COMPARATIVE EFFECT OF VARIOUS FORMS OF CHROMIUM ON SERUM GLUCOSE: AN ASSAY FOR BIOLOGICALLY ACTIVE CHROMIUM

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Abstract

This study was undertaken to develop an assay for biologically active chromium suitable for determining the best form of chromium for human supplementation studies. The assay involved monitoring the decrease in fasting serum glucose for 1-3 hours in normal human subjects following ingestion of 100 µg of chromium. The maximum decline occurred 1-2 hours after taking the chromium. The average maximum per cent decrease for 7 subjects was 6.0% for inorganic chromium, 5.7% for conventional brewer's yeast chromium and 16.8% for a high chromium yeast. A chromium-EDTA complex decreased serum glucose an average of 19.6% in 3 subjects. A placebo yeast and dose-response study demonstrated that the chromium in the high chromium yeast was the factor responsible for the serum glucose lowering effect. The high chromium yeast and CrEDTA had a significantly greater biologically active chromium than inorganic chromium. These findings demonstrate that inorganic chromium is biologically active in man, but chromium in the form of synthetic complexes or high chromium yeast is much more active.

Introduction

Chromium is recognised as a trace element for both animal and human nutrition. The dietary inorganic chromium must be converted into a biologically active form to function physiologically. This biologically active form was named glucose tolerance factor (GTF) by Mertz [1]. This agent isolated in crude form from brewer's yeast is able to reverse the impairment of glucose tolerance of chromium-deficient rats overnight. Inorganic chromium is without effect in these rats [2]. Some humans, however, are capable of converting inorganic chromium *in vivo* into a biologically active form as evidenced by the effectiveness of inorganic chromium in improving the glucose tolerance of three groups: normal infant [3], chromium deficient adults [4] and malnourished infants [5].

The mode of action of biologically active chromium (BAC) is a co-factor of insulin. BAC stimulates the oxidation of glucose *in vitro* in the presence of insulin, but is ineffective in the absence of insulin [6]. A BAC isolated from brewer's yeast differs from simple inorganic chromium compounds in rat studies of transport [7]. Thus, it is not surprising that there is no relationship between total chromium content in a diet, food or food extract and biological activity [8,9].

The most widely used assay of BAC is the *in vitro* insulin-dependent assay of Mertz and Roginski [10] which uses epidymal fat tissue from chromium-deficient rats. This tissue is incubated with different amounts of chromium, insulin and radioactive glucose and the rate of conversion to radioactive carbon dioxide is measured. A potentiation factor is then calculated. Anderson [11] has recently developed a more

sensitive assay using adiopocytes from epidermal fat tissue. Mirsky and co-workers [12] have developed a yeast fermentation assay, but it has not been widely used. All these *in vitro* assays are non-specific as they respond to factors other than chromium. Factors such as pH, osmolarity and compounds such as glucose, glutamate, glutathione and nicotinic acid are known to affect the production of carbon dioxide in the *in vitro* assay [13-15]. Some foods which contain high concentrations of chromium such as hops [16] actually inhibit insulation potentiation. Also, these *in vitro* assays are an extract and the activity depends on the pH and solvent used for extraction. The only published *in vivo* assay involves the injection of chromium samples into mice and monitoring decreases in serum glucose [17]. However, this assay does not respond to inorganic chromium. In addition, the kinetics are widely different for each BAC tested.

The clinical and public health significance of chromium has been recently reviewed by Mertz [18]. Nutritional risks result from long-term total parenteral alimentation, nutritional formulas and malnourishment. Pregnancy, ageing and diabetes can also increase the risk of chromium deficiency. Chromium deficiency should be considered in all clinical situations where an unexplained insulin resistance develops in patients. Chromium deficiency also influences three recognised risk factors for cardio-vascular disease: impaired glucose tolerance, elevated circulating insulin levels and elevated serum cholesterol. The following research is an effort to develop an *in vivo* assay for BAC which is applicable for determination of the type of chromium best suited for human supplementation.

Materials and Methods
Seven normal subjects, 6 males and 1 female, ages 22 to 42 (mean 26 7 years) volunteered to participate in this study with informed consent. Each subject, after an overnight fast of 12 hours, appeared for testing in the morning. A fingerprick sample was taken in the sitting position for the zero hour (baseline) sample. Each subject took one of three forms of chromium. The inorganic form was chromic (III) chloride. The brewer's yeast form was Formula 350 brewer's yeast, 1.25 μg chromium/g. This is a brewer's yeast grown on molasses to which 15% dried yoghurt is added by weight. The high chromium yeast was 2180 μg chromium/g. This yeast was grown in a chromium medium and was hydrolysed during processing. The chromium was taken by all subjects at a dose of 100 μg dissolved in 50 ml of water with the exception of the brewer's yeast form which required 500 ml of water for a palatable suspension. In addition, a placebo yeast containing < 1 g chromium/g was tested on one subject. A chromium-EDTA complex (CrEDTA) was prepared as described by Gonzalez-Vergara, et. al. [19]. Three subjects ingested 100 g of chromium in this form. A fingerprick sample was taken at 1, 2 and 3 hours post-dose for all forms of chromium.

The fingerprick blood samples were taken using an Autclat apparatus, with a Monolet lancet. Blood samples were collected in Microtainer Capillary Blood Serum Separators. The samples were centrifuged at 4°C and the serum kept at 4°C until analysis the same day. Serum glucose was measured by an ultraviolet enzymatic assay kit.

Chromium was measured in the commercial products after ashing at 500°C overnight

and reconstituted with 0.1 M HNO$_3$ using an atomic spectrophotometer. The actual concentration was very close to the label value. The chromic chloride and CrEDTA were measured in the neutralised solutions and diluted to 100 μg chromium/50 ml water before ingestion.

Results

The three forms of chromium were consumed by all 7 subjects and the results shown in Table 1. Chromium caused a decrease in serum glucose in all of the 21 experiments.

Table: Maximum per cent decrease in Fasting Serum Glucose as a result of ingestion of 100 μg of Chromium.

Maximum % Decrease in Serum Glucose			
Subject	Inorganic	Brewer's yeast	High chromium yeast
1	3.6	0.6	28.0
2	13.6	10.8	22.7
3	7.4	12.9	17.4
4	4.5	4.8	14.8
5	1.0	1.4	12.5
6	5.3	8.0	9.6
7	6.6	1.2	12.3
Mean S.D.	6.0 ± 3.9%	5.7 ± 5.0%	16.8 ± 6.5%
95% Confidence Interval	2.35 to 9.65%	1.07 to 10.3%	10.7 to 22.8%

The maximum decrease usually occurred after 1 or 2 hours, but there was variation in the timing from one form of chromium to another and from one subject to another. For all subjects, the decrease in serum glucose was greatest for the high chromium yeast of the three forms of chromium tested. There was no significant difference ($p > 0.5$) between the serum glucose response for the inorganic chromium and the brewer's yeast chromium as determined by analysis of variance. The high chromium yeast and inorganic chromium serum glucose response were very highly significantly different ($p < 0.005$). In addition, the brewer's yeast and the high chromium yeast were very highly significantly different ($p < 0.005$).

A blank water, placebo yeast and yeast dose-response study were done with one of the subjects and the results are shown in Table 2.

Table 2: The maximum per cent Decrease in Serum Glucose as a result of ingestion of Water, Placebo yeast and High Chromium yeast by a single subject.

Form Administered	Dose of Chromium	Maximum % Decrease in Serum Glucose
Water	0 μg	1.0
Placebo Yeast	< 1 μg	2.8
Yeast	100 μg	9.6
Yeast	200 μg	18.2

The 1% decrease produced by water is indicative of the precision of the glucose assay. The placebo yeast was given at the same time high chromium yeast dose (46 mg) as the 100 g chromium sample. The placebo caused only a slight decrease in serum glucose. The dose-response and placebo data indicate that the chromium was prepared by reacting chromium (III) with EDTA. This complex was tested in 3 subjects. The results are shown in Table 3.

Table 3: The maximum per cent in Serum Glucose in three subjects as a result of ingesting 100 g of Chromium in an Inorganic, High Chromium yeast and Complex form.

Subject	Maximum % Decrease in Serum Glucose		
	Inorganic	High Chromium yeast	CrEDTA
1	5.3	9.6	12.9
2	4.5	14.8	25.6
3	6.6	12.3	20.3

The average maximum per cent decrease in serum glucose for inorganic chromium was $5.5 \pm 1.1\%$ for these subjects. The average for the CrEDTA was $19.6 \pm 6.4\%$. The high chromium yeast caused an average decrease for serum glucose of $12.2 \pm 2.6\%$ which was not significantly different from the CrEDTA ($p > 0.1$) by analysis of variance. The CrEDTA and high chromium yeast were significantly different form the inorganic chromium ($p < 0.05$).

Discussion

There have been many human studies which have shown that inorganic chromium supplementation improves glucose tolerance in subjects with impaired glucose tolerance indirectly indicating a deficiency of BAC. Saner, in a study of normal new-born infants, found that a single acute dose of inorganic chromium doubled the glucose removal rate after intravenous glucose administration [3]. The present study demonstrates that inorganic chromium in normal adults affects glucose metabolism when taken in physiological doses.

A look at the data shows that there is considerable inter-subject variation. In spite of the variations, there is a statistically significant 258% greater biological activity for the high chromium yeast as compared with the inorganic chromium. This large difference corroborates a long-term human supplementation study which showed that changes in the body pools of chromium [20] and, lipid and glucose parameters [21] were equivalent when large doses of inorganic chromium or small doses of yeast chromium were given.

The failure of conventional brewer's yeast chromium to be more active than inorganic chromium is puzzling. This brand of brewer's yeast was shown to be highly active by the *in vitro* fat pad assay and it was for this reason that it was chosen for supplementation to diabetic men [20]. Our results point out that inherent limitations of an *in vitro* test for predicting *in vivo* results exist. The *in vivo* assay indicates that this brand of brewer's yeast contained essentially inorganic chromium.

Previous studies have shown that synthetic models of GTF containing chromium, glutathione and nicotinic acid have lower biological activity than isolated GTF by the *in vitro* assay [11]. However, this difference may not be real due to the problem of interferences in the assay from components of the GTF extract which are not part of this actual GTF complex. The results of the in vivo assay indicate that the synthetic CrEDTA complex was highly biologically active. This synthetic complex had significantly more BAC then inorganic chromium. Indeed, Mertz [22] has shown that *in vitro* chromium transport across the intestine was strongly stimulated by amino acids which form chelates with the chromium. The CrEDTA was 1.61 times more active than the high chromium yeast but the difference was not significant. The lower BAC content of the yeast product may be due in part to processing which may destroy

some of the BAC, which is presumably in an amino acid chelated form, into inorganic chromium.

The present study provides an interesting contrast to recent work by Ghafghazi, who found that acute administration of inorganic chromium produced hyperglycaemia in rats and an intolerance to a glucose load [23]. An *in vitro* study [24] by the same group also demonstrated that inorganic chromium inhibited secretion of insulin from pancreatic islets in a dose-dependent manner which may account for the hyperglycaemia in rats. The concentrations used in both these *in vivo* and *in vitro* studies were thousands of times higher than the physiological concentrations and thus these studies have no bearing on humans other than demonstrating the toxicology of high doses of chromium. Interestingly, Ghafghazi [23] found that a single injected dose of 5 mg/kg of inorganic chromium to rats followed by insulin had no effect compared to a control group. Although this is a high dose, the result points out the perils of using animal models since the humans in our study were capable of converting inorganic chromium into a biologically active form which had a hypoglycaemic effect.

The results of the present study showing a greater BAC in chromium yeast than inorganic chromium may appear to contradict recent reports by Anderson [16, 25] who found from urine chromium analysis that inorganic chromium and food chromium were absorbed to the same extent, 0.4% by humans. However, the BAC assay measures a combination of both absorption and conversion to a biologically active form. If inorganic chromium and high chromium yeast are absorbed to the same extent, as Anderson's data would seem to indicate, then the chromium yeast is more quickly converted to a biologically active form or is absorbed intact in an active form. The high chromium yeast is thus the only preferred form of chromium for human supplementation.

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