Original Article

Serum Chromium Level is Increased in Jordanian Smokers, Decreased in Jordanians with Prediabetes and type 2 Diabetes, But not Altered in Jordanians with Hypertension, With Obesity, or With Family History of Diabetes

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Abstract

Background: Chromium was found to be crucial for several biochemical processes in the human body, including, in particular, carbohydrate and lipid metabolism whereas the exact mechanisms of its actions have yet to be explored. Here, we asked whether low serum chromium levels are present in Jordanian smokers and Jordanians with prediabetes and type 2 diabetes (T2D), with hypertension, with overweight and obesity, and with a family history of diabetes. **Methods:** A total of 360 patients (120 with T2D, 120 with prediabetes, and 120 healthy controls) were recruited randomly based on the American Diabetes Association criteria. Smokers (n = 26), and patients with hypertension (n = 46), with overweight (n = 47) and obesity (n = 57), and with family history of diabetes (n = 63) were included in the tested population. Serum chromium concentration was measured using the graphite furnace atomic absorption spectrometry. **Results:** The results from this study revealed significant increase (P = 0.001 univariate, P = 0.038 multivariate) and significant decrease (P = 0.046 univariate, P = 0.038 multivariate) in serum chromium concentrations in smokers and people with T2D and prediabetes, respectively. In addition, serum chromium insignificantly altered ($P > 0.05$) in people with hypertension, with a family history of diabetes, and with overweight or obesity. **Conclusions:** Higher levels of serum chromium were observed in smokers, whereas lower levels were found to be present in patients with T2D and patients with prediabetes. In addition, serum chromium level may not be affected by hypertension, overweight and obesity, and family history of diabetes.

Keywords: *Obesity, overweight, serum chromium, smokers, type 2 diabetes, prediabetes, hypertension*

Introduction

Chromium is an element that the humans need in trace amounts, while these amounts required for optimal human health are not well determined. In addition, yet, its exact mechanisms of action in the body are not completely understood.[1] In the United States, in 2016, Cr (III) ion (Cr^{+3}), also termed as trivalent chromium, which is the biologically active form of chromium, was considered an essential nutrient in humans required for glucose and lipid metabolism.[2] However, in 2014, the European Food Safety Authority, which is acting for the European Union, concluded that there was no convincing and enough evidence for chromium to be recognized as an essential element.[3]

Various research studies have been conducted to confirm and explain the association between bodily chromium level and diabetes. A study by Morris *et al*. found that noninsulin dependent diabetes mellitus patients had 33% lower mean values of plasma chromium than those found among healthy individuals.[4] The study by Basaki *et al*. revealed that the mean values of chromium were significantly lower in the serum of patients with type 2 diabetes (T2D) compared to healthycontrols.[5] A recent case–control studiesss on Chinese population revealed that low levels of plasma chromium are associated with risk of T2D.[6,7] In addition, serum chromium was found to be involved in lipid metabolism. It has been shown that serum chromium concentrations are negatively correlated with triglycerides and positively correlated with cholesterol.[8]

Further, chromium supplements were used in various occasions to improve

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insulin resistance (IR) as well as lipid metabolism. For example, chromium picolinate (CrPic) supplementation at 200 μg twice a day for 3 weeks was found to significantly reduce the fasting blood glucose level by approximately 21%.[9] Very recent pooled analysis study conducted by Huang *et al*. suggested that patients with T2D supplemented chromium picolinate or chromium chloride had lower levels of fasting serum glucose.[10] However, earlier in 2016, Costello *et al*., in a meta-analysis evaluated the later randomized clinical trials, concluded that there is still little reason to recommend chromium supplements to achieve significant improvements in glycemic control.[11] Besides, oral chromium picolinate was found to improved lipid metabolism in obese rats,[12] and in humans.[11]

The evidence above supports the conclusion that chromium could be vital to glucose homeostasis and lipid metabolism. Even though, the exact mechanism of its actions in the body has yet to be explored.[13] Therefore, human studies in this context of research are still very significant. Here, for the first time, we asked whether low serum chromium levels are present in Jordanian smokers and Jordanians with T2D, with prediabetes, with hypertension, with overweight and obesity, and with a family history of diabetes.

Methods

Study population

A total of 360 patients (120 with T2D, 120 with prediabetes, and 120 healthy controls) were recruited from people who visited the central laboratories of King Abdullah University Hospital, and Princess Basma Teaching Hospital in the north of Jordan. Consecutive patients were selected in each group until we reached the predetermined sample size of 120 in each group. The tested population included smokers (n = 26), and patients with hypertension (n = 46), with overweight (n = 47), with obesity (n = 57), and with family history of diabetes (n = 63). Body Mass Index (BMI) was used to recruit overweight (BMI = 25.0–29.9) and obese (BMI >30) people.

The study was approved by the Institutional Review Board of Jordan University of Science and Technology (Irbid, Jordan). The researchers explained the study to all recruited patients and a written informed consent was obtained from each individual prior data or sample collection.

A questionnaire was administered to all recruited individuals to collect the required information such as gender, age, weight, height, health condition, smoking, and family history. Height and weight were measured according to the standard guidelines. All patients with T2D were on oral hypoglycemic drugs at the time of the study.

The inclusion criteria for patients with T2D were as follows: (a) no supplementation with vitamins or minerals, (b) no history of recent acute illness or clinical evidence suggestive of the kidney, liver, or endocrine diseases, and (c) absence of chronic diabetic complications such as retinopathy or diabetic foot. Prediabetes was defined as fasting serum glucose concentration, after 12 h fasting period, located within the range 100–125 mg/dL. Diabetes was diagnosed if fasting serum glucose concentration was within the range 126–200 mg/dL.[14] Fasting serum glucose results for all participants were confirmed, at least twice, on a different day before recruitment.

Sample collection

All samples were collected from recruited patients into plain tubes. Immediately after coagulations (~15–20 min), the samples were centrifuged at ×2000 g, and the serum was stored at −20°C for analysis.

Determination of serum glucose

Serum glucose was measured using standard methods using Roche kits (Roche Diagnostics, Mannheim, Germany), and Roche Chemistry Analyzer in the central laboratories at King Abdullah University Hospital and Princess Basma Teaching Hospital.

Determination of serum chromium

Chromium was analyzed using Unicam Atomic Absorption Spectrometer Model SOLAAR M5 fully equipped for graphite furnace atomization (ThermoElemental, Franklin, MA, USA). Chromium was measured at the maximum wavelength of 357.9 nm.

Calibration was performed by preparation of five standards (0.05, 0.5, 1.0, 2.5, and 5.0 μg/L) using chromium stock standard for atomic absorption spectroscopy of 1,000 μg/mL concentration (Sigma, USA), traceable to the National Institute of Standards and Technology [Figure 1]. The analytical accuracy was revealed using certified reference material from (UTAK, Valencia, CA, USA). Serum samples were prepared by 1:1 dilution using 1% Triton X-100, deionized water, and 2% magnesium nitrate as matrix modifier (Sigma, USA).

Statistical analysis

Data were described and analyzed using SPSS-IBM version 20 (SPSS Inc., Chicago, Illinois). Data were

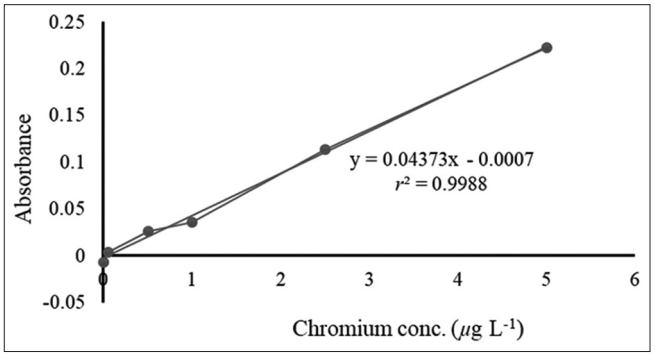

Figure 1: Calibration curve of serum chromium determination as evaluated by atomic absorption spectroscopy

described using means and percentages. The differences in the means of continuous variables between the three studied groups were tested using one-way ANOVA, and the differences between populations were tested using Chi-square test. The general linear model was used to test the differences in means of chromium between prediabetics, T2D, and healthy individuals after adjusting for important variables. A value of $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the demographic, anthropometric, and clinical characteristics of participants. The mean age was 53.5 years for patients with T2D, and 49.2 years for patients with prediabetes. Compared to controls and patients with prediabetes, patients with T2D were significantly older, were more likely to have a family history of diabetes, had higher BMI, and were less likely to be smokers.

Figure 2 reveals chromium level among study groups. People with Prediabetes, T2D, and healthy individuals had chromium levels of 0.62 (0.54) μg/L, 0.45 (0.33) μg/L, and 0.9 (0.5) μg/L, respectively. Both patients with diabetes and prediabetes had significantly lower chromium levels after adjusting for important variables. The difference in the mean chromium level between patients with prediabetes and diabetes was statistically significant.

Univariate and multivariate analysis of the difference in chromium level according to studied variables are shown in Table 2. There was no difference in chromium level according to gender, age, family history, hypertension, and BMI. Regarding smoking, there was a statistical difference in chromium level between smokers and nonsmokers. Smokers had significantly higher chromium levels compared to nonsmokers.

Discussion

To the best of our knowledge, this study is the first of its kind that directly link between serum chromium concentrations and smoking and hypertension. Moreover, it is the first in Jordan to assess chromium level in people with prediabetes, with T2D, and with overweight and obesity compared to

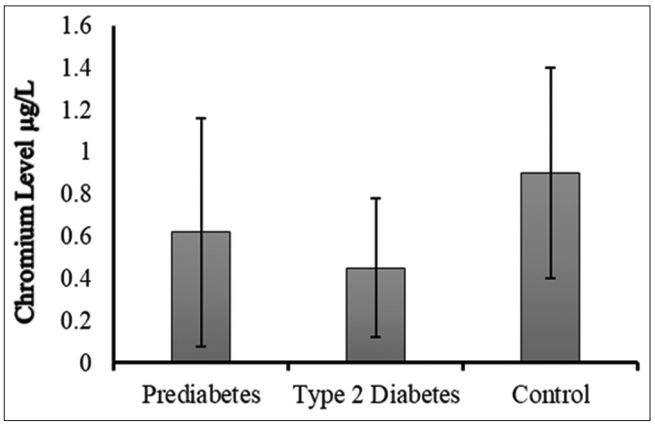

Figure 2: Chromium level among patients with prediabetes, type 2 diabetes, and healthy individuals. The mean difference was statistically significant among the study groups ($P < 0.05$). Values are given as mean ± standard deviation

Table 1: The demographic, anthropometric, and clinical characteristics of participants. values are given as mean (standard deviation)

Classification	Prediabetes *n* (%)	Type 2 diabetes *n* (%)	Control *n* (%)	*P*
Gender				
Male	48 (40)	51 (42.5)	50 (41.7)	0.923
Female	72 (60)	69 (57.5)	70 (58.3)	
Age (year), mean (SD)	49.2 (9.3)	53.5 (7.2)	42.6 (9.6)	
25-49.9	36 (30.0)	13 (10.8)	67 (55.8)	0.000*
50-54.9	48 (40.0)	51 (42.5)	40 (33.3)	
>55	36 (30.0)	56 (46.7)	13 (10.8)	
Hypertension				
Yes	46 (38.3)	61 (50.8)	0	0.051
No	74 (61.7)	59 (49.2)	120 (100)	
Family history of diabetes				
Yes	63 (52.5)	89 (74.2)	58 (48.3)	0.000*
No	57 (47.5)	31 (25.8)	62 (51.7)	
BMI (kg/m^2), mean (SD)	30.5 (5.4)	32.4 (6.2)	27.9 (4.4)	
Normal (18.5-24.9)	16 (13.3)	7 (5.8)	35 (29.2)	0.000*
Overweight (25.0-29.9)	47 (39.2)	38 (31.7)	45 (37.5)	
Obesity (>30)	57 (47.5)	75 (62.5)	40 (33.3)	
Smoking				
Smoker	26 (21.7)	16 (13.3)	31 (25.8)	0.049*
Nonsmoker	94 (78.3)	104 (86.7)	89 (74.2)	
Fasting blood glucose (mg/dL), mean (SD)	111 (5.0)	157 (22.0)	92 (6.0)	0.000

*P<0.05 was considered statistically significant. BMI=Body mass index, SD=Standard deviation

Table 2: Univariate, and multivariate analysis of the difference in chromium level among studied variables. Values are given as mean (standard deviation), *P* values are given as univariate and multivariate

Classification	Chromium Level µg/L, mean (SD)	Univariate (*P*)	Multivariate (*P*)
Group			
Prediabetes	0.62 (0.54)	0.046*	0.038*
Type 2 diabetes	0.47 (0.33)		
Control	0.9 (0.5)		
Gender			
Male	0.72 (0.55)	0.087	0.32
Female	0.62 (0.46)		
Age (year), mean (SD)			
25-49.9	0.73 (0.51)	0.105	0.212
50-54.9	0.66 (0.49)		
>55	0.59±0.49		
Hypertension		0.083	-
Yes	0.49 (0.43)		
No	0.59 (0.46)		
Family history of diabetes			
Yes	0.63 (0.45)	0.18	0.871
No	0.62 (0.47)		
BMI (kg/m^2), mean (SD)			
Normal (18.5-24.9)	0.79 (0.53)	0.055	0.419
Overweight (25.0-29.9)	0.67 (0.49)		
Obesity (>30)	0.61 (0.49)		
Smoking			
Smoker	0.83 (0.58)	0.001*	0.035*
Nonsmoker	0.62 (0.47)		

**P*<0.05 was considered statistically significant. SD=Standard deviation, BMI=Body mass index

healthy individuals, thus providing an important approach to reduce the prevalence of such disorders.

In this study, there was a significant depletion of serum chromium content in people with diabetes and prediabetes compared to healthy individuals. Tripathy *et al*. (2004) found that people with T2D diabetes had lower serum chromium concentrations compared to healthy individuals.[15] In addition, Makhlough *et al*. showed that patients with diabetic nephropathy had lower blood levels of chromium compared to healthy controls.[16] Moreover, in 2017, a study conducted on Pakistani population by Hajra *et al*. revealed that low serum concentrations of chromium were found in patients with T2D compared to nondiabetic patients.[17] Such decrease; in general, might be due to increased urinary chromium loss,[18] decreased chromium absorption, inadequate dietary intake, or poor glycemic control.[15,19] Similar results on different populations were also reported.[20-22] A study conducted on Iranians (2014), which recruited 132 participants, confirmed the existence of chromium deficiency in prediabetic individuals.[23] The same researchers recommended that investigating the effect of chromium on glucose hemostasis and insulin sensitivity should be focused on prediabetic people, which will be more operative afterward to manage the progression and the development of T2D.

In fact, chromium is a constituent of the low-molecular-weight protein (~1500 Da) chromodulin, which involves four types of amino acids (glycine, glutamate, cysteine, and aspartate).[24,25] It has been shown that chromium contributes to glucose homeostasis via potentiating the effects of insulin, and thus enhancing glucose uptake.[26] This occurs by facilitating insulin binding to its receptor and receptor kinase signaling resulting in an increase in insulin receptor phosphorylation and increasing the number of insulin receptors.[26]

Interestingly, it was found that T2D patients had urine chromium levels almost 100% higher than those seen among healthy individuals.[4] Very recently, Velmurugan *et al*. revealed a strong association between diabetes and urinary levels of certain metals such as chromium and arsenic.[27] Such evidence indicates that diabetes mellitus may lead to increased urinary loss of chromium ions (Cr^{+3}), which can lead over time to chromium deficiency.[25] Otherwise, a sequential randomized controlled cross-over study revealed that the loss of urinary chromium following the high glycemic-index diets, for 6 days, have not been observed in normal individuals.[28]

Another important finding in this study was the significant reduction in mean chromium concentration between smokers and nonsmokers among people with diabetes and prediabetes. Smoking is responsible for the transition of normoglycemia to impaired fasting glucose and rises the risk of T2D.[29] Increased blood glucose level associated

with diabetes leads to the subsequent production of reactive oxygen species as a result of spontaneous glycosylation.[30] This consequence was found to reduce insulin secretion, and infrequently to *β*-cell apoptosis.[31-33] Furthermore, in healthycontrols, smoking was found to reduce the expression of transferrin receptor compared to ferritin one, which increases the risk of T2D.[34] Zhu *et al*. found that trivalent chromium is the main species of cigarette and it is partially oxidized to hexavalent one during smoking.[35] Therefore, it is logical that smokers with T2D and prediabetes have higher chromium levels compared to nonsmokers, which is in line with our results in the present work. However, data regarding the relationship between smokers, T2D, and chromium deficiency is not available in the literature, thus making it a hot spot area for research.

Ravina and Slezack revealed that chromium level is lower in females compared to males.[36] However, Ding *et al*. study presented an insignificant difference in the chromium level between both genders;[37] this finding is consistent with the present study results. While, the contradictory with Ravina and Slezack study may be due to the differences in the environmental (i.e., nutritional) and geographical factors in addition to the difference in sample number.

Duncan concluded that increasing age and family history of diabetes led to IR in diabetic patients, due to depletion of chromium levels.[38] In the current study, about one-third of the population was >55 years, and 58% of the total population was with the family history of diabetes, however, in the current study, the age was not correlated with decreased chromium level.

A randomized clinical trial included 63 patients with metabolic syndrome revealed no effect of chromium picolinate on insulin sensitivity, glucose metabolism, body weight, or serum lipids.[39] Another randomized controlled trial of 40 patients with impaired glucose tolerance, concluded a negative effect in 1 and 2 h glucose tolerance, fasting plasma glucose, fasting insulin homeostatic model assessment of IR, and lipid measures over 3 months using 800 μg of chromium.[40] Moreover, a randomized, double-blinded, placebo-controlled trial revealed no evidence of high dose of chromium treatment in obese western patients with T2D, the same study also suggested that chromium supplements had no effect on blood pressure, HbA1c, or lipid profile.[41] A double-blind clinical trial found that chromium nicotinate at 50 and 200 μg did not improve glycemic control or increase insulin sensitivity in patients with T2D.[42] A systematic literature search of EMBASE, Pubmed, and the Cochrane Library conducted in 2015 by Yin and Phung concluded that chromium picolinate did not significantly affect HbA1c in diabetic people.[43]

On the other hand, Albarracin *et al*. (2008) stated that a combination regimen of chromium picolinate and biotin significantly lowered fasting plasma glucose, HbA1c compared to placebo.[44] Furthermore, Martin *et al*. showed a significant decrease in HbA1c and fasting plasma glucose levels after 6 months following supplementation of 1000 μg/day.[45] A single-blind randomized trial performed of 71 patients with T2D revealed a reduction in HbA1c levels after 1 month following 600 μg/day CrPic.[46]

Moreover, another, double-blind, placebo-controlled trials in individuals without diabetes found that chromium supplementation showed a decrease in weight and fat in three out of eight larger studies,[47] while Lukaski *et al*. concluded that supplementation of 200 μg of Cr as CrPic did not affect the body weight.[48] These contradictory results may due to the difference in sample size, deferent CrPic dose, diet, race, and geographical differences. However, our results show that chromium level is not affected by gender, age, BMI, family history, or hypertension.

Conclusions

Jordanian smokers were found to have higher serum chromium means compared to nonsmokers. While Jordanians with T2D and prediabetes have lower serum chromium concentrations compared to healthy individuals. In addition, the results from this study show that serum chromium concentration may not be affected by hypertension, overweight and obesity, family history of diabetes, age, and gender.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Panchal SK, Wanyonyi S, Brown L. Selenium, vanadium, and chromium as micronutrients to improve metabolic syndrome. Curr Hypertens Rep 2017;19:10.
2. National Institutes of Health. Office of Dietary Supplements UNIoH. Bethesda, MD, USA: Chromium; 2016.
3. EFSA Panel on Dietetic Products NaAN. Scientific opinion on dietary reference values for chromium. EFSA J 2014;12:1-25.
4. Morris BW, MacNeil S, Hardisty CA, Heller S, Burgin C, Gray TA, *et al.* Chromium homeostasis in patients with type II (NIDDM) diabetes. J Trace Elem Med Biol 1999;13:57-61.
5. Basaki M, Saeb M, Nazifi S, Shamsaei HA. Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. Biol Trace Elem Res 2012;148:161-4.
6. Li XT, Yu PF, Gao Y, Guo WH, Wang J, Liu X, *et al.* Association between plasma metal levels and diabetes risk: A case-control study in China. Biomed Environ Sci 2017;30:482-91.

7. Chen S, Jin X, Shan Z, Li S, Yin J, Sun T, *et al.* Inverse association of plasma chromium levels with newly diagnosed type 2 diabetes: A case-control study. Nutrients 2017;9. pii: E294.
8. Lima KV, Lima RP, Gonçalves MC, Faintuch J, Morais LC, Asciutti LS, *et al.* High frequency of serum chromium deficiency and association of chromium with triglyceride and cholesterol concentrations in patients awaiting bariatric surgery. Obes Surg 2014;24:771-6.
9. Rabinovitz H, Friedensohn A, Leibovitz A, Gabay G, Rocas C, Habot B, *et al.* Effect of chromium supplementation on blood glucose and lipid levels in type 2 diabetes mellitus elderly patients. Int J Vitam Nutr Res 2004;74:178-82.
10. Huang H, Chen G, Dong Y, Zhu Y, Chen H. Chromium supplementation for adjuvant treatment of type 2 diabetes mellitus: Results from a pooled analysis. Mol Nutr Food Res 2018;62. [Ahead of print]. DOI: 10.1002/mnfr.201700438.
11. Costello RB, Dwyer JT, Bailey RL. Chromium supplements for glycemic control in type 2 diabetes: Limited evidence of effectiveness. Nutr Rev 2016;74:455-68.
12. Cefalu WT, Wang ZQ, Zhang XH, Baldor LC, Russell JC. Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. J Nutr 2002;132:1107-14.
13. Ajlouni K, Jaddou H, Batieha A. Diabetes and impaired glucose tolerance in Jordan: Prevalence and associated risk factors. J Intern Med 1998;244:317-23.
14. American Diabetes Association. Standards of medical care in diabetes-2011. Diabetes Care 2011;34 Suppl 1:S11-61.
15. Tripathy S, Sumathi S, Raj GB. Minerals nutritional status of type 2 diabetic subjects. Int J Diab Dev Countries 2004;24:27-8.
16. Makhlough A, Makhlough M, Shokrzadeh M, Mohammadian M, Sedighi O, Faghihan M, *et al.* Comparing the levels of trace elements in patients with diabetic nephropathy and healthy individuals. Nephrourol Mon 2015;7:e28576.
17. Hajra B, Orakzai BA, Faryal U, Hassan M, Rasheed S, Wazir S, *et al.* Insulin sensitivity to trace metals (Chromium, manganese) in type 2 diabetic patients and non diabetic individuals. J Ayub Med Coll Abbottabad 2016;28:534-6.
18. Rhodes NR, McAdory D, Love S, Di Bona KR, Chen Y, Ansorge K, *et al.* Urinary chromium loss associated with diabetes is offset by increases in absorption. J Inorg Biochem 2010;104:790-7.
19. Cunningham JJ. Micronutrients as nutriceutical interventions in diabetes mellitus. J Am Coll Nutr 1998;17:7-10.
20. Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N, *et al.* Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trace Elem Res 2008;122:1-8.
21. Abou-Seif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. Clin Chim Acta 2004;346:161-70.
22. Ekmekcioglu C, Prohaska C, Pomazal K, Steffan I, Schernthaner G, Marktl W, *et al.* Concentrations of seven trace elements in different hematological matrices in patients with type 2 diabetes as compared to healthy controls. Biol Trace Elem Res 2001;79:205-19.
23. Rafiei R, Habyby Z, Fouladi L, Najafi S, Asgary S, Torabi Z, *et al.* Chromium level in prediction of diabetes in pre-diabetic patients. Adv Biomed Res 2014;3:235.
24. Yamamoto A, Wada O, Ono T. Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. Eur J Biochem 1987;165:627-31.
25. Devlin TM. Textbook of Biochemistry. USA: John Wiley & Sons; 2011.
26. Vincent JB. The biochemistry of chromium. J Nutr 2000;130:715-8.
27. Velmurugan G, Swaminathan K, Veerasekar G, Purnell JQ, Mohanraj S, Dhivakar M, *et al.* Metals in urine in relation to the prevalence of pre-diabetes, diabetes and atherosclerosis in rural India. Occup Environ Med 2018. pii: oemed-2018-104996.
28. Hajifaraji M, Leeds AR. The effect of high and low glycemic index diets on urinary chromium in healthy individuals: A cross-over study. Arch Iran Med 2008;11:57-64.
29. Fagard RH, Nilsson PM. Smoking and diabetes – The double health hazard! Prim Care Diabetes 2009;3:205-9.
30. Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010;2010:453892.
31. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802-12.
32. Sakai K, Matsumoto K, Nishikawa T, Suefuji M, Nakamaru K, Hirashima Y, *et al.* Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta-cells. Biochem Biophys Res Commun 2003;300:216-22.
33. Tanaka Y, Tran PO, Harmon J, Robertson RP. A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. Proc Natl Acad Sci U S A 2002;99:12363-8.
34. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB, *et al.* Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711-7.
35. Zhu X, Hu B, Jiang Z. Cloud point extraction combined with graphite furnace atomic absorption spectrometry for the determination of chromium species and their distribution in cigarette and cigarette ash. Int J Environ Anal Chem 2004;84:927-34.
36. Ravina A, Slezack L. Chromium in the treatment of clinical diabetes mellitus. Harefuah 1993;125:142-5, 191.
37. Ding W, Chai Z, Duan P, Feng W, Qian Q. Serum and urine chromium concentrations in elderly diabetics. Biol Trace Elem Res 1998;63:231-7.
38. Duncan MG. The effects of nutritional supplements on the treatment of depression, diabetes, and hypercholesterolemia in the renal patient. J Ren Nutr 1999;9:58-62.
39. Iqbal N, Cardillo S, Volger S, Bloedon LT, Anderson RA, Boston R, *et al.* Chromium picolinate does not improve key features of metabolic syndrome in obese nondiabetic adults. Metab Syndr Relat Disord 2009;7:143-50.
40. Komorowski J, Juturu V. Chromium supplementation does not improve glucose tolerance, insulin sensitivity, or lipid profile: A randomized, placebo-controlled, double-blind trial of supplementation in subjects with impaired glucose tolerance: Response to Gunton *et al.* Diabetes Care 2005;28:1841-2.
41. Kleefstra N, Houweling ST, Jansman FG, Groenier KH, Gans RO, Meyboom-de Jong B, *et al.* Chromium treatment has no effect in patients with poorly controlled, insulin-treated type 2 diabetes in an obese western population: A randomized, double-blind, placebo-controlled trial. Diabetes Care 2006;29:521-5.
42. Guimarães MM, Martins Silva Carvalho AC, Silva MS. Chromium nicotinate has no effect on insulin sensitivity, glycemic control, and lipid profile in subjects with type 2 diabetes. J Am Coll Nutr 2013;32:243-50.
43. Yin RV, Phung OJ. Effect of chromium supplementation on

glycated hemoglobin and fasting plasma glucose in patients with diabetes mellitus. Nutr J 2015;14:14.

44. Albarracin CA, Fuqua BC, Evans JL, Goldfine ID. Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled overweight to obese patients with type 2 diabetes. Diabetes Metab Res Rev 2008;24:41-51.
45. Martin J, Wang ZQ, Zhang XH, Wachtel D, Volaufova J, Matthews DE, *et al.* Chromium picolinate supplementation attenuates body weight gain and increases insulin sensitivity in subjects with type 2 diabetes. Diabetes Care 2006;29:1826-32.
46. Paiva AN, Lima JG, Medeiros AC, Figueiredo HA, Andrade RL, Ururahy MA, *et al.* Beneficial effects of oral chromium picolinate supplementation on glycemic control in patients with type 2 diabetes: A randomized clinical study. J Trace Elem Med Biol 2015;32:66-72.
47. Campbell WW, Joseph LJ, Anderson RA, Davey SL, Hinton J, Evans WJ, *et al.* Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women. Int J Sport Nutr Exerc Metab 2002;12:125-35.
48. Lukaski HC, Siders WA, Penland JG. Chromium picolinate supplementation in women: Effects on body weight, composition, and iron status. Nutrition 2007;23:187-95.