

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

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

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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 healthy controls.^[5] A recent case-control study 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 $\times 2000$ 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

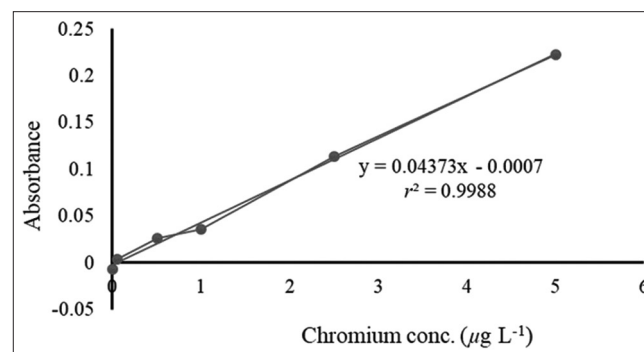


Figure 1: Calibration curve of serum chromium determination as evaluated by atomic absorption spectroscopy

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) $\mu\text{g/L}$, 0.45 (0.33) $\mu\text{g/L}$, and 0.9 (0.5) $\mu\text{g/L}$, 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

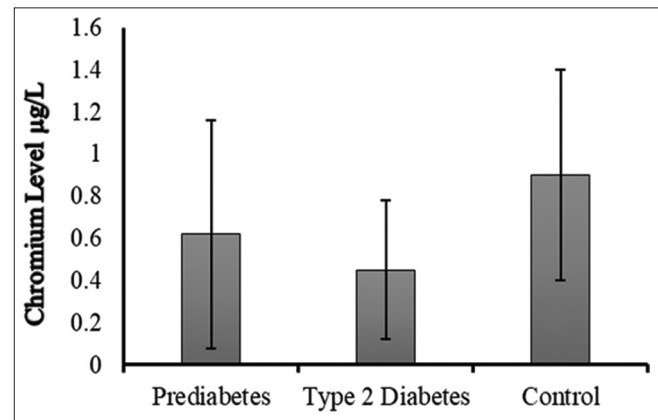


Figure 2: Chromium level among patients with prediabetes, type 2 diabetes, and healthy individuals. The mean difference was statistically significant among the study groups ($P < 0.05$). Values are given as mean \pm standard deviation

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) | 0.000* |
| 25-49.9 | 36 (30.0) | 13 (10.8) | 67 (55.8) | |
| 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) | 0.000* |
| Normal (18.5-24.9) | 16 (13.3) | 7 (5.8) | 35 (29.2) | |
| 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, Makhrough *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 healthy controls, 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 μ g 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 μ g 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 μ g/day.^[45] A single-blind randomized trial performed of 71 patients with T2D revealed a reduction in HbA1c levels after 1 month following 600 μ g/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 μ g 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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