The Effect of High and Low Glycemic Index Diets on Urinary Chromium in Healthy Individuals: A Cross-Over Study

Majid Hajifaraji PhD*, Anthony R. Leeds RNutr**

Background: Insufficient dietary intake of chromium as an essential nutrient leads to signs and symptoms that are similar to those observed for diabetes and cardiovascular diseases. We postulate that in healthy individuals, urinary chromium excretion following a high glycemic index diet is higher than after a low glycemic index diet.

Methods: A sequential randomized controlled cross-over study was carried out at the Metabolic Unit of Nutrition Department of King’s College London. Sixteen healthy individuals aged 18 – 60 years were recruited from 26 volunteers. A low or high glycemic index diet (as the main meals/day) was given to the volunteers over six days. Fasting blood glucose and insulin changes were determined and 24-hr urine samples were collected to measure chromium excretion before intervention and every second day within each treatment with a one wash-out period using Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometer.

Results: During the six-day diet study, there were no significant differences in 24-hr urinary chromium losses between the two groups following the low glycemic index (0.58 ± 0.08µg/24 hr) and high glycemic index diets (0.48 ± 0.06µg/24 hr). However, during day six there was a trend towards greater loss of chromium after the high glycemic index diet (0.73 ± 0.1 µg/24 hr) in comparison with the low glycemic index diet (0.54 ± 0.07 µg/24 hr).

Conclusion: Evidence that urinary chromium loses following the high glycemic index diets have not been clearly demonstrated in normal subjects. These results suggest that chromium excretion may need to be observed for longer than six days to address this question.

Keywords: Chromium • glycemic index • insulin resistance

Introduction

Chromium (Cr) is an essential nutrient required for glucose and fat metabolism. Insufficient dietary intake of Cr leads to signs and symptoms that are similar to those observed in diabetes and cardiovascular diseases.1,2

A low glycemic index (GI) diet can reduce postprandial glucose levels, and may increase insulin sensitivity.3 The mechanism for Cr transport (involving transferrin and chromodulin) may explain the link between increases in serum glucose and insulin and the accompanying increases in urinary Cr excretion.4

An apparent loss of Cr from the plasma compartment with excretion of Cr after oral glucose tolerance test (OGTT) has been previously reported by Morris and coworkers.5 They found a significant inverse relationship between plasma Cr and plasma insulin concentrations both over a 24-hr period after consuming the meals (P<0.001) and after a 75 g glucose load (P<0.01). This observation, suggesting the removal of Cr from the plasma compartment after meals, was not explained simply by increased urinary loss but might be explained by transient changes in uptake or binding of Cr by insulin sensitive tissues.

In our earlier study on British adults who consumed different GI foods, no significant difference was found between urinary Cr excretion and the GI diet groups, suggesting that it could be
The effect of high and low GI diets on urinary Cr in healthy individuals

Archives of Iranian Medicine, Volume 11, Number 1, January 2008

58

due to limitations of the study, or there might truly be no difference. However, the high urinary Cr seen in the low GI diet group (expressed per gram CHO) may reflect higher total body concentration, or a higher dietary intake.

Cr deficiency may be promoted by increasing Cr losses in response to physiologic stressors such as physical trauma, acute exercise, lactation, and consumption of a diet high in sugars. In this regards, our second study was done to find out whether a postprandial glucose load can affect Cr excretion. The study showed the peak time of Cr excretion was at four hours after glucose load.

According to published studies, a low GI diet can reduce postprandial glucose levels. It helps keep blood glucose levels within a near normal range, and may increase insulin sensitivity. Insulin resistance may occur with a high GI diet because of the direct effects of hyperglycemia and increased late postprandial serum free fatty acid levels. Therefore, reduction of the insulin binding to the cells as a result of reducing the number of receptors could be related to depletion or reduction of body Cr stores, because chromodulin needs to bind to insulin receptor for activating the receptor kinase activity.

It is possible that persistent consumption of high GI meals causes a gradual depletion of body tissue Cr concentrations by increasing urinary excretion. Therefore, we postulated that in healthy individuals, urinary Cr excretion after a high GI diet was higher than after a low GI diet.

Materials and Methods

An interventional (a randomized controlled cross-over design) study was carried out to investigate the effect of persistent consumption of low and high GI diets on urinary Cr excretion in healthy individuals by blood sampling combined with urine collections.

After approving the study by Research Ethics Committee of King’s College London (KCL), 16 healthy individuals aged 18 – 60 years were recruited from 26 volunteers. The participants were recruited by KCL circular e-mail followed by further information. They were questioned for the history of any experience of diabetes, renal, liver, or cardiovascular diseases, and anemia. The volunteers were asked to fill in their records and sign the informed consent form. A letter was sent to the volunteer’s general practitioner asking him/her for any medical condition that would prevent the participant from the study. This study was conducted on both men and women. Those participants with normal fasting blood glucose (3.9 – 5.8 mmol/L in serum), and those who did not have any experience of systemic diseases were included in the study.

Those with body mass index (BMI) >30, diabetes or impaired glucose tolerance (IGT) [i.e., normal fasting plasma glucose >5.8 mmol/L], systemic diseases such as cardiovascular, renal, or liver diseases, hypertension, hyperlipidemia, or family history of these diseases, under medication or taking minerals and vitamins supplements, or those who chronically ingest yeast supplements (a source of Cr) were excluded.

Ten-milliliter blood samples were collected into a 4-mL plain (no additive) vacutainer for full lipid profiles, a 2-mL ethylenediaminetetraacetic acid (EDTA) vacutainer for full blood count, and a 4-mL fluoride oxalate vacutainer, coated for blood glucose after an overnight fast (10 – 12 hr). Blood samples were shipped to the Department of Clinical Biochemistry at King’s College Hospital for analysis in less than three hours. BMI was calculated from weight (kg)/height (m²).

Food recipes and diets with various glycemic indices

The foods were cooked or prepared for the breakfast, lunch, and dinner of a week period study as follows: chicken curry with basmati rice was cooked as a moderate GI diet for the dinner of the night before the start of the study. Forty grams All-Bran cereal or 30 g corn flakes (both Kellogg’s brand) as the low and the high GI, respectively with 130 mL semi-skimmed milk were prepared for the breakfast. Three different sandwiches such as tuna mayonnaise, egg, and burger (own recipe) with multiple seeded whole meal or white bread were prepared, respectively as the low and the high GI plus a low fat Muller light fruit yoghurt for lunch. One moderate GI, and seven different low GI, and seven high GI recipes, including ready made and own recipes were prepared and cooked. The glycemic index of the meals in both low and high GI diets was estimated with computing the mixed GI. To find out the Cr content of the two GI diets and match them before starting the experiment, a dish of fifteen different food meals was cooked according to the recipes. Each food was mixed and homogenized using a Mulinex Masterchif-35 equipped with a nylon-coated bowl to avoid sample contamination. The
mixed food samples were transferred into the 50 mL polypropylene containers and weighed using a Precisa 80A200M (Swiss Quality) balance, and then stored at -40ºC until they were analyzed. The frozen food samples were dried using a Jouan-LP3 freeze dryer at -50ºC in five days. Approximately 0.5 g of each homogenized sample weighed on a Precisa 80A-200M (Swiss Quality) balance, was digested using 10 mL HNO₃ (density 1.4, Fluka Trace Select) and 1 mL of 100 volume H₂O₂ (density 1.11, BDH), and the rest of the liquid was 20 mL distilled water in a ETHOS PLUS Microwave Laboratory Station at 200ºC for 15 minutes. After cooling the samples they were diluted with 30 mL of ultrapure high purification (UHP) water, mixed, and transferred to a 10-mL trace metal free polypropylene tube. The weights of digestion tube, sample, and diluted digest were recorded. Then, the exact dilution factor was calculated. Samples were analyzed for Cr contents concentration using Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometer (DRC ICP-MS) at ICP laboratory of KCL.

Determination of chromium in food
An internal standard solution of 1% HNO₃ containing 10 ppb Gallium (Ga) was prepared. A 10-ppm Cr intermediate standard was prepared by diluting 0.5 mL of a 1000-ppm stock to 50 mL with 1% HNO₃ (added 0.5 mL of concentration HNO₃) in a centrifuge tube. Calibration standards of 0, 5, 10, and 20 ppb (µg/L) Cr were prepared in 25% v/v (mL solute/mL solution) of concentration HNO₃: to 50 mL metal free centrifuge tubes, then 12.5 mL of concentration HNO₃, and 0, 25, 50, and 1000 µL of 10 ppm intermediate were added. Two milliliter of samples/standard were diluted with 2 mL of internal standard solution. Perkin Elmer solution P/N 812 – 5035 (1ppb Cr in 0.5% HNO₃) was used for quality control (QC). Five milliliter of this solution was added to the coded chilled (in ice bucket) FX tube (fluoride oxalate), coated for blood glucose and 4 mL into chilled (in ice bucket) LH (lithium heparin) for insulin determination. All blood sample tubes were placed immediately on ice and then were centrifuged (at 2500 rpm/15 min at 4ºC). Plasma was separated into two aliquots. The plasma-coded vials were immediately stored at -80ºC until the study was completed. At the end of day seven, a one-week washout period commenced. During the washout period, the participants followed their individual diet. All subjects were asked to collect a 24-hr urine sample in a coded collection container (a Rolon 24-hr polythene lined specimen storage container, with a capacity of approximately 2500 mL), every second day of the study over six days (starting on days 2, 4, and 6). Each participant was given instructions for the completeness of collections. Also, they were instructed about the start and the time of collection (discarding the first sample in the morning and adding the last specimen in the following morning). A metal free container was provided for collecting 24-hr urine. The coded containers of collected urine samples were placed in the cold room, at 5ºC, (until transferring into small tubes) for a maximum of three days. After measuring the total volume of collected urine, 10-mL of thoroughly mixed sample was transferred into a labeled 15-mL metal free polypropylene tube. Urine sample was stored at -20ºC until Cr analysis. The participants were asked to collect a 24-hr urine sample starting on the last day of washout period (these samples were used to determine the Cr concentration before starting of both periods of the study). After crossing over the participants to the other
The effect of high and low GI diets on urinary Cr in healthy individuals

Archives of Iranian Medicine, Volume 11, Number 1, January 2008

Methods for the dietary assessment

A food diary was used to record a six-day food intake within the study (four week days and two weekend days) to determine nutrient intake such as carbohydrate (CHO), protein, fat, and also GI. Each volunteer was given a set of food scales and one recording document; a detailed record book for use with foods eaten or prepared at home or work. The scales used for the study were lightweight, battery powered Soehnle (Soehnle Ltd.), which give a digital display of weight and could be zeroed after each item was weighed. The scales were calibrated to within 1%. The participants were asked to weigh and record all foods consumed. During the six days they recorded every item consumed, including foods, snacks, and drinks provided. They also received instruction on how to record their food intake for six days. During the study none of the participants took any medication, especially supplements. Dietary instruction was provided (a list of some permissible and impermissible foods) on optional snacks for the low GI diet group.12,13 Foods intake data were entered to Microdiet (the British food composition analysis software) and analyzed for the nutrients.14

Laboratory methods

An enzymatic peroxidase antiperoxidase (PAP) method was used for determination of glucose using a Stat Analyzer (MIRA-S Ltd.). The material and method was carried out at Diabetes Research Laboratory of St. Thomas’ Hospital. A radioimmunoassay was used for measuring insulin. The plasma samples were assayed by competition binding with 125 I-Insulin.15 Nuclear Enterprises and NE1600 Gamma Counter were used for the measurement. The plasma samples were assayed by competition binding with 125 I-Insulin.15 Nuclear Enterprises and NE1600 Gamma Counter were used for the measurement. The assay was carried out at the Department of Metabolic Medicine of Hammersmith Hospital. Homeostatic Model Assessment (HOMA-IR) index was calculated for each subjects using the equation of fasting insulin time to fasting glucose divided by 22.5.16 Urine samples were evaluated for Cr contents concentration using DRC ICP-MS of Perkin-Elmer Ltd at ICP laboratory of KCL.

Statistical analysis

Collected data were analyzed using the code number of subjects in each group. Analyses were conducted using SPSS software version 11.5. Descriptive statistics (mean, standard deviation, standard error of mean, mean differences, and 95% confidence interval) were used to summarize the data. Within group comparisons were performed using the paired-sample t-test. We also used regression analysis and simple to examine significant relationships (α=0.05) among variables. Prism package was used to get the area under the curve (AUC) of plasma glucose, insulin, and urinary Cr.

Results

Sixteen healthy individuals (nine males and seven females) aged 18 – 61 years were recruited from 26 volunteers (Table 1). After determination of Cr contents of 15 low and high GI foods, we found no significant difference between the mean value of the high GI (0.58 ± 0.21 µg/g of dry sample) and the low GI (0.66 ± 0.12 µg/g of dry sample) diets (mean difference -0.075 [95% CI -0.62 to 0.47]). Therefore, it would be matched in both groups and should not influence on the results of GI diet effects.

There was no significant difference of nutrient intake between the high and the low GI groups (Table 2). Table 3 shows the changes of 24-hr urinary Cr excretion during consumption of high and low GI diets by normal subjects. Paired t-test showed no significant difference between the urinary Cr excretions on corresponding days. The results showed no significant difference of urinary Cr excretion from assumed baseline value after four days of consumption of the low GI diet, while a nonsignificant increase was shown following the high GI diet at day 6 (Figure 1). However, during

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of the participants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>SBP¹</td>
</tr>
<tr>
<td>DBP²</td>
</tr>
<tr>
<td>GI³ of LGI³</td>
</tr>
<tr>
<td>GI³ of HGI³</td>
</tr>
</tbody>
</table>

¹Systolic blood pressure; ²Diastolic blood pressure; ³Glycemic index; ³Low and high glycemic index meals.
day six there was a nonsignificant trend towards greater loss of Cr after the high GI diet in comparison with the low GI diet. After computing the insulin resistance index (HOMA-Insulin Resistance Index) a nonsignificant increase was found following four days consuming of the high GI diet, while there was still a nonsignificant steady decrease following the low GI diet (Figure 2).

There was a correlation between Cr excretion of high GI diet group \( (P=0.005) \) and no correlation in low GI \( (P=0.06) \) with HOMA-insulin resistance index in day 5 of study (Figure 3).

**Discussion**

In this study, we investigated whether or not consumption of low and high GI diet over six days may affect the urinary Cr excretion in healthy individuals. Because we matched the Cr content of the foods in both GI diets, it might not be expected to influence on Cr excretion, but simply equalizing the chemical content does not ensure that bioavailability was also equal.

There was no significant difference of nutrient intake either between the two diet groups or between males and females. Using a cross-over design helped us to keep control confounding variables, which might influence our results. In the present study, statistical analysis of variance did not show significant effect of time or diet alone or depends on basal Cr on Cr excretion within subjects over six days. Also statistical analysis indicated that urinary Cr excretion was not related to method (phase of study). This means that the washout period before crossing over the groups was enough for this study.

Since there was no published food composition table to assess Cr intake we adjusted the data for calorie intake, as a surrogate of Cr intake and the nonsignificant difference in Cr excretion between the two diets remained. Linear regression showed no correlation between mean Cr excretion and mean energy intake of subjects in two GI groups.

The present study showed no significant changes of plasma glucose and insulin concentrations during six days consumption of the low and high GI diets. This result might be due to consuming some other snacks or foods with different GI by participants, which have not been recorded in food diary intake. Another reason might be the limited time (short period) of the study. There have been several studies that have reported improved insulin sensitivity in response to improved Cr nutrition\(^{17,18}\); however, a number of studies have also reported no improvements in circulating insulin in response to supplemental Cr. A meta-analysis of the published studies failed to find a significant effect of Cr on insulin concentration\(^ {19} \) However, several positive studies were not included in the analysis because of the lack of specific data, inability to have access to the original data, or for other reasons.

We found a steady increase of urinary Cr excre-

| **Table 2. Nutrients intake of subjects in each step of study in both high and low GI groups.** |
|---------------------------------|---------------------------------|---------------------------------|------------------------------|
| **High-low GI** (n=16) | **Low-high GI** (n=16) | **Paired differences** | **Mean E%** |
| **Energy (KJ/day)** | 7030.8 ± 281.8 | 6716 ± 281.8 | 314.8 | 377.2 | (-494.1, 1123.8) | — |
| **Protein (g/day)** | 66.4 ± 1.7 | 68.4 ± 2.1 | -1.9 | 2.7 | (-7.7, 3.8) | 16.7 |
| **CHO (g/day)** | 231.6 ± 11.8 | 216 ± 11 | 15.5 | 13.6 | (-13.7, 44.8) | 52.1 |
| **Fat (g/day)** | 53.4 ± 1.4 | 53.9 ± 2.9 | -0.5 | 2.9 | (-6.6, 5.7) | 28.9 |
| **Alcohol (g/day)** | 7.7 ± 4.4 | 3.4 ± 1.5 | 4.3 | 3.1 | (-2.4, 11) | 2.4 |

Values are expressed as mean and standard error; \(^1\) Mean differences of two methods in each GI group; \(^2\) 95% confidence interval the difference.

| **Table 3. Changes of 24-hr urinary Cr before and after consumption of various GI diets in each treatment (n=16).** |
|---------------------------------|---------------------------------|---------------------------------|------------------------------|
| **High-low GI** | **Low-high GI** | **Mean difference** | **95%CI** | **P value** |
| **µg/24 hr** | **%Changes from baseline** | **µg/24 hr** | **%changes from baseline** | **Mean difference** | **95%CI*** | **P value** |
| Basal† | 0.62±0.12 | — | 0.62±0.12 | — | — | — | — |
| Day 2 | 0.51±0.08 | -17 | 0.44±0.08 | -28.1 | 0.07 | (-0.153, 0.261) | NS‡ |
| Day 4 | 0.52±0.08 | -16.2 | 0.46±0.1 | -25.1 | 0.05 | (-0.132, 0.521) | NS |
| Day 6 | 0.73±0.14 | 17.7 | 0.54±0.07 | -13.2 | 0.019 | (-0.226, 0.250) | NS |

\(^*\)Mean and SEM; \(^**\)Paired \( t \)-test (%changes from baseline); \(^***\)95% confidence interval of the difference; \( \dagger \) A 24-hr urine of the last day of the wash-out period was assumed as basal Cr; \( \ddagger \) Not significant (difference between two GI diet groups).
The effect of high and low GI diets on urinary Cr in healthy individuals

The Cr contents of the reference and high sugar diets were both approximately 16 µg per 1000 calories. Urinary Cr excretion increased significantly ($P<0.03$) from 0.24±0.01 µg/day while consuming the reference diet to 0.28±0.01 µg/day during the high sugar diet period. They demonstrated that consumption of diets high in simple sugars stimulated Cr losses; and this coupled with marginal intake of dietary Cr might lead to marginal Cr deficiency, which was associated with impaired glucose and ultimately impaired

Figure 1. Twenty-four-hour urine Cr excretion during six days low and high GI diets in normal subjects (values are expressed as mean and standard error, $n=16$).

Figure 2. Insulin resistance index during six days of consuming high and low GI diets in normal subjects ($n=16$).
insulin sensitivity.

Anderson proposed that stresses such as increased sugar intake had been shown to lead to increased losses of Cr and consequently might lead to a comprised Cr status.7 Interestingly finding from this study was the different changes of urinary Cr excretion in males and females especially after four days of consuming the diets. The results indicated the greater urinary Cr after the high GI diets in both sexes than after the low GI diet, but there was a trend forwards a greater effect in women. Our results showed that when insulin resistance reduced, insulin secretion was reciprocally reduced in the low GI diet group compared with the high GI group, which was not different, but was followed by reduced glucose levels. This positive relationship between insulin resistance and insulin secretion was consistence with Khan et al’s study, although their result was related to increased and not reduced glucose levels.21 However, it was inconsistence with their previous study showing that insulin resistance was followed by increased insulin secretion but a reduced circulating glucose.22 There are number of studies in a variety of groups of human subjects, which are consistent with the hypothesis that reducing diet’s GI improves insulin sensitivity. Kiens and Richter investigated the influence of dietary carbohydrate types on insulin action on seven healthy young men who ingested two isoenergetic diets with a high GI or a low GI for four weeks, in a cross-over design.23 This present study showed a nonsignificant steady decrease of insulin resistance after second day of consuming a low GI diet versus a high GI diet. Interestingly, there was a positive correlation between Cr excretion of day six and insulin resistance index of day 5 in the high GI diet group ($P=0.005$), but not in the low GI diet group ($P=0.06$). Previous review demonstrated that reduction of the insulin binding to the cells as a result of reducing the number of receptors could be related to depletion or reduction of body Cr stores.24

According to this result the fasting plasma insulin and also insulin resistance index tend to reduce after two days of a low GI diet versus a high GI diet. And there is a relationship between 24-hr Cr excretion and insulin resistance index after four days of the high GI diet. An animal study on the determination of Cr in different body tissues, following feeding the low and the high GI diets for three to four weeks is required to determine how Cr stores are affected by GI diets. Long-term studies (at least three to four weeks) on humans are required to find the difference of urinary Cr excretion following the consumption of low and high GI diets in both healthy individuals and in patients with type 2 diabetes. Determination of blood Cr concentration as well as blood glucose and insulin concentrations with improvement of

Figure 3. Linear regression between Cr excretion after consuming two GI diets in day 6 and HOMA-IRI in day 5 of the study in 16 normal subjects [Variables: Cr24hr-6 hg, Cr24-6 lg=Cr 24 hr day 6 of HGI and LGI groups, iri-5 hr, iri-5 $l=$HOMA-IRI day 5 of HGI and LGI groups] (Values are expressed as mean and standard error, n=16).
verification of 24-hr urine collection quality using a marker such as P-amino benzoic acid (PABA) to determine urinary Cr concentration are required. A noninvasive scan technique such as nuclear magnetic resonance spectroscopy (NMRS) might be helpful to determine the Cr stores in different organs and tissues in human. This has been discussed with a staff at the Medical Research Council (MRC)-NMRS, but techniques are not yet sensitive enough for the very low levels. When NMRS is more sensitive this technique might be used.

Acknowledgment

Thanks to all the participants who took part in our study. This research would not have been possible to run without cooperation of all subjects.

References