10. Zhou B, Wang H, Luo G, Niu R, Wang J. Effect of dietary yeast chromium and L-carnitine on lipid metabolism of sheep. Biol Trace Elem Res 2013;155:221-7.
11. Emami A, Ganjkhanlou M, Zali A. Effects of Cr methionine on glucose metabolism, plasma metabolites, meat lipid peroxidation, and tissue chromium in Mahabadi goat kids. Biol Trace Elem Res 2015;164:50-7.
12. Guerrero-Romero F, Rodriguez-Morán M. Complementary therapies for diabetes: The case for chromium, magnesium, and antioxidants. Arch Med Res 2005;36:250-7.
13. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: Vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 2001;101:294-301.
14. Anderson RA. Chromium and insulin resistance. Nutr Res Rev 2003;16:267-75.
15. Sahin K, Tuzcu M, Orhan C, Sahin N, Kucuk O, Ozercan IH, et al. Anti-diabetic activity of chromium picolinate and biotin in rats with type 2 diabetes induced by high-fat diet and streptozotocin. Br J Nutr 2013;110:197-205.
16. Vrtovec M, Vrtovec B, Briski A, Kocijancic A, Anderson RA, Radovancevic B. Chromium supplementation shortens QTc interval duration in patients with type 2 diabetes mellitus. Am Heart J 2005;149:632-6.
17. Hepburn DD, Vincent JB. Tissue and subcellular distribution of chromium picolinate with time after entering the bloodstream. J Inorg Biochem 2003;94:86-93.
18. Fang Z, Zhao M, Zhen H, Chen L, Shi P, Huang Z. Genotoxicity of tri- and hexavalent chromium compounds in vitro and their modes of action on DNA damage in vitro. PLoS One 2014;9:e103194.
19. Li F, Wu X, Zhao T, Zhang M, Zhao J, Mao G, et al. Anti-diabetic properties of chromium citrate complex in alloxan-induced diabetic rats. J Trace Elem Med Biol 2011;25:218-24.
20. Suzuki A, Sarangbin S, Kirimura K, Usami S. Direct production of citric acid from starch by a 2-deoxyglucose-resistant mutant strain of Aspergillus niger. J Ferm Bioen 1996;81:320-3.
21. Cordero-Herrera I, Martín MÁ, Goya L, Ramos S. Cocoa flavonoids attenuate high glucose-induced insulin signalling blockade and modulate glucose uptake and production in human HepG2 cells. Food Chem Toxicol 2014;64:10-9.
22. Mulik S, Sharma S, Suryawanshi A, Veiga-Parga T, Reddy PB, Rajasagi NK, et al. Activation of endothelial roundabout receptor 4 reduces the severity of virus-induced keratitis. J Immunol 2011;186:7195-204.
23. Terpilowska S, Siwicki A. The influence of chromium and iron interactions on cell viability. Toxicol Lett 2012;211:S103.
24. Sharma I, Pati PK, Bhardwaj R. Effect of 28-homobrassinolide on antioxidant defence system in Raphanus sativus L. under chromium toxicity. Ecotoxicology 2011;20:862-74.
25. Jana M, Rajaram A, Rajaram R. Chromium picolinate induced apoptosis of lymphocytes and the signaling mechanisms thereof. Toxicol Appl Pharmacol 2009;237:331-44.
26. Ma X, Tsuda S, Yang X, Gu N, Tanabe H, Oshima R, et al. Pu-erh tea hot-water extract activates Akt and induces insulin-independent glucose transport in rat skeletal muscle. J Med Food 2013;16:259-62.
27. Moore SF, van den Bosch MT, Hunter RW, Sakamoto K, Poole AW, Hers I. Dual regulation of glycogen synthase kinase 3 (GSK3)α/β by protein kinase C (PKC)ε and Akt promotes thrombin-mediated integrin αβ3 activation and granule secretion in platelets. J Biol Chem 2013;288:3918-28.
28. Leto D, Saltiel AR. Regulation of glucose transport by insulin: Traffic control of GLUT4. Nat Rev Mol Cell Biol 2012;13:383-96.
29. Yang X, Palanichamy K, Ontko AC, Rao MN, Fang CX, Ren J, et al. A newly synthetic chromium complex – Chromium (phenylalanine) 3 improves insulin responsiveness and reduces whole body glucose tolerance. FEBS Lett 2005;579:1458-64.
30. Feng W, Ding Y, Zhang W, Chen Y, Li Q, Wang W, et al. Chromium malate alleviates high-glucose and insulin resistance in L6 skeletal muscle cells by regulating glucose uptake and insulin sensitivity signaling pathways. Biometa 2018;31:891-908.
31. Liu K, Zhao W, Gao X, Huang F, Kou J, Liu B. Diosgenin ameliorates palmitate-induced endothelial dysfunction and insulin resistance via blocking IKKβ and IRS-1 pathways. Atherosclerosis 2012;223:350-8.
32. Klover PJ, Mooney RA. Hepatocytes: Critical for glucose homeostasis. Int J Biochem Cell Biol 2004;36:753-8.