Author's response to reviews

Title: Chromium supplementation in non-obese non-diabetic subjects is associated with a decline in insulin sensitivity

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Author's response to reviews: see over
Response to Reviewers:

We thank the reviewers for their insightful suggestions for improvement of the manuscript. The specific manners in which the manuscript has been revised to address the reviewer concerns are as follows:

Reviewer 1 (Dr Krejpcio):

1. Request that we include a power analysis.

We have included a power analysis. We agree with the reviewer that this is a small study which is why we stated in the discussion section (in the limitations paragraph) that the results should be viewed with caution and needs to be replicated. We have added a paragraph in the statistics section stating that the sample size has adequate power to detect an effect of f = .3 or higher which is equivalent to medium/large to large effect on insulin sensitivity. We reference previous reports that chromium picolinate in the dose studied may, in people with type 2 diabetes, have a medium size effect on insulin sensitivity.

“The group sample size of subjects included in the final data analyses (14 active and 15 placebo) had adequate power (.80) to detect an effect of f=.30 or higher. This is equivalent to a medium/large to large effect on insulin sensitivity and had been previously reported with chromium picolinate therapy [24, 25].”

2. Requested that we discuss in more detail the rationale for the dose of chromium picolinate used in this study.

Chromium picolinate is absorbed more efficiently than many other forms of chromium ~ 2% efficiency compared to dietary chromium which is absorbed with ~ 0.5% efficiency (references: Anderson RA et al. Journal of Trace Elements in Experimental Medicine. 1996; 9(1): 11.; Olin KL et al. Trace Elem Electroly. 1994; 11(4): 182). Supplementation with 200 to 1000 ug of chromium picolinate has been reported to improve glucose intolerance and improve insulin sensitivity without associated toxic effects. We now state this in the discussion section of the manuscript. The reviewer notes that Racek et al.2006 used 400 μg of chromium enriched yeast in their study; and Krol et al 2011 used 500 μg of brewer’s yeast. It has been reported that chromium in Brewer’s yeast may be absorbed with 5 to 10% efficiency...
Thus one can assume that at most about 20 to 50 μg of chromium was absorbed in these experiments with brewer’s yeast. This compares to absorption of ~ 20 μg of chromium in our experiment which was sufficient to raise serum and urine chromium levels.

“Supplementation with 200 to 1000 μg of chromium picolinate has been reported to improve glucose intolerance and lower circulating insulin levels[21, 48] with one study in type 2 diabetes patients showing a greater improvement with 1000 μg dose compared to 200 μg dose [21]. The 1000 μg daily has been used in a number of other clinical studies [22, 23] and not associated with any toxic effects.”

We have included many of the newer references requested and cite the references regarding chromium and renal function which had been inadvertently omitted in the original manuscript.

5. Additional information about the chromium analyses.

The analyses of urine and serum chromium were performed by Quest Diagnostics, Chantilly. We have included more details regarding the analyses:

“The urine chromium levels were measured by atomic absorption spectrometry with graphite furnace atomization (AAGF) with Zeeman Background Correction and the serum chromium levels were measured by Inductively Coupled Plasma Mass Spectrometry (ICPMS) _with Collision Cell Technology at Quest Diagnostics Nichols Institute (Chantilly VA). For AAGF, the sample was diluted with a "matrix modifier" that helped control the atomization of Chromium at a specific temperature. For ICPMS, the sample was diluted with a weak nitric acid solution. A linear calibration curve was obtained on blank samples and performed before and after the assays. Elevated values were repeated with a new sample set-up to check for contamination issues.”

Reviewer 2 (Dr. Setola):

1. Request inclusion of a power analysis

See note above.

2. Illustrate statistical significances in the tables and figures.

Table 1, column 5 illustrate that placebo and chromium groups are matched with respect to age, BMI, glucose levels, lipid values (column 5 documents the p values). The figure legends document the chromium levels and statistical significances.

3. Additional information about the euglycemic hyperinsulinemic clamp.

We have provided additional information about the clamp as requested:

“A hyperinsulinemic –euglycemic clamp [18] was performed at baseline and after 16 weeks treatment with chromium picolinate or placebo. A primed-continuous infusion of regular human insulin was administered at a rate of 40 mU/min/m2 body surface area for 120 minutes. This insulin infusion rate is sufficient to suppress hepatic glucose production in a normal non-obese
Bedside blood glucose levels were measured at 5 minute intervals and the glucose level was maintained at approximately basal level with a variable infusion of 20% glucose. Glucose disposal values (M/LBM/I) were calculated as mg glucose infused per min per kg lean body mass (LBM) during the steady state period between 90 and 120 minutes divided by steady state insulin (SSI) levels (in μU/ml x 100).”

4. Additional information regarding assessment of diet and physical activity.

Physical activity was assessed using a questionnaire by Baecke. A detailed diet history was obtained by the clinical research center nutritionist. We have added this information in the methods section of the manuscript:

“Exercise and general physical activity pattern were determined using the questionnaire developed by [17]. This questionnaire generates a physical-activity index score from 3 to 15 based on work, sport and leisure time energy expenditure, with each category scored from 1 to 5 (lowest to highest activity level). Subjects with scores greater than 10 (population mean approximately 8.3) were not be enrolled in the study.

Subjects underwent a dietary history at enrollment by the clinical research center nutritionist and were placed on a weight maintenance diet in order to avoid the confounding effect of weight loss on insulin sensitivity. Subjects could not be on nutritional supplements for at least three months prior to enrollment.”

Yours sincerely,

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