PRENULIN™

NATURAL GLUCOSE SUPPORT

Science Dossier

November 2013

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PRENULIN™
NATURAL GLUCOSE SUPPORT

Product Overview

How Sweet It Is…

It's no secret that Americans commonly overindulge in sugary foods. On average, Americans eat much more sugar than their grandparents did 50 years ago. This sugar can be natural, such as sugar from milk (lactose) and sugar from fruit (fructose), or it can be refined such as table sugar (sucrose). Refined sugars are added to foods at the table or during processing, and they're known by a variety of different names such as dextrose, corn sweetener, maltose, etc. From a nutritional standpoint, there are huge differences between natural and refined sugars: fruit contains calories from its natural sugar, fructose, but also fiber and nutrients that are important for overall health; whereas, refined sugars are high in calories, but almost devoid of nutrients.

Today, excessive body weight is a major problem in society in general, and in the area of health care in particular. Over-consumption of added sugars – and the "empty" calories they deliver -- is a key factor in our societal weight problem. More calories consumed means more weight gained…unless there's a corresponding increase in physical activity, which itself is often problematic in our increasingly sedentary culture.

The US government's Dietary Guidelines for Americans 2005 explains that the healthiest way to reduce caloric intake is to decrease consumption of added sugars and other sources of empty calories. Sugar-sweetened beverages, such as soft drinks, make up a huge proportion of the added sugar in the American diet. Recent studies, published in scientific journals such as JAMA, The Journal of Pediatrics and Obesity (formerly Obesity Research), indicate a link between sugar-sweetened beverage intake and weight.

Some physicians and nutrition experts point to sugar as a key contributor in chronic ailments such as heart disease, hypertension, and certain cancers, in addition to obesity and diabetes. Recently, Dr. Robert Lustig, a specialist on pediatric hormone disorders and the leading expert in childhood obesity at the University of California, San Francisco, School of Medicine, gave a lecture in which he described sugar (specifically the fructose found in table sugar and high-fructose corn syrup) as having toxic effects in the body. He echoed these sentiments on a recent segment of the television news magazine "60 Minutes". Like most other health professionals, Dr. Lustig believes that since sugar supplies the body with only empty calories, devoid of nutrients, it is detrimental because Americans eat so much of it at the expense of foods with higher nutrient values. He also believes that it is how fructose is processed by the liver that can make it harmful to consume too much of it, especially in the form of sweetened beverages.

"Blocking" Calories from Sugar

Cutting back on sugar isn't easy. Anyone who has attempted a low-calorie diet knows about the challenges of reducing sugar intake. But what if there was another way to control body weight and blood sugar levels, a plan that involved controlling the body's ability to absorb and utilize sugar?

For years, researchers in the US, Japan and several other countries have been studying a substance known as L-arabinose that functions as a "sucrose blocker". L-arabinose is a simple sugar that is commonly found in plants such as corn, sugar beets, apples, etc. For many years it has been used as a food-grade material and as a precursor in pharmaceutical production.

Animal studies have shown that L-arabinose works by inhibiting the digestive enzyme sucrase, delaying the digestion and absorption of sucrose. This means that although there is an intake of sugar calories, L-arabinose prevents the sugar from being broken down so it won't be turned into fat.

In 1995, an animal study conducted by researchers in Japan and published in the journal *Metabolism* found that L-arabinose inhibited sucrase activity and thereby reduced blood sugar levels (also known as glycemic response).

In 2000, another animal study was conducted by Japanese researchers to determine the effect of L-arabinose on the ability to make fat (a process known as lipogenesis). The study, published in the *Journal of Nutrition*, found that inhibiting sucrase activity leads to reduced sucrose utilization, which in turn, helps to stop sugar from turning into fat.

In the United States, an animal study was conducted by Dr. Harry Preuss of Georgetown University Medical Center and several colleagues. The goal was to test the effectiveness of various natural sucrose and starch blockers – L-arabinose, white bean extract, and hibiscus – used separately and together. The study, published in the *International Journal of Medical Sciences*, found that L-arabinose, when used on its own, and in combination with the other ingredients, was very effective in lowering blood sugar.

Like L-arabinose, chromium is a naturally occurring element that has been proven effective in regulating insulin so that blood sugar levels are balanced. This balance ensures that blood sugar is more often used for immediate energy by the body, rather than going into fat cells for storage. Chromium is obtained from food, but some experts believe most people aren't getting enough through their diets.

In 2006, a randomized, double-blind, placebo-controlled study, published in the journal *Metabolism*, evaluated the effect and safety of chromium-containing milk powder in patients with type-2 diabetes. Results showed that subjects who took the chromium had lower fasting plasma glucose, fasting insulin, and improvement of metabolic control.

Also in 2006, a randomized, double-blind study, published in *Biological Trace Element Research*, was conducted to determine the effect of chromium-enriched yeast on blood glucose and insulin variables in persons with type-2 diabetes. Results suggest that supplementation of well-controlled type-2 diabetics with chromium-enriched yeast can result in improvements in blood glucose variables.

Pharmachem Introduces Prenulin™

After reviewing the animal studies on L-arabinose, Pharmachem researchers became very interested in studying a combination formula of L-arabinose and high-quality forms of chromium. Such a formula would combine both the glucose absorption benefits of L-arabinose and the insulin control capability of chromium. This unique formula is known as Prenulin™ Natural Glucose Support*.

Although it is an essential mineral, chromium is not easily absorbed into the body. Prenulin is available with two patented varieties of chromium to choose from. The first is Pharmachem's Food-Bound Chromium, which is a unique, patented form of pre-chelation for this hard-to-digest mineral. Food-Bound Chromium was developed using a proprietary, multi-stage fermentation process that transforms chromium, yeast and probiotics from a simple admixture into a fully enrobed, food-bound system. The safety of Food- Bound Chromium was confirmed in both acute and chronic toxicity studies, which showed no signs of toxicity.

The second option is Chromax® Chromium Picolinate from Nutrition 21. The picolinic acid in Chromax enhances the absorption and bioavailability of chromium. Chromax is designated GRAS for nutritional bars and beverages. It has been the subject of more than 40 human clinical studies with a wealth of positive findings in the area of insulin utilization for metabolic health, and features an FDA qualified health claim.

To properly study the Prenulin formulation, Pharmachem sponsored a clinical trial conducted by a research team which included Drs. Gil Kaats and Harry Preuss. In two separate studies, consumption of Prenulin was shown to significantly lower both circulating glucose and insulin levels after consumption of a 70-gram sucrose challenge, compared to placebo. The studies are described in a peer-reviewed article, "A Combination of L-arabinose and Chromium Lowers Circulating Glucose and Insulin Levels After an Acute Oral Sucrose Challenge," in the May 2011 issue of *Nutrition Journal*.

Prenulin is available for use in nutritional supplements, and as a functional ingredient for foods and beverages. Pharmachem's technical support and development teams will work closely with you to ensure Prenulin meets your specific requirements for application.

For more information on Prenulin, please contact Mitch Skop, toll-free 1-800-526-0609, 201-246-1000, cell 201-220-7137; or e-mail **sales@pharmachemlabs.com**.

*Formerly known as Phase 3 Sugar Controller.

Research Milestones

Below is a brief summary of research studies conducted on L-arabinose and Chromium, the key ingredients in Prenulin™, as well as studies on Prenulin itself.

Prenulin Clinical Studies

2011 -- Two double-blind, placebo-controlled studies were conducted to examine the effects of a formula containing l-arabinose and trivalent chromium (also known as Prenulin) on circulating glucose and insulin responses to sucrose challenge. In both studies, consumption of Prenulin was shown to significantly lower both circulating glucose and insulin levels after consumption of a 70-gram sucrose challenge, compared to placebo. ("A Combination of L-Arabinose and Chromium Lowers Circulating Glucose and Insulin Levels After an Acute Oral Sucrose Challenge," Gilbert R. Kaats, Samuel C. Keith, Patti L. Keith, et al., *Nutrition Journal*, 2011; 10:42).

2009 -- A 28-day, pilot study of 10 human subjects showed that consumption of an ingredient formula containing L-Arabinose and a patented chromium (Chromium + GPM), known as "L-A/Cr," or Prenulin, had a statistically significant inhibitory effect on sucrose of 25%. There was no evidence of short-term adverse effects or with changes in blood chemistries, body composition as measured by DEXA, or self-reported quality of life measures. ("A Pilot Study of the Effects of L-A/Cr: A Novel Combination of L-Arabinose and a Patented Chromium Supplement on Serum Glucose Levels After Sucrose Challenges," Gilbert R. Kaats, Harry Preuss, Joel E. Michalek, et al., 2009.)

L-Arabinose Studies

2007 -- This study assessed the ability of various natural substances, commonly referred to as "CHO blockers," to influence starch and sucrose absorption in vivo in ninety-six rats and two pigs. Groups of nine rats were fed water or water plus rice starch and/or sucrose; and circulating glucose was measured at timed intervals thereafter. For each variation in the protocol a total of at least nine different rats were studied with an equal number of internal controls on three different occasions. The pigs rapidly drank CHO and inhibitors in their drinking water. The results of the study support the hypothesis that the enzyme inhibitors examined at reasonable doses can safely lower the glycemic loads starch and sucrose. ("Inhibition by Natural Dietary Substances of Gastrointestinal Absorption of Starch and Sucrose in Rats and Pigs: Acute Studies," Harry Preuss, Bobby Echard, Debasis Bagchi, et al., *Int'l Journal of Medical Sciences* 2007; 4: 196-202.)

2000 -- In this animal study, rats were fed 0-30g sucrose/100g diets containing 0-1g L-arabinose/100g for 10 days. Lipogenic enzyme activities and triacylglycerol concentrations in the liver were significantly increased by dietary sucrose, and arabinose significantly prevented these increases. The results suggest-that L-arabinose inhibits intestinal sucrase activity, thereby reducing sucrose utilization, and consequently decreasing the amount of sugar that the body turns into fat. ("L-Arabinose Feeding Prevents Increases Due to Dietary Sucrose in Lipogenic Enzymes and Triacylglycerol Levels in Rats," Shigemtisu Osaki, Tomoe Kimura, Tomomi Sugimoto, et al., *Journal of Nutrition* 2001; 131: 796-799.)

1995 -- A study was conducted to investigate the effects of L-arabinose and related pentoses on the activities of intestinal alpha-glucosidases and pancreatic amylase in vitro, and to evaluate the effects of L-arabinose on glycemic responses using several experimental animals in vivo. The results showed that L-arabinose selectively inhibits intestinal sucrase activity in an incompetitive manner and suppresses the glycemic response after sucrose ingestion by inhibition of sucrase activity. ("L-Arabinose Selectively Inhibits Intestinal

Sucrase in an Uncompetitive Manner and Suppresses Glycemic Response After Sucrose Ingestion in Animals," Kenji Seri, Kazuko Sanai, Noriki Matsuo, et al., *Metabolism* 1996; 45(11): 1368-1374).

Chromax Studies

2007 -- A study was conducted to examine acute Cr absorption, based on 24 h urinary Cr values, for picolinate, two types of nicotinate, and chloride in young adult, non-overweight females. College-aged women were given 200 mg of Cr as each of the four supplement types in random order accompanied by a small standardized meal, separated by at least a week washout. Cr picolinate produced significantly higher 24 h urinary Cr than either of two nicotinate supplements or Cr chloride given in a multivitamin–mineral supplement. This difference was seen for absolute values of the urinary Cr and for percent increases. In conclusion, based on an indirect measure of acute absorption, Cr picolinate was superior to three other Cr complexes commonly sold as supplements. ("Comparison of Acute Absorption of Commercially Available Chromium Supplements," Robert A. DiSilvestro, Emily Dy, *Journal of Trace Elements in Medicine and Biology* 2007; 21: 120–124.)

2004 -- The effects of short-term Cr supplementation were studied using a randomized crossover design. Thirteen healthy men of normal body mass index performed three trials each separated by one week. Test meals, providing 75 g of available carbohydrates, consisted of white bread with added Cr (400 or 800 µg as Cr picolinate) or placebo. After the addition of 400 and 800 µg Cr incremental area under the curve (AUC) for capillary glucose was 23% ($p = 0.053$) and 20% ($p = 0.054$), respectively, lower than after the white bread meal. These differences reached significance if the subjects were divided into responders ($n = 10$) and non-responders ($n = 3$). Researchers concluded that acute chromium supplementation showed an effect on postprandial glucose metabolism in most but not all subjects. The response to Cr may be influenced by dietary patterns. ("Effects of Acute Chromium Supplementation on Postprandial Metabolism in Healthy Young Men," Marc T. Frauchiger, Caspar Wenk, Paolo C. Colombani. *Journal of the American College of Nutrition* 2004; 23:4, 351–357.)

1999 -- A double-blind, randomized, placebo-controlled trial was conducted on 29 subjects at high risk for Type 2 diabetes because of family history and obesity in order to assess the effect of chromium supplementation on insulin sensitivity and body composition. The 8-month trial used chromium picolinate (1000 µg/day) or placebo. Clinical and metabolic evaluations consisted of insulin sensitivity and glucose effectiveness; measurement of glucose tolerance and insulin response to an oral glucose tolerance test (75 g OGTT); and 24-hr glucose and insulin profiles. Abdominal fat distribution was also assessed. The CrPic group showed a significant increase in insulin sensitivity at midpoint ($P < .05$) and end of study ($P < .005$) compared with controls, which had no significant changes. CrPic significantly improved insulin sensitivity in those obese subjects with a family history of Type 2 diabetes. Improvement in insulin sensitivity without a change in body fat distribution suggests that Cr may alter insulin sensitivity independent of a change in weight or body fat percentage, thereby implying a direct effect on muscle insulin action. ("Effect of Chromium Picolinate on Insulin Sensitivity in Vivo," William Cefalu, Audrey Bell-Farrow, Jane Stegner, et al., *The Journal of Trace Elements in Experimental Medicine* 1999; 12: 71-83.)

1998 -- A randomized, double-masked, placebo-controlled study was conducted with 122 subjects who received either chromium picolinate 400 µg ($n = 62$) or placebo ($n = 60$). After controlling for differences in caloric intake and expenditure, as compared with the placebo group, subjects in the active treatment group lost significantly more weight and fat mass, and had a greater reduction in percent body fat, without any loss of fat-free mass. It was concluded that this study replicated earlier findings that supplementation with chromium picolinate can lead to significant improvements in body composition. ("A Randomized, Double-Masked, Placebo-Controlled Study of the Effects of Chromium Picolinate

Supplementation on Body Composition: A Replication and Extension of a Previous Study," Gilbert Kaats, Kenneth Blum, Dennis Pullin, et al., *Current Therapeutic Research* June 1998; 59:6, 379-388.)

Chromium Picolinate Studies

2006 -- A review was conducted of 15 clinical studies on chromium picolinate supplementation in subjects with diabetes mellitus. Twelve of the 15 studies were randomized, controlled trials. Three were open label trials. Thirteen of 15 clinical studies (including 11 randomized, controlled studies) involving a total of 1,690 subjects (1,505 in CrPic group) reported significant improvement in at least one outcome of glycemic control. All 15 studies showed salutary effects in at least one parameter of diabetes management, including dyslipidemia. Collectively, the data support the safety and therapeutic value of CrPic for the management of cholesterolemia and hyperglycemia in subjects with diabetes. ("Clinical Studies on Chromium Picolinate Supplementation in Diabetes Mellitus—A Review," C. Leigh Broadhurst and Philip Domenico, *Diabetes Technology & Therapeutics* 2006; 8(6): 677-687)

2002 -- An expert panel evaluated the product specifications of Chromax® Chromium Picolinate. They determined the safety of consumption of Chromax when used as an ingredient in food is based on scientific procedures by comparing the estimated daily intake (EDI) of trivalent chromium under the intended conditions of use of Chromax with the acceptable daily intake (ADI) of trivalent chromium derived from animal and/or human toxicity date. The panel reviewed the publicly available toxicity data on trivalent chromium, clinical efficacy studies employing chromium tripicolinate, and published chronic animal studies of other trivalent chromium compounds. They concluded that Chromax in a cumulative daily intake of no more than 600 mcg trivalent chromium is safe and GRAS by scientific procedures. ("Summary and Conclusion of the Expert Panel Regarding the Generally Recognized As Safe Status of Chromax® Chromium Picolinate as a Nutrient Supplement in Food," *Environ International Corporation*, June 2002.)

2001 -- A clinical study tested the effect of carrot juice on blood sugar. During the study researchers measured the glycemic index of carrot juice to be 86, on a scale where the glycemic index of bread is 100. The glycemic response of carrot juice was lowered to 66 by consuming oil along with the juice. Chromium was also found to be beneficial for 4 of 6 people who participated in a 1-week supplement test. Carrot juice is likely to cause fewer problems to individuals struggling to lower their blood sugar than animal fats, refined sugar, bread, and flour products. ("Let's Juice! The Glycemic Index of Carrot Juice and Controlling Blood Glucose Levels," Michael Donaldson, Hallelujah Acres Foundation.)

2000 -- A short-term study was conducted on five patients newly diagnosed with Type 2 diabetes who maintained their condition using diet alone. The patients received 400 µg/day chromium picolinate for 12 weeks. All patients showed significantly increased glucose utilization when taking chromium with a mean increase of 60% which returned to pre-supplementation levels when chromium was withdrawn. Insulin resistance calculated using a HOMA technique from fasting insulin and glucose concentrations improved significantly after 6 weeks of chromium supplementation remaining so until supplementation ceased after which IR returned toward pre-supplementation values. The results of this study indicate that chromium supplementation improves insulin sensitivity in patients with diet-controlled Type 2 diabetes comparable to that seen during treatment with thiazolidinediones. In the absence of a change in weight the likeliest explanation is a direct effect of chromium on insulin action in line with previous in vitro studies reported form our laboratory. ("Chromium Supplementation Improves Insulin Resistance in Patients with Type 2 Diabetes Mellitus," B.W. Morris, S. Koutat, R. Robinson, et al., *Diabetic Medicine* 2000; 17: 684-686.)

1999 -- A survey was conducted as a follow-up to at 1997 study involving 180 subjects with type 2 diabetes. In the initial study, supplemental chromium was shown to improve fasting glucose, post-prandial glucose, insulin, hemoglobin A1c, and cholesterol. In the follow-up survey, the fasting glucose, postprandial glucose, and diabetic symptoms of 833 people with type 2 diabetes were monitored for up to 10 months following Cr supplementation (500 μg/d Cr as chromium picolinate). Fasting and postprandial glucose improved in >90% of the subjects, and similar improvements occurred after 1-10 months. Results confirm the safety and beneficial effects of supplemental Cr and demonstrate that beneficialeffects of supplemental Cr observed in a few months are also present after 10 months. ("Follow-up Survey of People in China with Type 2 Diabetes Melllitus Consuming Supplemental Chromium," Nanzheng Cheng, Xixing Zhu, Hongli Shi, et al., *The Journal of Trace Elements in Experimental Medicine* 1999; 12: 55-60.)

1999 -- A 16-week, randomized, double-blind, placebo-controlled study was conducted with human subjects to test the hypothesis that the elevated intake of supplemental chromium is involved in the control of type 2 diabetes. Subjects being treated for type 2 diabetes (180 men and women) were divided into three groups and supplemented with: 1) placebo; 2) 100 μg Cr as chromium picolinate two times per day; or 3) 500 μg Cr two times per day. Results demonstrate that supplemental chromium had significant beneficial effects on HbA1c, glucose, insulin, and cholesterol variables in subjects with type 2 diabetes. The beneficial effects of chromium in individuals with diabetes were observed at levels higher than the upper limit of the Estimated Safe and Adequate Daily Dietary Intake. ("Elevated Intakes of Supplemental Chromium Improve Glucose and Insulin Variables in Individuals with Type 2 Diabetes," Richard A. Anderson, Nanzheng Cheng, Noella A. Bryden, et al., *Diabetes* 1997; 46: 1786-1791.)

Nutrition Journal

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A combination of l-arabinose and chromium lowers circulating glucose and insulin levels after an acute oral sucrose challenge

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A combination of l-arabinose and chromium lowers circulating glucose and insulin levels after an acute oral sucrose challenge

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ABSTRACT

Background: A growing body of research suggests that elevated circulating levels of glucose and insulin accelerate risk factors for a wide range of disorders. Low-risk interventions that could suppress glucose without raising insulin levels could offer significant long-term health benefits.

Methods: To address this issue, we conducted two sequential studies, the first with two phases. In the first phase of Study 1, baseline fasting blood glucose was measured in 20 subjects who consumed 70 grams of sucrose in water and subsequently completed capillary glucose measurements at 30, 45, 60 and 90 minutes (Control). On day-2 the same procedure was followed, but with subjects simultaneously consuming a novel formula containing l-arabinose and a trivalent patented food source of chromium (LA-Cr) (Treatment). The presence or absence of the LA-Cr was blinded to the subjects and testing technician. Comparisons of changes from baseline were made between Control and Treatment periods. In the second phase of Study 1, 10 subjects selected from the original 20 competed baseline measures of body composition (DXA), a 43-blood chemistry panel and a Quality of Life Inventory. These subjects subsequently took LA-Cr daily for 4 weeks completing daily tracking forms and repeating the baseline capillary tests at the end of each of the four weeks. In Study 2, the same procedures used in the first phase were repeated for 50 subjects, but with added circulating insulin measurements at 30 and 60 minutes from baseline.

Results: In both studies, as compared to Control, the Treatment group had significantly lower glucose responses for all four testing times (AUC=$P<0.0001$). Additionally, the Treatment was significantly more effective in lowering circulating insulin after 60 minutes from baseline

(AUC=*P*=<0.01). No adverse effects were found after acute sucrose challenge or in those who consumed LA-Cr daily for four weeks.

Conclusions: As compared to a placebo control, consumption of a LA-Cr formula after a 70-gram sucrose challenge was significantly more effective in safely lowering both circulating glucose and insulin levels.

Trial Registration: Clinical Trials.gov, NCT0110743

BACKGROUND

A growing body of research suggests that elevated circulating levels of glucose and insulin accelerate risk factors for a wide range of pathological disorders [1-4]. Accordingly, interventions with low-risk dietary supplements that suppress glucose levels without raising insulin levels could offer significant long-term health benefits [5]. Animal studies and a single clinical trial previously reported that consumption of l-arabinose (LA), a poorly-absorbed, readily-available sweet-tasting pentose sugar, led to significant suppression of the circulating glucose and insulin after sucrose challenge [6-8]. This appears to be related to l-arabinose's ability to lessen the rapid absorption of sucrose thereby preventing elevation of circulating levels of glucose and insulin [5] typically found in modern diets. Similarly, other animal and human studies have also reported suppression of circulating glucose levels without elevating insulin with the consumption of various forms of chromium (Cr) [9-13]. This appears to be related to chromium's ability to enhance insulin sensitivity. The purpose of this study was to examine the effects of a formula containing l- arabinose and trivalent chromium (LA-Cr) on circulating glucose and insulin responses to sucrose challenge.

METHODS

All subjects gave written informed consent in compliance with the Helsinki Declaration as approved by the researchers' ethics committee.

Study 1, Phase 1: A total of 20 non-diabetic subjects were enrolled in this phase from a pool of subjects who had participated in previous studies and had demonstrated a high compliance with study protocols. Their relevant characteristics are set out in Table 1. All 20 completed a DXA total body composition scan, the 50-item Quality of Life (QOL) inventory shown in Table 2 and the 43-chemistry blood test panel shown in Table 3**.** Blood chemistries were drawn at a Quest service Center of the subject's choice (www.quest.com).

On test day-1 (Control), after fasting for 10 hours, subjects completed a baseline "finger-stick" capillary blood sample, and consumed 70 grams of sugar dissolved in 150 grams of bottled water. Blood glucose levels were retested at 30, 45, 60 and 90 minutes. On test day 2 (Treatment), subjects followed the same procedure, but with a LA-Cr supplement containing 1,000 mg of l-arabinose and 200 mcg of a patented food-source chromium. All glucose measurements were obtained on-site using a glucometer (*ACCU-CHEK Aviva* meter, *ACCU-CHEK Multiclix*, and *Multiclix Pen*, Roche Diagnostics, Indianapolis IN).

For each subject and each timed test period, a change from baseline was obtained by subtracting the values for the corresponding baseline from the values of the four test periods. A glucose "suppression score" was obtained for each subject by subtracting the treatment change score from the control change score. The suppression scores were averaged over the 20 subjects and the changes from control scores to treatment scores were expressed as a percentage of control scores. Decreases were shown as negative percentages. The area under the curve (AUC) scores were obtained by using *KaleidaGraph*, *graphing and data*

analysis, Version 3.6. The AUC scores for glucose treatment periods for each subject were compared to the control AUC scores for each subject using a paired, 2-tailed t-test. Significance was defined as $P<0.05$**.**

Study 1, Phase 2: Ten of the 20 subjects were randomly chosen and asked to consume a daily serving of LA-Cr for four weeks. Subjects provided daily tracking information on adverse effects. At the conclusion of each week, subjects completed the same Treatment sucrose challenge as described in Phase 1 and repeated the Blood, DXA, and QOL inventory at the end of the 4^{th} week.

Study 2: Fifty new non-diabetic subjects were recruited, and completed the same DXA and QOL inventory used in phase 2 of Study 1. In addition to glucose measurements, fasting insulin measurements were also obtained at baseline, 30 and 60 minutes from baseline with and without simultaneous consumption of the LA-Cr supplement at baseline. Insulin measurements were performed by Quest Laboratories, San Antonio, TX. The same procedures and instruments used in the pilot study were used to obtain glucose and insulin suppression scores. Significance was defined as $P<0.05$. All 50 subjects completed the glucose measurements. The phlebotomist was unable to draw blood from one subject and accordingly 49 subjects completed the insulin tests. To examine the relationship between the total glucose suppression or the total insulin suppression and baseline factors, each suppression score was compared with each baseline factor as follows: The data were ranked in order of suppression score and separated into quartiles, with Q1 representing the most suppression and Q4 representing the least suppression. An analysis of variance (ANOVA) was conducted across the 4 quartiles. Significance was defined as $P<0.05$.

RESULTS

The data for each of the groups and each of the time periods are shown in Table 4. As shown in Table 5, consuming La-Cr simultaneously with a 70 gram sucrose challenge (treatment) suppressed the glucose response in both Study 1 and 2 in all four time measurements as compared to control. These differences were statistically significant for all four time periods. Circulating insulin levels were also statistically lower at 60 minutes in the treatment group. Although not shown, weekly reductions in glucose were essentially the same in each of the four weeks as were found for these subjects in the first phase of Study 1. In addition, no significant changes were found in comparisons between baseline and ending blood chemistries and self-reported QOL scores.

To further examine the association between suppression scores and baseline measures, glucose and insulin suppression scores were divided into four equal quartiles. An ANOVA revealed that there were no statistically significant relationships between glucose suppression scores and baseline measures of glucose, insulin, age, gender, ethnicity, scale weight, height, bone mineral density, total body fat, total body lean, and body mass index. However, there was a significant association between glucose suppression scores and % body fat (P=0.038). A further comparison of the quartiles of glucose suppression scores and % body fat revealed that the greater the suppression score, the lower the % body fat (Q4=42.5%, Q3=40.8%, Q2=40.0%, Q1=32.5%). A Student t-test between the highest (n=12) and lowest (n=12) glucose suppression quartiles was also significant (P=0.025).

DISCUSSION

This study compared the acute effects of the simultaneous ingestion of a combined l-arabinose and the trivalent chromium formulation (LA-Cr) after a 70 gram oral challenge of sucrose. Sucrose absorption was estimated by the appearance of increased levels of circulating glucose after the sucrose challenge [6]. Data from two separate studies found an 18% to 31% reduction in glucose when taking LA-Cr supplement compared to ingesting the sucrose alone. In the second study, we also found a 28% reduction in circulating insulin concentrations 60 minutes after taking the formulation. With regard to safety, other than some discomfort with the capillary measurements, no adverse effects were reported. Nor were any adverse effects reported among the 10 subjects who consumed the LA-Cr daily for the 4-week study period.

When the effects of the LA-Cr were measured weekly with the acute oral sucrose challenge, the glucose-lowering response of the combination remained over the 28-day period. Additionally, there were no significant differences between baseline and ending values on any of the 43 blood chemistries, DXA body composition measures, or the self-reported Quality of Life Inventory after using the LA-Cr daily for 28 days.

We devised our protocol with the thought that we were essentially examining the l-arabinose in the formula. Findings similar to ours have been reported in a well-controlled rat model, i.e., l-arabinose works quickly when taken prior to a sucrose challenge and continues to work effectively over a sub chronic period of time that may provide insights into the mechanisms of action. The data support the hypothesis that l-arabinose worked by blocking sucrose absorption [6,13]. In rats, l-arabinose did not influence circulating glucose levels when no sucrose, but rather saline, was given. Under these circumstances, it did not lower glucose via enhancing uptake or metabolism of glucose. Further, l-arabinose did not affect glucose

appearance when glucose replaced sucrose as the challenging sugar. Finally, *in vitro* studies have shown that l-arabinose blocks sucrase in an uncompetitive manner [14].

This was unlike effects with chromium that influence circulating glucose levels through its ability to enhance insulin sensitivity and its removal from the circulation. While chromium could have influenced the results of our sub-chronic study, it is unlikely to have done so in the acute studies since chromium does not work acutely after initial intake [8-12]. Our studies examined the product with both ingredients without partitioning the individual or interactive effects of chromium and l-arabinose.

To explore individualized reactions, we examined the association between suppression scores and baseline measures by sub-grouping glucose and insulin suppression scores into four equal quartiles. An ANOVA revealed that there were no statistically significant relationships between glucose suppression scores and baseline measures of glucose, insulin, age, gender, ethnicity, scale weight, height, bone mineral density, total body fat, total body lean, and body mass index. However, there was a significant association between glucose suppression scores and % body fat (P=0.038). A further comparison of the quartiles of glucose suppression scores and % body fat revealed that the greater the suppression score, the lower the % body fat (Q4=32.5%, Q3=40.8%, Q2=40.8%, Q1=42.5%). A Student t-test between the highest (n=12) and lowest (n=12) glucose suppression quartiles was also significant (P=0.025). This could suggest that the higher the subject's % fat, the more LA-Cr may be required to obtain the same glucose suppression results.

An ANOVA of the insulin suppression score quartiles failed to reach statistical significance on any of the baseline measures, including % body fat. However, a t-test between the highest and lowest age quartiles (Q4=33.9 yrs, Q3=40.1 yrs, Q2=40.6 yrs, Q1=46.5 yrs)

revealed a significant relationship between age and insulin suppression suggesting the insulin suppression effect may be greatest in younger people. However, the irregular pattern of Q2-Q4 calls this interpretation into question, suggesting it may be a statistical artifact as a function of the multiple ANOVA analyses conducted.

The data from these two separate studies reveal that a formula containing l-arabinose and chromium (LA-Cr) can facilitate a consistent suppression of both circulating glucose and insulin without adverse side effects. The replication of the suppressive effect observed in the two sequential studies increases the confidence of the formula's efficacy. Furthermore, the percentage of subjects for whom the supplement had at least some suppressive effect, 78% for glucose and 70% for insulin, is particularly noteworthy since we had little control over how many subjects actually fasted for the required 10 hours prior to being tested.

There is a widespread belief that we are undergoing a global "epidemic" of obesity and diabetes [14-16]. Some studies have suggested that an important contributing factor is the greater intake of rapidly absorbed or simple carbohydrates, particularly sugar [17-19]. At least one study [19] suggests that rapidly absorbed carbohydrates are more harmful than those that are more slowly absorbed, perhaps due to the difference in their effects on the glucose-insulin system. The data derived from this study suggest that it may be feasible to suppress the harmful effects of glucose and insulin associated with intake of rapid carbohydrates with a low- or no-risk dietary supplement. Since even small reductions of circulating glucose and insulin can have significant health benefits, this study suggests that longer-term and dose-related studies need to be conducted.

Abbreviations

CHO = carbohydrates, DXA = Total Body Dual-energy X-ray Absorptiometry, LA-Cr = 1.0 grams of L-arabinose and 200 μg of a patented proprietary chromium, QOL = an 86-item Quality of Life questionnaire, SG = United States Surgeon General

Competing Interests

All authors declare that they have no competing interests.

Authors Contributions

GRK was principal investigator contributing to the design of the study, supervision of the conduct of all testing and drafted and edited the final manuscript. HAP contributed to the study design, data interpretation, research and writing of the relevant scientific literature, and review and publication of the manuscript. HAC and NP reviewed the study design, manuscript and medical testing. SCK provided information technology support, acquired and maintained data, and provided audited data to the principle investigator. PLK reviewed and explained the informed consent form, enrolled and scheduled all subjects, supervised or conducted all DXA and glucose testing, administered on-line orders or provided subjects with requisitions for off-site blood testing, and edited and reviewed the manuscript. RBL aided in the interpretation of the data, the statistical analyses, and the preparation, editing and review of the final manuscript. All authors read and approved the final manuscript.

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Table 1: Baseline demographics for Study 1, Phases 1 and 2, and Study 2

	Study-1, Phase-1		Study-1, Phase-2		Study 2	
Number Subjects	**20**		**10**		**50**	
Males	**6**		**3**		**20**	
Females	**14**		**7**		**30**	
Blacks	**2**		**1**		**11**	
Hispanics	**3**		**2**		**12**	
Whites	**4**		**7**		**25**	
	0		**0**		**2**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age	**53.8**	**11.3**	**53.7**	**11.8**	**40.2**	**14.8**
Weight	**195.8**	**46.8**	**195.8**	**48.1**	**182.2**	**44.8**
Height	**67.3**	**4.1**	**67.3**	**4.2**	**65.8**	**4.4**
BMI	**29.9**	**6.0**	**30.2**	**6.1**	**29.6**	**6.0**
Bone Mineral Density	**1.189**	**0.102**	**1.189**	**0.104**	**1.202**	**0.092**
% Body Fat	**42.4%**	**7.8%**	**42.4%**	**8.1%**	**38.9%**	**9.4%**
Fat Mass	**83.0**	**22.3**	**83.0**	**22.9**	**70.9**	**28.8**
Fat-free Mass	**112.8**	**35.6**	**112.8**	**36.6**	**111.3**	**27.4**

Table 2. Quality of Life Inventory

1	Headaches	26	Irregular heartbeat
2	Irritable bowel syndrome	27	Shortness of breath
3	Arthritis	28	Constipation
4	Premenstrual syndrome	29	Stomach gas or indigestion
5	Recurring sinus infections	30	Feeling weak
6	Tension fatigue syndrome	31	Eating too rapidly
7	Recurrent anxiety	32	Eating after being full
8	Recurrent depression	33	Embarrassed about overeating
9	Insomnia	34	Depressed over eating habits
10	Low self esteem	35	Depressed about my weight
11	Binge eating	36	Difficult to stop eating
12	Chronic tension	37	Worrying about the future
13	Lack of energy	38	Unable to concentrate
14	Food allergies	39	Forgetfulness
15	Feeling under stress	40	Bad temper or quick to anger
16	Cancer	41	Indigestion
17	Prostate problems	42	Diabetes
18	Overeating	43	Vomiting
19	Stomach pain	44	Heartburn
20	Back pain	45	Esophageal reflux
21	Pain in arms, legs, or joints	46	Control over my appetite
22	Menstrual pain or problems	47	Ability to relax
23	Chest pain	48	Heart disease
24	Dizziness	49	Fibromyalgia
25	Diarrhea	50	Difficulty in falling asleep

Subjects Rated Magnitude of Problems Occurring Over the last 30 Days Using a Scale of 0=None, 1=Minor, 2=Major and 3=Severe

Table 3. The 43-Panel Blood Chemistry Test Completed by Subjects in Pilot Study, Phase-1

LIPID PANEL	CBC (INCLUDES DIFF/PLT)
TRIGLYCERIDES	WHITE BLOOD CELL COUNT
CHOLESTEROL, TOTAL	RED BLOOD CELL COUNT
HDL CHOLESTEROL	HEMOBLOBIN
LDL CHOLESTEROL	HEMATOCRIT
CHOL/HDLC RATIO	MCV
METABOLIC PANEL	MCH
GLUCOSE	MCHC
UREA NITROGEN (BUN)	RDW
CREATININE	PLATELET COUNT
BUN/CREATININE RATIO	ABSOLUTE NEUTROPHILS
SODIUM	ABSOLUTE LYMPHOCYTES
POTASSIUM	ABSOLUTE MONOCYTES
CHLORIDE	ABSOLUTE EOSINOPHILS
CARBO DIOXIDE	ABSOLUTE BASOPHILS
CALCIUM	NEUTROPHILS
PROTEIN, TOTAL	LYMPHOCYTES
ALBUMIN	MONOCYTES
GLOBULIN	EOSINOPHILS
ALBUMIN/GLOBULIN RATIO	BASOPHILS
BILIRUBIN, TOTAL	OTHER MEASURES
ALKALINE PHOSPHATASE	CARDIO CRP
AST & ALT	TSH W/REFLEX TO FT4

Table 4. Capillary Glucose and Venous Insulin Levels After a 70g Sucrose Challenge With and Without Simultaneous Consumption of LA-Cr for the Pilot and Clinical Studies

Pilot Study N=20 (Glucose Measurements Only)					
Minutes from Baseline (glucose)	:0	:30	:45	:60	:90
Mean glucose levels in the control group (sugar only)	100.4	157.9	161.3	151.4	121.5
Standard deviations of glucose in control group (sugar only)	16.1	23.8	18.5	24.7	17.0
Mean glucose levels in the treatment group	104.5	149.1	149.5	133.4	116.4
Standard deviations of glucose levels in treatment group	12.0	15.9	16.8	20.7	13.7
Clinical Study N=50 (Glucose measurements)					
Minutes from Baseline (glucose)	:0	:30	:45	:60	:90
Mean glucose levels in the control group (sugar only)	97.2	150.3	151.4	141.8	120.5
Standard deviations of glucose in control group (sugar only)	10.3	22.2	25.0	25.6	22.2
Mean glucose levels in the treatment group	99.9	142.8	140.0	133.5	116.9
Standard deviations of glucose levels in treatment group	11.5	15.8	16.8	19.1	18.8
Clinical Study N=49 Insulin measurements)					
Minutes from Baseline (insulin)	:0	:30	n/a	:60	n/a
Mean insulin levels in the control group (sugar only)	4.4	32.3		32.2	
Standard deviations of insulin in control group (sugar only)	3.9	24.7		19.9	
Mean insulin levels in the treatment group	4.4	29.0		24.4	
Standard deviations of insulin scores in thetreatment group	3.9	20.6		16.5	

Table 5: Changes from Baseline in Capillary Glucose and Venous Insulin Levels After a 70g Sucrose Challenge With and Without Simultaneous Consumption of LA-Cr for Pilot Study (N=20) and Clinical (N=50).

Pilot Study N=20 (Glucose Only)					
Minutes from Baseline	**30**	**45**	**60**	**90**	**AUC**
% Difference Between Treatment vs Control	-22.3%	-26.0%	-43.2%	-43.5%	-31.4%
Significance Levels	P<0.007	P<0.001	P<0.001	P<0.031	P<0.0001

Clinical Study N=50 (Glucose Only)					
Minutes from Baseline (Glucose)	**30**	**45**	**60**	**90**	**AUC**
% Difference Between Treatment vs Control	-19.1%	-26.1%	-24.8%	-27.1%	-18.4%
Significance Levels	P<0.01	P<0.001	P<0.01	P<0.05	P<0.0001
Clinical Study N=49 (Insulin Only)					
Minutes from Baseline (Insulin)	**30**		**60**		**AUC**
% Difference Between Treatment vs Control	-11.9%		-28.3%		-28.3%
Significance Levels	NS		P<0.001		P<0.01

***P* values are from repeated measures t-test and Ar**

A Pilot Study of the Effects of L-A/Cr: A Novel Combination of L-Arabinose and a Patented Chromium Supplement on Serum Glucose Levels After Sucrose Challenges

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Abstract

Animal and human studies have previously reported that consumption of L-Arabinose, a poorly absorbed readily available sweet-tasting pentose, and various forms of chromium (picolinate, polynicotinate, etc.) separately led to a significant suppression of serum glucose levels after a sucrose challenge. However, no studies have been reported on the effects of using both of these ingredients in a single supplement nor when using the unique form of chromium used in this study. This pilot study was conducted to provide this information and to explore the methodological challenges, potential for adverse effects and feasibility of conducting a larger clinical trial examining the safety and efficacy of a final version of the supplement.

Changes from baseline in fasting serum glucose levels were measured on 10 human subjects on 9 test days over a 35-day study period with (treatment) and without (control) consuming a novel supplement initially containing 500 mgs of L-Arabinose and 100 mcg of a patented proprietary chromium (Chromium+GPM; Pharmachem Laboratories, Kearny, NJ). The final version of the supplement, hereafter referred to as "L-A/Cr", contained 1,000 mgs of L-Arabinose + 200 mcgs of Chromium GPM per 1.1 grams. To assess safety and to explore individual differences in reaction to the supplement, all subjects completed a fasting 43-item blood chemistry test, an 84-item Quality of Life inventory, and a DEXA body composition test before and after the study period. Capillary blood samples were drawn on each of the 9 test days at baseline and 15, 30, 45, 60, 90,120 and 180 minutes from baseline and were analyzed by two different glucometers. Control group measurements were taken on day-0 and day-35 after a 70 gm sucrose challenge without L-A/Cr. Since there were no significant differences between these two control group measurements, the data were also combined into a single control group and compared with the changes occurring in the L-A/Cr treatment group.

After an initial comparison between treatment and control, all subjects consumed one daily serving of the L-A/Cr each day for 28 days and completed the 70 gm sucrose challenge with L-A/Cr on days, 7, 14, 21, and 28. The outcome measure was the increase in glucose levels from baseline. Data were analyzed using repeated measures Students *t*-tests, Area under the Curve with ANOVA using Dunnett's *t*-test, and repeated measures mixed effect linear model with no adjustment for multiple testing. In all comparisons, simultaneous consumption of L-A/Cr with sucrose consistently led to decreased glucose responses supporting its inhibitory effect. In spite of the small number of subjects (10) and irrespective of the type of statistical analysis used, the preponderance of evidence suggests that consumption of the L-A/Cr supplement has a statistically significant inhibitory effect on sucrose of ~25%. There was no evidence of short-term adverse effects or with changes in blood chemistries, body composition as measured by DEXA or self-reported quality of life when subjects consumed the product daily over 28 days. In view of the potential of the product to also reduce circulating insulin after a sucrose challenge, a future study with a larger subject sample measuring both blood glucose and insulin is strongly suggested

INTRODUCTION

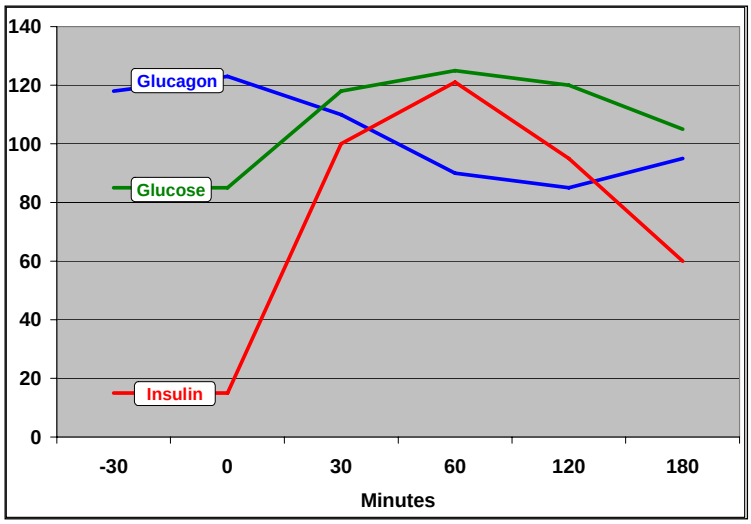
One of NIH's Office of Dietary Supplements' five scientific goals is to "Evaluate the role of dietary supplements in the prevention of disease and reduction of risk factors associated with disease" by stimulating research on:

- "…how dietary supplements moderate, alter, or enhance metabolic, physiological, and psychological processes associated with maintenance or lack of optimal health"
- "…validation of the accuracy, sensitivity, and specificity of unique biomarkers of dietary supplement effects on known endpoints and their surrogates associated with chronic diseases, optimal health, and improved performance." [1]

Poor glucose control and insulin resistance are two of the most pervasive biomarkers and end points associated with chronic diseases, diseases that affect of over 80% of Americans. Any dietary supplement that could contribute to the reduction of glucose levels and circulating insulin could make an enormous contribution to these major healthcare problems.

In view of the increasing number of diabetics and pre-diabetics throughout the world, dietary supplements that can improve glucose control and reduce circulating insulin will meet the ODS goals and could have a profound positive effect on healthcare throughout the globe. According to a recent Associated Press article the value of improved glucose control could be helpful to diabetics who are increasingly less able to purchase medication and doctor visits with the downturn in the current economy. The AP's analysis reports that doctor visits, insulin, medicines, blood-sugar testing, and drug sales have all declined due to the economy, in spite of reports that the number of diabetics and pre-diabetics continues to increase.

But benefits of glucose control extend beyond diabetes. For example, concluding that heart disease is primarily a disease of the elderly, a recent American Heart Association report [2] reported that the incidence of heart failures is 10 cases per 1,000 at age 65, and doubles every decade thereafter. People over 65 represent more than 75% of heart failures in the US [3] and in Europe, people over 70 accounted for 88.5% of heart failures [4]. A 2009 study examined the risk factors for heart failure, [5] reporting that among the 21 risk factors studied, the three strongest predictors of heart failure (P<0.001), were fasting glucose levels, blood pressure and diagnosed hypertension. Their findings confirm a 2008 study that found that assessment of fasting glucose levels and blood pressure were more predictive of mortality in older men and women than metabolic syndrome [6] and a 2008 study that led to inclusion of fasting glucose as a component of the Health ABC Heart Failure Score [7]. In fact, as one writer concludes, dietary fat is not a cause of obesity, heart disease, or any other chronic disease of civilization [8], but rather it is the effects of simple carbohydrates, particularly sugars, that ultimately lead to the storage of fat and the problems with insulin resistance. The diagram below describes the relationship between **glucose**, **insulin** and **glucagon**, demonstrating there is little change during initial 30 minute fasting period. However, when this person



consumes a food that contains high levels of sucrose or sugar, the sugar is quickly converted into blood **glucose** which begins to rise sharply and continue to rise for about an hour. The pancreas detects this sudden rise to an unhealthy level and starts a "counter-attack" by excreting **insulin** and **glucagon** into the blood stream. When **insulin** levels rise, the body stops burning stored fat and burns circulating **glucose**. As **glucagon** levels drop, the liver converts stored glycogen into **glucose** and releases it into the bloodstream. The action of **glucagon** is thus opposite to that of **insulin,** which instructs the body's cells to take in **glucose** from the blood in times of satiation. The net result of these changes is that until **glucose** levels return to normal, the body becomes less efficient at burning stored fat and more efficient at storing fat.

L-Arabinose (LA). L-A is one dietary supplement that has the potential for improved glucose control. L-A is a poorly absorbed, readily available sweet-tasting monosaccharides pentose. Monosaccharides (from Greek monos: single, sacchar: sugar) are the most basic unit of carbohydrates. They are the simplest form of sugar and are usually colorless, water-soluble, crystalline solids. Some monosaccharides have a sweet taste. Examples of monosaccharides include glucose (dextrose), fructose (levulose), galactose, xylose and ribose. Monosaccharides are the building blocks of disaccharides such as sucrose and polysaccharides (such as cellulose and starch.) Further, each carbon atom that supports a hydroxyl group (except for the first and last) is chiral, giving rise to a number of isomeric forms all with the same chemical formula. For instance, galactose and glucose are both aldohexoses, but have different chemical and physical properties. Animal [9-11] and human studies have reported that consumption of L-A led to a significant suppression of serum glucose levels after a sucrose challenge. One of the studies [9] reported that L-A reduced adipose tissue. In one of the human studies [12] it was found that L-A attenuated sucrose-induced hyperglycemia in diabetic and non-diabetic subjects when the L-A was consumed in conjunction with 30 grams of sucrose. Glucose measurements were taken 30, 60, 90 and 120 minutes after the challenge. The effect appears to have peaked 30 minutes after the challenge, dissipated after 60 minutes and was completely absent after 120 minutes when both groups' glucose levels returned to baseline.

Chromium+GPM (CG). Chromium (Cr) is an essential trace element required for normal carbohydrate, protein and fat metabolism [13]. Some studies have reported that different forms of supplemental dietary Cr have improved glucose control [14-16] while others have not found this effect [17]. It may be that individuals with impaired glucose tolerance may benefit more from acute Cr supplementation than people with normal glucose tolerance [16], a finding that has been reported for three month supplementation with Cr [18]. If, in fact, the beneficial effects of Cr supplementation are associated with impaired glucose control, Cr could make a significant contribution to the obesity epidemic, since virtually all morbidly obese adults have impaired glucose tolerance and 36% of people with impaired glucose tolerance will progress to type 2 diabetes within 10 years [19] and glucose concentration is significantly correlated with the BMI [20].

This study used a unique form of Cr, Chromium+GPM (CG) reported by the manufacturer to improve the absorption and bioavailability through the use of a proprietary blend of sacchromyces cervisiae yeast and probiotics bifidobacterium bidifum and lactobacillus acidophillus. The manufacturer also reports that acute and chronic LD-50 toxicity studies support the clinical safety of CG.

A consistent finding in these and other studies suggests that supplementation with Cr and LA appear to have positive effects on insulin levels as well as contributing to improve glucose control suggesting that a combination of the two could magnify the positive effects of either nutrient alone. It was for this reason that the supplement in this study was a combination of both LA and CG.

RESEARCH DESIGN AND METHODS

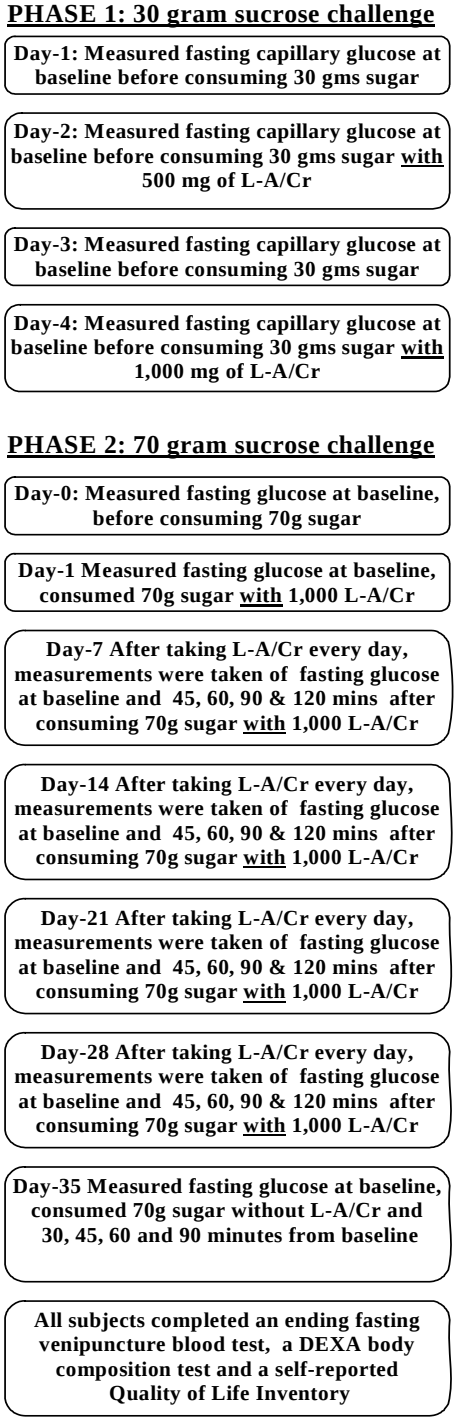
Objective

This pilot study was conducted to examine the feasibility of conducting a larger clinical trial on the extent to which L-A/Cr could reduce capillary glucose levels after consuming 30 and 70 grams of sucrose (cane sugar.) Assessment of product safety was completed by examining the effects of the supplement on 43

blood chemistries, self-reported quality of life and DEXA-derived body composition after consuming one serving of the product over a 28-day study period. A secondary objective was to examine the association between changes in glucose levels as a function of individual differences in the three safety measures.

Design/Method

Ten non-diabetic adults, aged 35-69, 3 males (avg. % fat=29.7%) and 7 females (avg. % fat =41.8%, gave informed consent, completed a fasting venous 43-chemistry blood test, DEXA total body composition test, and a Quality of Life (QOL) inventory at baseline and the end of the study. With the exception of fasting for 12 hours for each of the testing days, no restrictions were made with regard to subjects' diet and exercise activities. To avoid concerns about a potential influence from unpaid funds, all funds were provided before grantor was provided with test results. A flow diagram of the study design is shown below.



After fasting for 12 hours, a fasting capillary blood finger stick sample were obtained and measured using two different glucometers to provide two baseline measurements from the same blood sample. Immediately after the baseline measurement, subjects consumed either 30 or 70 grams of pure cane sugar with or with taking either 500 mg version of L-A/Cr or a 1,000 mg version) as shown in the flow diagram above. Thus, each subject generated seven "change from baseline scores" scores when taking 30 grams of sugar or 70 grams of sugar. We then repeated the procedure except having each subject take either 500 or 1,000 mg versions simultaneously with the sugar. To calculate the benefits (or the inhibitory effect) of L-A/Cr, we subtracted the change from baseline scores for each subject with and without taking L-A/Cr. These scores were then combined, an average for each time period was calculated and the % difference with and without L-A.

Outcome Measures

For each test, a single capillary (finger-stick) fasting glucose measurements was obtained from each subject and subsequently analyzed using two different glucometers thus providing two measurements at baseline and for each subsequent period. Using the subject's two baseline glucose measurements, all subsequent measurements were subtracted from the baseline measurement to obtain two "change from baseline" measures for each time period as shown in the example in Table 1.

Table 1. Example of Change from Baseline Outcome Measurements

	Capillary (Finger-stick) Measurements							
	Baseline	**15 mins**	**30 mins**	**45 mins**	**60 mins**	**90 mins**	**120 mins**	**180 mins**
Test 1	**100**	**125**	**140**	**150**	**135**	**110**	**95**	**90**
Test 2	**105**	**120**	**145**	**148**	**139**	**115**	**100**	**95**
				Change from baseline calculations				
Change 1	**N/A**	**125**	**140**	**150**	**135**	**110**	**95**	**90**
Change 2	**N/A**	**120**	**145**	**148**	**139**	**115**	**100**	**95**

Statistical Analyses

For each test, changes and cumulative changes from baseline were calculated for each testing time period. For cumulative, the 30-minute change was the average of all changes scores from baseline to 30 minutes, the 45-minute change was the average from baseline to 45 minutes, etc. The data were analyzed using the area under the curve (AUC) and an ANOVA using Dunnett's tests. Additional analyses were conducted using a repeated measures mixed effects linear model with no adjustment for multiple testing

RESULTS

A summary of the data for each testing day and time is Table 2 below. All calculations are based on cumulative changes from baseline using two measurements obtained for each subject for each testing time. Thus, the mean shown for minute 30 is the mean of all cumulative changes from the baseline measurement baseline through 30 minutes, through 45 minutes, etc. Day 35 (line 29) shows the results of a second 70gm control compared to the results of the first 70gm control, i.e. line 29 with line 1).

Table 2. Comparisons of Glucose Changes From Baseline After Sucrose Challenges With and Without L-A/Cr

Ref	Times of Measurements	15	30	45	60	90	120	180
1	30gm Sucrose Control	26.1	29.9	27.2				
2	500mg L-A/Cr	16.5	25.2	25.4				
3	Difference	-9.6	-4.7	-1.9				
4	% diff	-36.8%	-15.7%	-6.8%	0%	0%	0%	0%
5	Times of Measurements	15	30	45	60	90	120	180
6	30gm Sucrose Control	25.1	34.3	33.1	28.4	23.5	18.5	14.9
7	1,000 L-A/Cr	21.8	29.2	29.8	25.4	20.6	15.5	12.5
8	Difference	-3.3	-5.2	-3.3	-3.0	-3.0	-3.1	-2.4
9	% diff	-13.2%	-15.0%	-9.8%	-10.5%	-12.6%	-16.5%	-16.2%
10	Times of Measurements	15	30	45	60	90	120	180
11	Day-0: 70gm Sucrose Control	30.8	44.1	50.0	50.3	45.0	36.8	30.3
12	Day-1: 1,000 mg L-A/Cr	26.0	35.3	38.6	36.2	31.3	25.1	20.0
13	Difference	-4.8	-8.8	-11.5	-14.1	-13.7	-11.7	-10.3
14	% diff	-15.4%	-19.9%	-22.9%	-28.1%	-30.5%	-31.8%	-34.1%
15	Day-7: 1,000 mg L-A/Cr	n/a	n/a	51.3	46.8	35.1	26.2	19.6
16	Difference	n/a	n/a	-10.6	-9.6	-10.4	-7.0	-5.2
17	% diff	n/a	n/a	-17.1%	-17.0%	-22.9%	-21.1%	-20.8%
18	Day-14: 1,000 mg L-A/Cr	n/a	n/a	46.9	43.0	30.9	21.0	14.4
19	Difference	n/a	n/a	-15.0	-13.4	-14.7	-12.2	-10.4
20	% diff	n/a	n/a	-24.3%	-23.8%	-32.2%	-36.8%	-42.1%
22	Day-21: 1,000 mg L-A/Cr	n/a	n/a	52.6	46.9	37.5	28.0	20.7
23	Difference	n/a	n/a	-9.3	-9.6	-8.0	-5.2	-4.1
24	% diff	n/a	n/a	-15.0%	-16.9%	-17.6%	-15.6%	-16.5%
25	Day-28: 1,000 mg L-A/Cr	n/a	n/a	53.8	49.0	38.1	27.9	20.1
26	Difference	n/a	n/a	-8.1	-7.4	-7.5	-5.3	-4.7
27	% diff	n/a	n/a	-13.1%	-13.1%	-16.4%	-15.9%	-18.9%
28	Times of Measurements	15	30	45	60	90	120	180
29	Day-35: 70gm Control	n/a	n/a	54.6	55.0	49.4	38.3	28.9
30	Difference (Day-0 vs Day-35)	n/a	n/a	-4.6	-4.7	-4.4	-1.4	1.5
31	% diff	n/a	n/a	-9.1%	-9.4%	-9.8%	-3.9%	4.8%

AUC analyses of the effects of a 30gm sucrose challenge (Fig 1) and a 70 gm sucrose challenge (Fig 2) with and without consuming the 1,000 L-A/Cr supplement are shown above. The trending *P* value for the 30gm challenge was based on an ANOVA using the Dunnett's *t*-test. Although this trending difference failed to reach statistical significance in this pilot study, using these same data to project the results of a 50-subject clinical trial would change the *P* value from $P = 0.0959$ to $P = 0.00093$.

The AUC analysis in Fig 2 reveals that L-A/Cr had a highly significant effect, $P = 0.014$, on a 70 gm sucrose challenge using the ANOVA and Dunnet's *t*-test. Using these same data to project an estimated *P* value for a 50-subject clinical trial would change the *P* value from 0.01355 to $P < 0.0001$.

Fig 1. Area Under the Curve Analysis of the Difference Between Capillary Glucose Changes From Baseline After a 30gm Sucrose Challenge With and Without Taking L-A/Cr

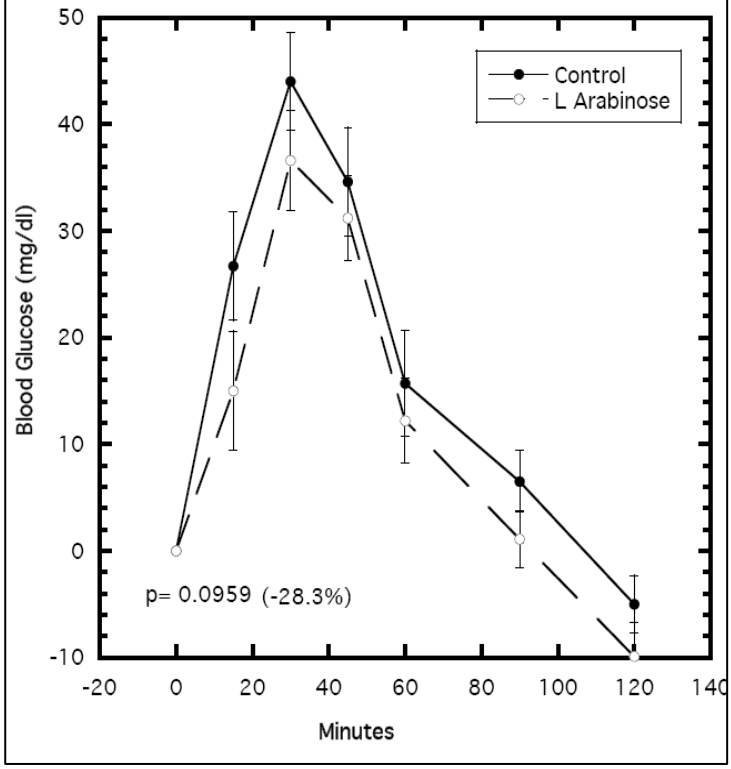


Fig 2. Area Under the Curve Analysis of the Difference Between Capillary Glucose Changes From Baseline After a 70gm Sucrose Challenge With and Without Taking L-A/Cr

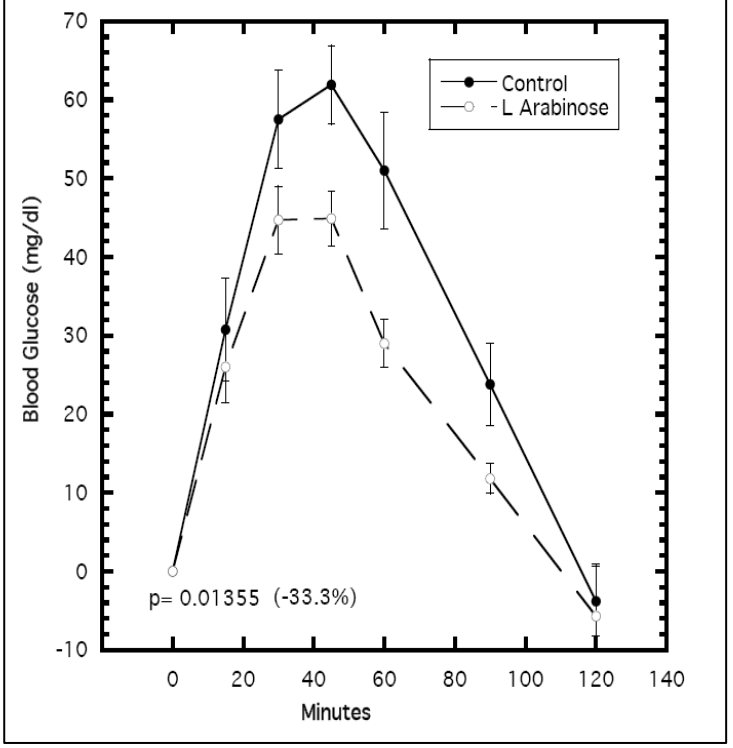


Fig 3 shows the AUC comparisons between the control group without L-A/Cr and the five treatment groups with L-A/Cr.

Fig 3. Area Under the Curve Analyses Comparing Responses of 10 Subjects to a 70gm Sucrose Challenge With (Treatment) and Without (Control) Simultaneous Consumption

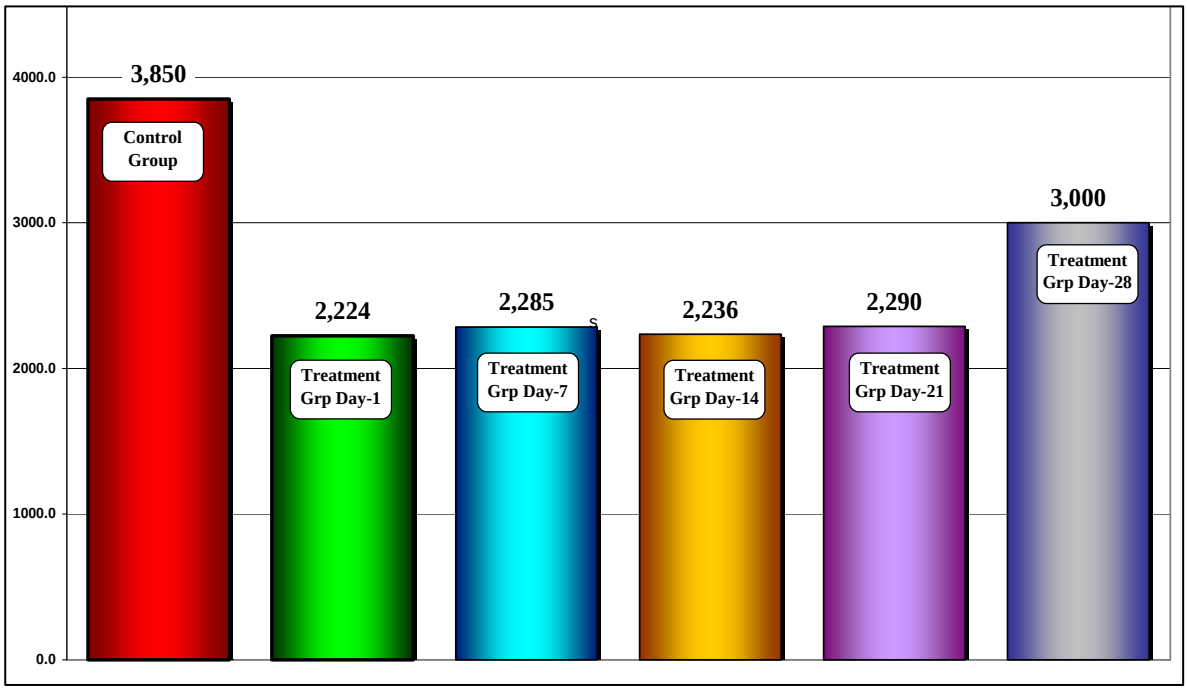


Fig 4 shows the % difference between the control group and the treatment group calculated from the cumulative change from baseline to 90 minutes from baseline.

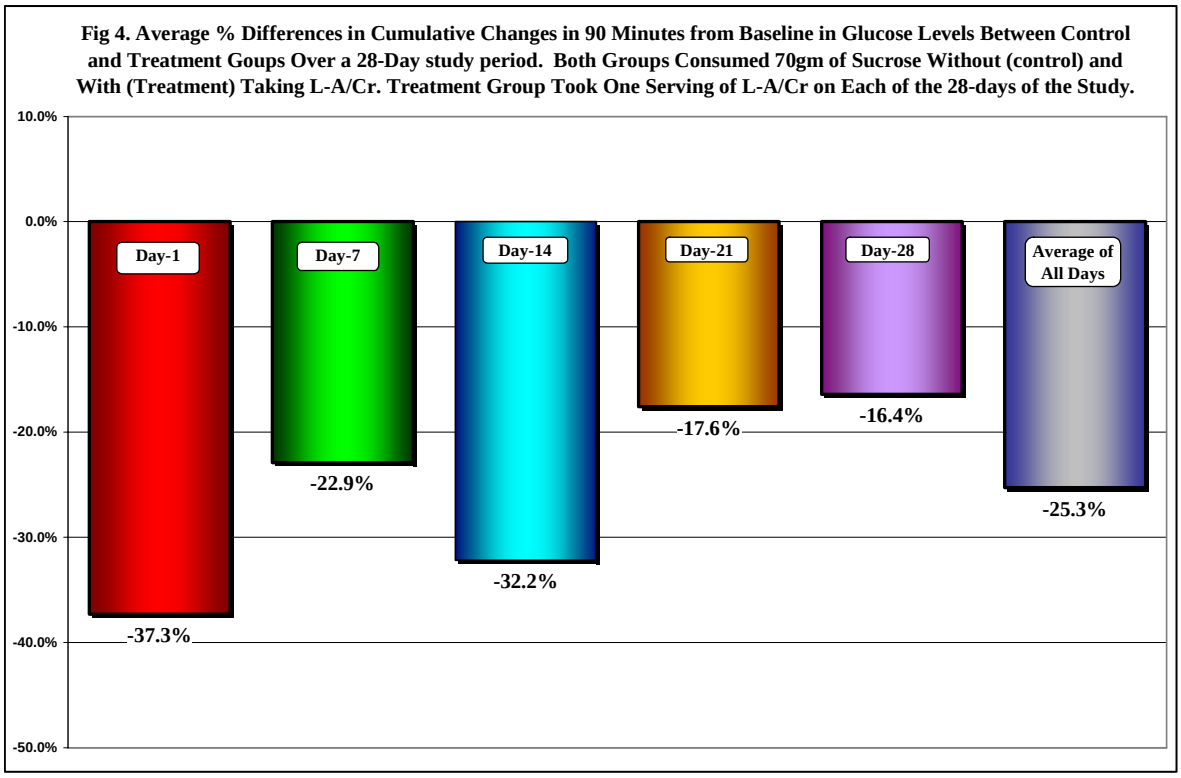


Table 3 shows a comparison between the control group who took 70 gm of sucrose with and without the L-A/Cr using a repeated measures mixed effects linear model with no adjustment for multiple testing.

Table 3. Glucose change[1] by treatment and minute in which subjects in the Control group received a 70gm sucrose challenge and those in the Treated group received the same 70gm challenge while simultaneously consuming L-A/Cr

Minute		Control (70gm)	Treated (L-A/Cr)	p-value[2]
30	N	20	20	0.14
	Mean(SD)	54.2 (25.4)	44.7 (14)	
	Median	54	47	
	Range	4 ,93	10 ,68	
45	N	20	20	0.01
	Mean(SD)	61.9 (16.1)	45.1 (11.5)	
	Median	60	43	
	Range	34 ,91	23 ,69	
60	N	20	20	<0.001
	Mean(SD)	51 (23.4)	29 (12.7)	
	Median	47.5	25.5	
	Range	22 ,109	15 ,68	
90	N	20	20	0.06
	Mean(SD)	24 (17.1)	11.9 (6.8)	
	Median	23	11.5	
	Range	-5 ,61	1 ,25	
All	N	80	80	<0.001
	Mean(SD)	47.7 (25)	32.6 (17.8)	
	Median	47.5	33.5	
	Range	-5 ,109	1 ,69	

1. Value at minute 30, 45, 60 or 90 minus the value at minute 0
2. From a repeated measures mixed effects linear model with no adjustment for multiple testing

Table 4 shows a comparison between combining data from the two control groups taking 70gm of sucrose with and without the L-A/Cr using a repeated measures mixed effects linear model with no adjustment for multiple testing

Table 4. Glucose change[1] by treatment and minute combining data from two control groups, 0 and 35, ("Control") in which subjects received a 70gm sucrose challenge. The "Treated" group consumed the same 70gm sucrose challenge, but simultaneously consumed L-A/Cr.

Minute		Control (70 gm)	Treated (L-A/Cr)	p-value[2]
30	N	40	20	0.03
	Mean(SD)	54.4 (19.3)	44.7 (14)	
	Median	55.5	47	
	Range	4 ,93	10 ,68	
45	N	40	20	0.02
	Mean(SD)	58.6 (23.9)	45.1 (11.5)	
	Median	60	43	
	Range	9 ,101	23 ,69	
60	N	40	20	0.01
	Mean(SD)	44.6 (25.7)	29 (12.7)	
	Median	42	25.5	
	Range	-7 ,109	15 ,68	
90	N	40	20	0.65
	Mean(SD)	14.5 (21.1)	11.9 (6.8)	
	Median	15	11.5	
	Range	-37 ,61	1 ,25	
All	N	160	80	0.003
	Mean(SD)	43 (28.3)	32.6 (17.8)	
	Median	45.5	33.5	
	Range	-37 ,109	1 ,69	

1. Value at minute 30, 45, 60 or 90 minus the value at minute 0
2. From a repeated measures mixed effects linear model with no adjustment for multiple testing

Table 5. Pre- and Post-study Blood Tests

Chemistry	Pre	Post	Chg	P Value
LIPID PANEL				
TRIGLYCERIDES	107.2	115.8	8.6	0.624
CHOLESTEROL, TOTAL	174.1	178.7	4.6	0.474
HDL CHOLESTEROL	57.1	56.6	-0.5	0.865
LDL CHOLESTEROL	95.5	98.8	3.3	0.271
CHOL/HDLC RATIO	3.2	3.3	0.1	0.223
METABOLIC PANEL				
GLUCOSE	94.5	92.2	-2.3	0.389
UREA NITROGEN (BUN)	14.6	14.5	-0.1	0.914
CREATININE	0.9	0.9	0.0	0.356
SODIUM	140.3	139.7	-0.6	0.489
POTASSIUM	4.3	4.2	-0.1	0.211
CHLORIDE	24.4	24.4	0.0	1.000
CARBO DIOXIDE	105.0	105.4	0.4	0.606
CALCIUM	9.4	9.4	0.0	0.739
PROTEIN, TOTAL	7.3	7.2	-0.1	0.458
ALBUMIN	4.4	4.4	0.0	0.394
GLOBULIN	2.9	2.8	-0.1	0.544
ALBUMIN/GLOBULIN RATIO	1.5	1.6	0.0	0.591
BILIRUBIN, TOTAL	0.7	0.7	0.0	0.555
ALKALINE PHOSPHATASE	81	78	-3.0	0.301
AST	18.4	16.3	-2.1	0.384
ALT	17.2	18.1	0.9	0.732
CBC (INCLUDES DIFF/PLT)				
WHITE BLOOD CELL COUNT	6.4	6.7	0.3	0.659
RED BLOOD CELL COUNT	4.6	4.5	-0.1	0.037
HEMOBLOBIN	13.8	13.6	-0.2	0.173
HEMATOCRIT	41.1	40.2	-0.9	0.110
MCV	89.1	89.9	0.8	0.195
MCH	33.7	34.0	0.3	0.261
MCHC	30.0	30.6	0.5	0.016
RDW	14.0	14.1	0.1	0.645
PLATELET COUNT	251.8	247.6	-4.2	0.334
ABSOLUTE NEUTROPHILS	3728	4117	390	0.578
ABSOLUTE LYMPHOCYTES	2019	1983	-36	0.734
ABSOLUTE MONOCYTES	452.0	442.1	-9.9	0.760
ABSOLUTE EOSINOPHILS	145.3	155.7	10.4	0.637
ABSOLUTE BASOPHILS	36.0	31.6	-4.4	0.503
NEUTROPHILS	57.9	59.7	1.8	0.503
LYMPHOCYTES	32.1	30.8	-1.3	0.560
MONOCYTES	7.1	6.7	-0.4	0.280
EOSINOPHILS	2.4	2.4	0.0	1.000
BASOPHILS	0.6	0.5	-0.1	0.475
CARDIO CRP	5.5	3.1	-2.4	0.196
TSH W/REFLEX TO FT4	2.0	2.2	0.3	0.163
HEMOGLOBIN A1C	5.6	5.6	0.1	0.177

As shown in Table 5, the only significant changes in the blood chemistries were in red blood cell count (P=0.037) and MCHC (P=0.016), neither of which is clinically significant nor did either exceed the normal ranges.

DISCUSSION

This pilot study was designed to explore the methodological challenges, potential for adverse effects and feasibility of conducting a larger clinical trial examining the safety and efficacy of L-A/Cr. Comparisons were made between taking either a 30 or 70 gram sucrose challenge with or without a 500 or 1,000 mg version of L-A/Cr. Altogether, seven different comparisons were made between the different challenges and amounts of the supplement as shown in the figures above. In all instances, notwithstanding statistical significance, changes in glucose levels when taking the L-A/Cr supplement were less than the changes in the control groups. This level of consistency over seven trials is rather remarkable and rarely observed in pilot studies with only 10 subjects. In spite of the small number of subjects and the type of statistical analysis used, (AUC, ANOVA, or repeated measurements mixed effects linear model with no adjustments for repeated measures), the preponderance of evidence suggests that consumption of the L-A/Cr supplement has an inhibitory effect on sucrase. Using changes from baseline data, L-A/Cr reduced capillary glucose levels over 25%. With regard to safety, no adverse effects other than some discomfort with the finger-sticks were reported on any of the test days, nor during the 28 day period when subjects took one serving of the supplement on a daily basis. Additionally, there were no significant differences between baseline and ending measurements on any of the 43 blood chemistries measured, the DEXA body composition test, or the self-reported Quality of Life Inventory.

Mechanism of Action

Much of the benefit of lowered glucose seems to be negated by increasing insulin resistance since the body progressively adapts to higher and higher levels of insulin. Continually revved up insulin production slowly dulls the body's response to insulin. As a result, blood sugar levels start to creep up, setting the stage for diabetes-associated complications such as blindness, stroke and renal failure. To make matters worse, chronically elevated blood sugar concentrations exacerbate insulin resistance. Over time, it takes more and more insulin to get the same lowering effect on blood glucose, much like an addiction process. As the body becomes more and more resistant to the effects of insulin, more and more insulin is needed for controlling blood glucose levels. And, the longer this insulin remains in the blood stream, the less effective the body becomes at burning stored fat, and the more effective the body becomes at storing the fat.

As one reviewer concludes, [8] type-2 diabetes are more likely the result of consumption of simple carbohydrates, particularly sugar and its effect on insulin secretion, and thus the hormonal regulation of homeostasis—the entire harmonic ensemble of the human body. Insulin is the primary regulator of fat storage, this reviewer concludes, and when insulin levels rise, fat is accumulated in fat tissue, when insulin levels fall, fat is released and used for fuel.

The vicious circle gets rolling, researchers at the Salk Institute for Biological Studies discovered, [21] when out-of-control blood sugar levels disable the molecular switch that normally shuts off sugar production in the liver in response to rising levels of insulin. Their findings suggest that appropriate inhibitors of the enzymatic pathway that blocks the "sugar-off"-switch might be useful in lowering glucose levels in diabetic individuals and reducing long-term complications associated with the disease. The human body can switch between different types of fuel: during the day the body mostly burns glucose, and during the night or prolonged fasting, it burns primarily fat. Three years ago these researchers discovered a "fasting switch" called CRTC2 that flips on glucose production in the liver when blood glucose levels run low during the night. After a meal, the hormone insulin normally shuts down CRTC2 ensuring that blood sugar levels don't rise too high. In many patients with type II diabetes, however, CRTC2 no longer responds to rising insulin levels and as a result the liver acts like a sugar factory on overtime, churning out

glucose throughout the day, even when blood sugar levels are high. In addition to diabetes, women with the highest insulin levels were found to be nearly 50 % more likely to have developed breast cancer compared with women who had the lowest insulin levels. When the researchers controlled for insulin, the association between obesity and breast cancer became much weaker suggesting that insulin levels may mediate a major component of the obesity-cancer relationship.

Recent studies [22-24] conducted at Columbia University's Medical Center suggests that maintaining blood sugar levels, even in the absence of disease, may be an important strategy for preserving cognitive health and memory. The Columbia research suggests failing memory could be blamed, at least in part, on rising blood glucose levels as we age. The researchers suggest that even for people without diabetes, blood glucose levels tend to rise as we grow older reports that the research suggests that improving glucose metabolism could help some of us avert the cognitive slide that occurs with aging.

If validated on a larger clinical trial, L-A/Cr could make a substantial contribution to healthcare throughout the world in view of the association between impaired glucose control and multiple disease risks, particularly with the marked increase in type 2 diabetes in the United States [25] and globally [26]. Type 2 diabetics have almost twice the risk of death from cardiovascular diseases compared to non-diabetics [27], and diabetic retinopathy occurs in more than 60% of diabetics who have had the disease for 20 years [28]. However, the methods by which glucose is reduced, not the simple reduction of glucose, can obviate or even reverse the benefits of glucose reduction. For example, the NIH recently discontinued a glucose-lowering study after 3.5 years when the study unexpectedly found an increased death rate in subjects randomized to intensive treatment of high blood glucose levels. A recent reviewer concludes that "…the results of these trials were predictable. For almost 40 years there has been evidence that intensive lowering of glucose levels in patients with type 2 diabetes mellitus (DM) can lead to significant harm and has limited benefits" [29]. This reviewer reports that the pharmaceutical companies who sold glucose-lowering drugs so aggressively promoted these drugs that it took a special review committee convened by the NIH to set the record straight. He also reports that, although two groups being treated with different amounts of insulin were continued in the study, while there were no suggestions that the insulin led to increased macro- or micro-vascular complications, there was also no evidence that it led to improved these conditions. Notwithstanding these results, a recent review in JAMA (April 15, 2009) concludes the glucose control in diabetics is still worthwhile and worth pursuing stating "…most importantly, glucose control must still be undertaken…reaffirming the need to achieve optimal glycemic control." [30].

In view of the above, it could be that optimal glycemic control can best achieved by lowering glucose without a corresponding increase in insulin, or, ideally, by simultaneously lowering insulin levels. Since some preliminary evidence suggests that L-Arabinose and chromium may both lower insulin levels, the combination of the two offered by L-A/Cr underscores the importance of conducting further research with a larger subject sample and with corresponding measurement of insulin levels.

CONCLUSIONS

This pilot study was designed to explore the methodological challenges, potential for adverse effects and feasibility of conducting a larger clinical trial examining the safety and efficacy of L-A/Cr. Multiple comparisons were made between changes in glucose from baseline after different sucrose challenges and two amounts of L-A/Cr. In all comparisons, simultaneous consumption of L-A/Cr with sucrose consistently led to decreased glucose responses supporting its inhibitory effect. In spite of the small number of subjects (10) and irrespective of the type of statistical analysis used, the preponderance of evidence suggests that consumption of the L-A/Cr supplement has a statistically significant inhibitory effect on sucrose of ~25%. There was no evidence of short-term adverse effects or with changes in blood chemistries, body composition as measured by DEXA or self-reported quality of life when subjects consumed the product daily over 28 days. In view of the potential of the product to also reduce circulating insulin after a sucrose challenge, a future study with a larger subject sample measuring both blood glucose and insulin is strongly suggested.

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Research Paper

Inhibition by Natural Dietary Substances of Gastrointestinal Absorption of Starch and Sucrose in Rats and Pigs: 1. Acute Studies

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Rapid gastrointestinal absorption of refined carbohydrates (CHO) is linked to perturbed glucose-insulin metabolism that is, in turn, associated with many chronic health disorders. We assessed the ability of various natural substances, commonly referred to as "CHO blockers," to influence starch and sucrose absorption *in vivo* in ninety-six rats and two pigs. These natural enzyme inhibitors of amylase/sucrase reportedly lessen breakdown of starches and sucrose in the gastrointestinal tract, limiting their absorption. To estimate absorption, groups of nine SD rats were gavaged with water or water plus rice starch and/or sucrose; and circulating glucose was measured at timed intervals thereafter. For each variation in the protocol a total of at least nine different rats were studied with an equal number of internal controls on three different occasions. The pigs rapidly drank CHO and inhibitors in their drinking water. In rats, glucose elevations above baseline over four hours following rice starch challenge as estimated by area-under-curve (AUC) were 40%, 27%, and 85% of their internal control after ingesting bean extract, hibiscus extract, and l-arabinose respectively in addition to the rice starch. The former two were significantly different from control. L-Arabinose virtually eliminated the rising circulating glucose levels after sucrose challenge, whereas hibiscus and bean extracts were associated with lesser decreases than l-arabinose that were still significantly lower than control. The glucose elevations above baseline over four hours in rats receiving sucrose (AUC) were 51%, 43% and 2% of control for bean extract, hibiscus extract, and L-arabinose, respectively. Evidence for dose-response of bean and hibiscus extracts is reported. Giving the natural substances minus CHO challenge caused no significant changes in circulating glucose concentrations, indicating no major effects on overall metabolism. A formula combining these natural products significantly decreased both starch and sucrose absorption, even when the CHO were given simultaneously. These results support the hypothesis that the enzyme inhibitors examined here at reasonable doses can safely lower the glycemic loads starch and sucrose.

Key words: starch blockers, bean and hibiscus extracts, sucrose blockers, L-arabinose, hibiscus extract

1. INTRODUCTION

The overweight state and obesity are now recognized as attaining epidemic proportions in the United States and throughout the world [1-5]. Although the potential for excess fat accumulation and perturbed metabolism from ingesting diets high in refined CHO content has been recognized for many years [6-9], it is only recently that the general public, medical community, and food industry have taken this possibility to heart [10-12]. Seeking a remedy, many of the afflicted have turned to caloric-restricted diets proportionately low in refined carbohydrates (CHO) [13-15]. Some individuals have successfully lost weight on "low carb diets," others are not prepared to accept this life style change. Issues ranging from the wisdom of replacing CHO with fat to the palatability of a diet severely depleted in CHO have led to procrastination. Accordingly, continual emergence of data supporting a positive correlation between excess refined CHO intake and obesity has made many investigators seek more practical means to duplicate results found with the stringent depletion of CHO in the diet. One alternative is to reduce the rapid gastrointestinal absorption of CHO in a manner similar to reports of decreased fat absorption with various fibers [16-18].

Numerous natural dietary substances possess inhibitory effects on enzymes that influence CHO absorption in the gastrointestinal tract -- the theory being that ingested starches and sucrose not broken down into smaller units will pass through the small intestines instead of being reabsorbed. Subsequently, the unabsorbed CHO are fermented distally by intestinal microbiota that can lead to a multitude of effects – some that may be beneficial toward body fat loss [19]. While the approach seems simple, what appears to be a sound hypothesis remains an elusive one to prove. Conclusive, difficult-to-refute results concerning the inhibitory and/or hypoglycemic effects of natural constituents such as bean extract, hibiscus extract, and L-arabinose are limited. Bean and hibiscus

extracts have been reported to inhibit amylase [20-25], while L-arabinose inhibits sucrase [26-28].

The major purpose of the present study is to examine the potential of certain natural substances alone and combined in a formula to decrease or at least slow the gastrointestinal absorption of CHO. As a first approximation, we examined the ability of three natural ingredients known to inhibit amylase and/or sucrase – bean extract, hibiscus extract, and L-arabinose, as well as a formulation containing these three ingredients to influence starch and sucrose absorption in Sprague-Dawley rats.

2. METHODS AND PROCEDURES

Animals:

The Animal Welfare Board at Georgetown University Medical Center approved the protocol for the investigation. Ninety-six male Sprague-Dawley rats (SD) were obtained from Taconic Laboratories (Germantown, NY). Rats ate regular rodent chow and drank water *ad libitum* and were maintained in a facility with constant temperature and a 12 hour light-dark phase. Adult rats, obtained at varying times, weighed between 344-442 grams at the start of this acute study. Two Yorkshire pigs, initially weighing approximately 20 Kg, were obtained from Thomas D. Morris, Inc., Reisterstown, MD and were allowed free access to food and water.

Protocols:

In the studies, there were two variables. The first variable was the oral CHO challenge that consisted of no CHO (control), rice starch, sucrose, or combined rice starch and sucrose. The second factor was the potential blocker to be examined such as bean extract, hibiscus extract, L-arabinose, or a formula containing these three ingredients.[1]

Rats were deprived of food the night before each testing (approximately 17 h). A baseline blood was then drawn. One half hour prior to the CHO challenged, SD were gavaged with either two ml of water alone of two ml of water containing the inhibitor(s), i.e., 0.5 grams of each ingredient(s) (bean and hibiscus extracts, L-arabinose, and the formulation described below) were given. At the moment of CHO challenge, rats again received either a gavage of two milliliters of water alone or two milliliters of water containing the same inhibitor(s) as in the preceding one-half hour plus either two grams rice starch, sucrose, or combined rice starch (2 g) and sucrose (2 g). Thus, each test rat received a total of one gram of an inhibitor or the formulation. A drop of blood was obtained from the tail at baseline (time 0), 1 hour, 2 hours, 3 hours and 4 hours after the final challenge for glucose determinations. The total amount of blood drawn in a rat for a given study was below 0.5 ml. Glucose was estimated using commercial glucose strips (Lifescan, One Touch Ultra, Melitas, CA).

In a given daily procedure, three rats were examined in a test situation. Three additional SD received a comparable volume of water and served as internal controls to account for any daily variations in test results. Since each test situation was examined at three different time intervals, nine datum points were obtained for both control and test in any given situation. The same rat was not tested more than once during a three-week interval, or more than four times in all.

Two Yorkshire pigs, weighing approximately 70 and 90 kg at the initiation of study, were deprived of food and water for 2 hours at the time of study. Then, they were given challenges of 200 g sucrose (table sugar) and/or 100 g rice starch individually or combined in enough drinking water to solubilize the constituents. This fluid mixture was consumed totally within minutes. To complete an investigation on each challenge, two separate procedures were run on the two pigs. In the first, pig 1 was control and pig 2 was the test animal receiving the CHO blocker. In the second, the roles were reversed. Thus, each pig could serve as his own control. When a pig served as test, it was given the contents of four capsules of the formulation described below in the drinking water along with the CHO challenges. At baseline and the selected times, a drop of blood from the ear was used to measure circulating glucose concentrations. The total amount of blood obtained at a single testing amounted to less than 0.5 ml.

Ingredients:

The individual test ingredients as well as the formulation were obtained from AdvoCare International, Carrollton, Texas. The formula was composed of w/w: dry bean extract (seed - *Phaseolus vulgaris*) 19%, hibiscus extract (flower - *Hibiscus sabdariffa*) 31%, L-arabinose 31%, gymnema extract ((leaf - *Gymnema sylvestre*) 12%, green tea extract leaf - (*Camellia sinensis*) 6%, and apple extract (fruit - *Malus sylvestris*) 1% plus the addition of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.

Statistical Analyses:

Results are presented as mean ± SEM. Where a significant effect of regimen was detected by ANOVA (repeated measures) ($p<0.05$), the Dunnett t test was used to establish which differences between means reached statistical significance [29]. When the data from two columns of data were analyzed at a single time point, Student's t test was used. Statistical significance was set at $p < 0.05$.

3. RESULTS

To develop a testing procedure, rice starch or sucrose challenges were carried out individually on SD rats and compared to the control situation in which rats received a similar volume of fluid (water only) (Fig. 1). Following the respective challenges of rice starch or sucrose, the appearance of glucose above baseline (delta) increased significantly, the highest measured point at one hour with a decrease over the remaining three hours. The circulating glucose levels

[1] Carb-Ease™, Advocare International, Dallas, TX

decreased below baseline over the course of the four hours in the control rats, which had been fasted overnight and received only water, i.e., no CHO challenge.

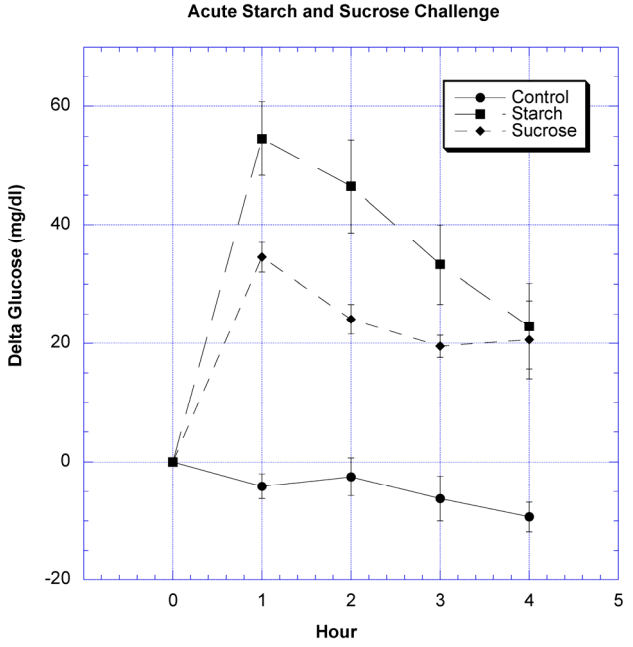

Fig. 1. The changes in circulating glucose at timed intervals after challenges with water (control), rice starch and sucrose are shown. Mean ± SEM is depicted for a minimum of 9 rats in each group.

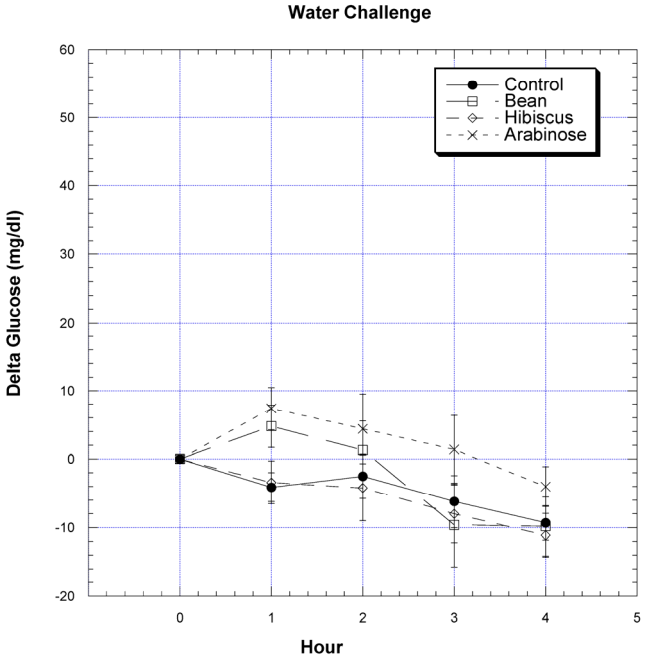

Fig. 2. All rats were gavaged with 2 ml water – no CHO challenge. One half hour prior to the water challenges and at the time of challenges a total of 2 ml of water (control), or 1 gram of bean extract, hibiscus, or L-arabinose in 2 ml water was given. The change in circulating glucose at timed intervals after various challenges is depicted. Mean ± SEM is depicted for nine rats. * Significantly different at that time point when compared to control.

Bean extract, hibiscus extract, and L-arabinose were tested for their effects on rats receiving only water (no CHO challenge) (Fig. 2). In these rats starved overnight and deprived of food for the four hour study, the blood glucose levels of rats receiving only water tended to decrease, resembling the earlier findings depicted in Fig. 1. The circulating glucose pattern was essentially no different than control after the SD rats had been given bean extract, hibiscus extract, or L-arabinose.

The effects of three natural elements, bean extract, hibiscus extract, and L-arabinose, on glucose appearance in the circulating blood after sucrose challenge are depicted in Table 1. The average circulating glucose level after the 17 h deprivation of food was 88.4 mg/dl ± 1.4 (SEM) with a range of 72 mg/dl to 105 mg/dl. L-Arabinose proved to be very effective, i.e., the appearance of glucose in the blood stream after gavage of sucrose was essentially non-existent. Area under the curve was only 2% of control. Interestingly, both hibiscus and bean extracts also decreased glucose appearance compared to control after sucrose challenge over the first three hours, although at comparable doses, bean and hibiscus extracts were not as effective as L-arabinose. The glucose elevations above baseline at two hours (mg/dl ± SEM) were: 24.1±2.5 for control, -5.7±3.7 for L-arabinose, 9.8±8.5 for bean, and 8.1±2.3 for hibiscus. All interventions were statistically significantly different from control. Areas under the curve averaged 51% for bean extract and 43% for hibiscus extract compared to control.

The effects of three natural products (bean extract, hibiscus extract, and L-arabinose) on glucose appearance in the circulating blood after rice starch challenge are also depicted in Table 1. L-Arabinose had only a small, insignificant effect on the appearance of blood glucose after the rice starch challenge, i.e., there were no statistically significant differences at any of the time points compared to control. Area under the curve was 85% of the control. In contrast, both bean and hibiscus extracts significantly lowered the appearance of circulating glucose compared to control following the rice starch challenge -- at the first and second hours for bean extract and at the first, second and third hours for hibiscus. The glucose elevations above baseline at two hours (mg/dl ± SEM) were: 46.5±7.9 for control, 14.7±10.0 for bean, 5.9±3.3 for hibiscus, and 39.0±8.7 for L-arabinose. Findings for the bean and hibiscus extracts were statistically significantly different from control. Area under the curve was 40% for bean and 27% for hibiscus extracts after starch challenge compared to the control situation in which no natural inhibitor was given.

In additional studies, effects of increasing the doses of bean and hibiscus extracts by 50% to 100% compared to the original doses were examined (Table 2). For bean extract, a 50% increase and a doubling of the initial dose caused further lowering of the absorption of rice starch compared to the standard dose after one and two hours as estimated by the

appearance of circulating glucose. Although glucose appearance for all doses was statistically lower than control, the differences among the various doses did not prove statistically significant. Results with hibiscus extract were somewhat similar in these studies, except at the original dose (1X) the value at the two hour period was not different from the one hour period, unlike the previous studies. This was not the case for the higher doses.

Table 1 Carbohydrate Challenge Tests in Rats Using Different CHO Blockers

Challenge	Sucrose (200g)				Starch (100g)			
Time (h)	Control	Bean	Hibiscus	L Arab	Control	Bean	Hibiscus	L Arab
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	34.5±2.5	12.5±6.2*	17.1±4.9*	5.7±3.7*	54.6±6.2	18.4±9.0*	19.1±7.1*	39.8±7.6
2.0	24.1±2.5	9.8±3.5*	8.1±2.3*	-5.7±3.7*	46.5±7.9	14.7±10.0*	5.9±3.3*	39.0±8.7
3.0	19.5±1.9	11.3±2.7*	8.1±2.4*	0.1±3.5*	33.3±6.7	20.1±8.7	7.9±2.3*	32.7±7.4
4.0	20.6±6.6	23.2±3.6	8.8±3.7	-4.1±4.5*	22.9±7.2	11.6±7.6	11.8±5.1	25.6±5.7

Circulating glucose levels above or below baseline after CHO challenge specified in heading.

Each number represents the average change in glucose concentrations (mg/dl) ± SEM of 9 rats.

*Statistically significantly different from control at that time point.

Table 2 Dose-Response for Bean and Hibiscus Extracts in Rats One and Two Hours after Challenge

	Bean Extract		Hibiscus Extract	
Time (h) Dose	1	2	1	2
0	56.0±6.0	36.1±7.0	51.0±2.1	29.3±4.7
1.0 X	28.3±3.5*	19.2±2.1*	28.3±6.2*	30.3±3.0
1.5 X	19.3±6.4*	10.0±3.4*	25.3±3.2*	14.7±2.6*
2.0 X	11.7±0.7*	0.4±5.4*	21.7±3.8*	14.7±5.8*

Circulating glucose levels above baseline after starch challenge at specified times.

Each value represents the average change in glucose concentrations (mg/dl) ±SEM of 9 rats.

* Statistically significantly different from control (Zero Dose).

Two doses of a formula of natural ingredients containing bean and hibiscus extracts and L-arabinose were examined, and these data are presented in Table 3. A one gram dose, designated "low dose", and a "high dose", composed of twice as much, were studied. Concerning the rice starch challenge, the higher dose was so effective that there was virtually no elevation of circulating glucose levels following the starch challenge. The area under the curve was negative to baseline. Despite not being as effective as the high dose, the lower dose of the formulation was still effective over the first two hours, but inexplicably the circulating glucose levels were higher than the control situation by the fourth hour. The area under the curve was 48% of control. After sucrose challenge, both doses were effective over three hours. At one and three hours, the higher dose caused a statistically greater lowering than the low dose. Similar to the case with the rice starch challenge, the high dose virtually prevented any rise in the circulating glucose levels after sucrose challenge. Area under the curve for the low dose was 47% and for the high dose was 6% of control.

The contents of four capsules of the CHO enzyme-inhibiting formulation were given when the pigs were challenged. The human dose is three to four capsules at one time. In Table 4, it can be seen that the addition of the formula containing the enzyme inhibitors significantly lowered the appearance of glucose in the circulating blood whether the challenge was starch alone, sucrose alone, or a combination of the two CHO. For example, 30 minutes after the starch challenge, the blood glucose increased above baseline by an average of approximately 25 mg/dl in the pigs in the absence of the enzyme-inhibiting formula, with essentially no increase in blood glucose when the formula was co-administered with the starch. Similar results were observed at the one hour time points following the sucrose challenge and combined starch plus sucrose challenge.

Table 3 Carbohydrate Challenge Tests in Rats Using Two Doses of Formula

Challenge	Sucrose			Starch		
Time (h)	Control	Low Dose	High Dose	Control	Low Dose	High Dose
0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	37.8+4.6	13.0+2.4*	0.5+5.7*	57.2+11.6	21.5+5.4*	-5.3+2.0*
2.0	23.3+3.2	10.0+4.7*	6.3+7.1*	39.8+5.2	13.8+11.4*	-10.8+4.5*
3.0	19.8+2.8	11.3+1.9*	-1.7+5.8*	30.2+8.8	16.2+6.7*	-0.8+2.2*
4.0	14.5+6.6	15.0+2.1	0.8+9.0*	9.5+5.5	23.8+4.1*	-3.7+3.7*

Circulating glucose levels above or below baseline after CHO challenge in control rats and two groups receiving different doses of formula
Each number represents the average change in glucose concentrations (mg/dl) ± SEM of 9 rats.
* Statistically significantly different from control.

Table 4 Carbohydrate Challenge Tests on Two Pigs

CHO Challenge	Sucrose (200g)		Starch (100g)		Sucrose/Starch (200g/100g)	
Time (h)	Control	Test	Control	Test	Control	Test
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	24.0	-3.5	25.0	0.5	22.0	-5.5
1.0	22.0	-11.5	14.5	0.0	23.5	-2.0
2.0	10.0	-4.5	14.0	6.0	15.0	-7.5
3.0	2.0	-7.0	-3.0	-3.0	0.0	-9.5
4.0	-2.0	-13.0	-4.5	2.0	-4.0	-5.5

Circulating glucose levels above or below baseline (mg/dl) after CHO challenge specified in heading.
Test pigs received the equivalent of 4 capsules of the formula.
Each number represents the average values of the two pigs.

4. DISCUSSION

Few well-controlled animal studies *(in vivo)* of so-called CHO blockers are available [26,27,30,31]. Even less information exists comparing different inhibitors and examining dose-responses. The gavage of rice starch or sucrose causes a rapid appearance of glucose in the blood as depicted in Fig. 1. We chose this appearance to estimate the gastrointestinal breakdown of rice starch and sucrose. The hypothesis tested was that natural starch and sucrose enzyme inhibitors (amylase and sucrase) would diminish and/or slow the breakdown of starch and sucrose in the gastrointestinal tract, diminishing glucose entrance into the blood stream.

The actions of the bean and hibiscus extracts and L-arabinose on CHO absorption in the gastrointestinal tract differed somewhat. After the rice starch challenge, bean and hibiscus extracts at the same dose significantly decreased the appearance of glucose in the circulating blood to a reasonably similar extent (Table 1). In contrast, L-arabinose had no significant effect on this appearance after the starch challenge. The results were different when sucrose provided the challenge. L-Arabinose proved to be highly effective in preventing a rise in circulating glucose after sucrose challenge (Table 1). In fact, there was virtually no appearance of glucose after sucrose challenge when L-arabinose was given. Although less effective than L-arabinose, both bean and hibiscus decreased the absorption of sucrose significantly. When the doses of bean and hibiscus extract were increased, less glucose appeared in the circulation over the first and second hour following the higher doses compared to the lower doses (Table 2). These data suggest that there is a dose-response with bean and hibiscus extracts on circulating glucose after rice starch challenge.

Just as postulated, we believe that changes in the appearance of circulating glucose are due to the effects on CHO breakdown in the gut [20-28]. This concept was strengthened when it was shown that these natural ingredients did not affect circulating glucose levels unless the rats were challenged with rice starch or sucrose, i.e., these natural ingredients did not affect circulating glucose levels after a water challenge (Fig. 2). The fact that bean and hibiscus extracts blocked the appearance of glucose after sucrose challenge suggests the possibility that they may also have the additional

ability to inhibit sucrase.

When a formula containing the above three ingredients was given to the rats, the acute appearance of glucose was diminished significantly whether the challenge was rice starch or sucrose (Table 3). When the dose of the formulation was doubled, i.e., two grams, the appearance of glucose was essentially nonexistent. In the latter case, the amounts of L-arabinose and hibiscus extract in the formula were only about one-third of the amounts in the direct challenge. Bean extract was only 19% by weight of the straight dosage. Therefore, combining ingredients might be useful to increase the over all effects. The formulation contained other ingredients not examined here (*Gymnema sylvestre*, apple extract, and green tea). We cannot state what role they played in the results.

In calculating human doses based on the doses used in our rats, the levels of inhibitors seemed unreasonable for common use. Therefore, we examined two pigs that possessed weights in a range common for human adults. In these studies, we accomplished significant decreases in glucose appearance in the blood stream from starch and/or sucrose challenge when using doses equivalent to those recommended in humans. Thus, our studies indicate that gastrointestinal absorption of starches and sugars can be lessened significantly with reasonable doses of CHO enzyme inhibitors.

In conclusion, examining extracts from bean and hibiscus at similar doses in rats shows them to be comparably effective in blocking rice starch absorption in rats. A positive dose-response was noted. Interestingly, these same ingredients also delayed sucrose absorption based on their ability to influence the appearance of circulating glucose after sucrose challenge. L-Arabinose slowed the absorption of sucrose, but not that of rice starch. The inability of any of these agents to influence circulating glucose when there was no CHO challenge confirms that they work mostly via affecting CHO absorption rather than on overall metabolism. When combined in a formula, these ingredients could slow absorption after the simultaneous challenge of sucrose and starch. When the formula was given to large pigs at the suggested human dosing, the inhibitors were quite effective in lowering the appearance of glucose in the circulation after sucrose and starch challenges alone and in combination. Accordingly, these findings lend support to the concept that natural, safe supplements can influence the glycemic load favorably and perhaps be beneficial for many aspects of health.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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L-Arabinose Feeding Prevents Increases Due to Dietary Sucrose in Lipogenic Enzymes and Triacylglycerol Levels in Rats[1]

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ABSTRACT L-Arabinose is a natural, poorly absorbed pentose that selectively inhibits intestinal sucrase activity. To investigate the effects of L-arabinose feeding on lipogenesis due to its inhibition of sucrase, rats were fed 0–30 g sucrose/100 g diets containing 0–1 g L-arabinose/100 g for 10 d. Lipogenic enzyme activities and triacylglycerol concentrations in the liver were significantly increased by dietary sucrose, and arabinose significantly prevented these increases. Arabinose feeding reduced the weights of epididymal adipose tissue. Moreover, plasma insulin and triacylglycerol concentrations were significantly reduced by dietary L-arabinose. These findings suggest that L-arabinose inhibits intestinal sucrase activity, thereby reducing sucrose utilization, and consequently decreasing lipogenesis. J. Nutr. 131: 796–799, 2001.

KEY WORDS: • L-arabinose • sucrose • lipogenic enzymes • triacylglycerol levels • rats

L-Arabinose is a pentose with a sweet taste. It is absorbed from the intestinal tract in rats (1,2) and chicks (3,4) but at a lower rate than glucose. A portion of the ingested L-arabinose is excreted in the urine (1). Although widely present in nature, L-arabinose is rarely used, and its physiological effects in vivo have received little attention. Seri et al. (5) demonstrated that L-arabinose selectively inhibits intestinal sucrase activity in a noncompetitive manner and suppresses the plasma glucose increase due to sucrose ingestion. Neither D-arabinose nor the disaccharide L-arabinobiose inhibits sucrase activity. Sanai et al. (6) also examined the effects of L-arabinose on gastrointestinal digestion and absorption of ^{14}C-labeled sucrose in rats. After the oral administration of ^{14}C-labeled sucrose, cumulative expiratory $^{14}CO_2$ was significantly and dose-dependently reduced by L-arabinose, and a large quantity of undigested ^{14}C-labeled sucrose and its metabolites was observed in the cecum of the arabinose-treated rats. The authors also observed suppressive effects of L-arabinose on the increase in blood glucose after sucrose loading in rats. Because the intestinal absorption of sucrose is inhibited in the presence of L-arabinose, the absorption of sucrose should be reduced by arabinose ingestion. Therefore, L-arabinose may also be useful in preventing excess sucrose utilization. In the present experiment, we investigated the effects of arabinose ingestion on the activities of lipogenic enzymes, which are involved in long-chain fatty acid synthesis, and on the plasma and liver triacylglycerol levels in rats.

MATERIALS AND METHODS

Materials. [1-^{14}C]Acetyl coenzyme A (CoA)[3] (1.85–2.22 MBq/mmol) was purchased from Morevek Biochemicals (Brea, CA). [^{14}C]Sodium bicarbonate (0.21 GBq/mmol) was obtained from New England Nuclear (Boston, MA). An insulin radioimmunoassay kit was obtained from Eiken Chemical Company (Tokyo, Japan). A glucose assay kit (Glucose CII test) was from Wako (Osaka, Japan). Most other reagents were obtained from Wako or Sigma Chemical Co. (St. Louis, MO). L-Arabinose and pregelatinized cornstarch were from Sanwa Starch (Nara, Japan).

Animals. Male 5-wk-old Wistar rats (Japan SLC, Hamamatsu, Japan) were deprived of food for 1 d and then fed synthetic diets for 10 d. Four basal synthetic diets with different sucrose concentrations were used: C (no sucrose), CS10 (containing 10 g sucrose/100 g by weight), CS20 (containing 20 g sucrose/100 g by weight) and CS30 (containing 30 g sucrose/100 g by weight). For a comparison, another dietary group was added: the sucrose in the CS20 diet was replaced with 10 g glucose and 10 g fructose (CGF20 diet). The composition of the C diet was 713.5 g pregelatinized cornstarch, 180 g casein, 50 g cellulose, 24.5 g salt mixture (7), 1 g choline chloride and 1 g vitamin mixture (7) per 100 g. When sucrose was added to the diet, pregelatinized cornstarch was replaced with sucrose by weight. In the 0.5 or 1 g L-arabinose/100 g diets, cellulose was replaced with 0.5 or 1 g L-arabinose/100 g. All of the experiments for these dietary groups (except the CGF20 diet) were repeated at least three times, and typical results are shown in **Table 1** and **Figs. 1** and **2**. The CGF20 diet containing 0–1 g L-arabinose/100 g was studied once in comparison with the CS20 diet.

The rats were individually housed in wire-bottomed cages in a temperature-controlled room (24°C) with an automatic lighting schedule (0800–2000 h). They had free access to water and were fed equal energy-containing diets relative to body mass in all groups. The amount of diet consumed by the rats was measured at 1700 h every day. Based on the measurement, the expected average amount of food consumed by rats fed the C, CS10, CS20 and CS30 diets containing 0–1 g L-arabinose/100 g was fed the next day. Only the rats consuming similar energy levels during the experimental period were used for the study.

Rats were killed by decapitation while under anesthesia with diethyl ether. An aliquot of liver was quickly removed and homogenized with three volumes of 0.25 mol sucrose/L. The liver homog-

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[3] Abbreviations used: C, pregelatinized cornstarch diet; CS10, CS20 and CS30, C diet containing 10, 20 or 30 g sucrose/100 g; CGF20, C diet containing 10 g glucose and 10 g fructose/100 g.

TABLE 1

Effects of arabinose feeding on the weights of body, liver, adipose tissue, cecum with wet content, pH of cecum contents and plasma glucose concentration in rats fed CS30, CS20, CS10, C or CGF20 diet[1,2]

Diet and L-arabinose content, %	Body	Liver	Epididymal adipose tissue	Cecum with wet contents	Cecum contents pH	Plasma glucose
	g				pH	mmol/L
CS30						
0	225 ± 9.5	11.4 ± 1.33	2.04 ± 0.19	2.12 ± 0.17	7.55 ± 0.36	13.2 ± 0.33
0.5	216 ± 4.5	10.3 ± 1.70	1.77 ± 0.23	8.68 ± 2.14	5.00 ± 0.37	11.3 ± 1.05
1	218 ± 4.5	9.20 ± 0.55	1.35 ± 0.05	13.4 ± 2.65	4.90 ± 0.10	12.0 ± 0.55
CS20						
0	219 ± 12	11.7 ± 1.16	1.98 ± 0.36	2.28 ± 0.28	7.80 ± 0.22	11.4 ± 0.74
0.5	212 ± 11	10.7 ± 0.98	1.36 ± 0.13	6.50 ± 1.22	5.32 ± 0.11	11.6 ± 0.95
1	212 ± 9.1	10.1 ± 0.80	1.44 ± 0.18	10.8 ± 1.42	4.60 ± 0.22	10.9 ± 1.29
CS10						
0	219 ± 6.9	11.3 ± 0.33	1.98 ± 0.35	3.03 ± 0.56	7.87 ± 0.25	10.8 ± 0.19
0.5	226 ± 21	11.1 ± 0.60	1.71 ± 0.16	4.54 ± 1.47	6.40 ± 0.14	10.9 ± 0.44
1	222 ± 9.3	10.8 ± 0.19	1.80 ± 0.29	6.67 ± 1.09	5.28 ± 0.47	11.5 ± 1.25
C						
0	223 ± 9.7	10.7 ± 0.53	1.83 ± 0.21	3.06 ± 0.57	7.87 ± 0.19	11.0 ± 1.00
0.5	218 ± 2.6	11.2 ± 0.70	1.88 ± 0.13	2.91 ± 0.03	7.30 ± 0.10*	10.9 ± 0.27
1	218 ± 9.2	10.5 ± 0.77	1.75 ± 0.10	3.05 ± 0.81	6.70 ± 0.10*	11.0 ± 1.25
ANOVA *P*-values						
Sucrose (S)	0.367	0.249	0.093	<0.001	<0.001	<0.05
L-Arabinose (A)	0.518	<0.01	<0.001	<0.001	<0.001	0.256
S × A	0.827	0.510	<0.01	<0.001	<0.001	0.554
C						
2	215 ± 1.5	10.1 ± 1.04	1.70 ± 0.23	5.65 ± 0.66*	6.05 ± 0.17*	10.0 ± 0.31
5	212 ± 6.2	10.2 ± 0.68	1.41 ± 0.10*	7.91 ± 0.75*	6.04 ± 0.15*	10.9 ± 0.96
CGF20						
0	214 ± 13	10.4 ± 0.61	1.79 ± 0.22	2.59 ± 0.18	7.90 ± 0.10	11.7 ± 0.55
0.5	212 ± 12	10.9 ± 1.10	1.98 ± 0.12**	2.27 ± 0.21**	7.00 ± 0.14**,***	11.8 ± 1.07
1	211 ± 26	10.6 ± 0.72	1.74 ± 0.09**	2.47 ± 0.24**	6.60 ± 0.14**,***	11.6 ± 0.87

[1] CS10, CS20 and CS30 diets contained 10, 20 and 30% (by weight) sucrose, respectively. C diet contained pregelatinized cornstarch. CGF20 diet contained 10% glucose and 10% fructose.

[2] Values are means ± SD, $n = 4$. Two-way ANOVA was followed by inspection of data in each column for C, CS10, CS20 and CS30 diets containing 0–1% L-arabinose. Means with a different superscript are significantly different in each column ($P < 0.05$).

In C diets containing 0–5% L-arabinose, * different from no arabinose by *t* test ($P < 0.05$).

In CGF20 diets containing 0–1% L-arabinose, ** different from the corresponding CS20 diet, *** different from no arabinose by *t* test ($P < 0.05$).

enate was centrifuged at 10,000 × *g* for 10 min, and then the supernatant was centrifuged at 105,000 × *g* for 45 min (model L5, type 40 rotor; Beckman Instruments, Palo Alto, CA). The 105,000 × *g* supernatant was used for measurement of lipogenic enzyme activities. Another aliquot of liver was immediately frozen in liquid nitrogen and stored at −80°C for subsequent extraction of total lipids and measurement of triacylglycerols. The care and treatment of experimental animals were in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (8).

Lipogenic enzyme activities. Acetyl-CoA carboxylase (EC 6.4.1.2) activity was assayed according to the $H^{14}CO_3^-$ fixation method (9). To attain full activity, the enzyme was first preincubated with 10 mmol citrate/L. Fatty acid synthase (EC 2.3.1.85) activity was assayed according to Hsu et al. (10). Adenosine triphosphate (ATP) citrate-lyase (EC 4.1.3.8) activity was assayed as described by Takeda et al. (11). The enzyme activities in the supernatant of the liver homogenates are shown as mU/mg protein, where 1 mU is the amount that catalyzes the formation of 1 nmol product/min at 37°C. Protein was determined according to the method of Lowry et al. (12).

Plasma glucose and insulin analyses. Plasma glucose concentrations were determined according to the glucose-oxidase method (13). Plasma insulin concentrations were measured with a two-antibody system radioimmunoassay according to the method of Morgan and Lazarow (14).

Statistical analysis. For CS30, CS20, CS10 and C diets containing 0–1 g L-arabinose/100 g, two-way ANOVA was followed by an inspection of all differences between pairs of means using the least significant difference test (15). For the C diets containing 0–5 g L-arabinose/100 g, comparisons were made with the diet without arabinose by *t* test. The CGF20 diets containing 0–1 g L-arabinose/100 g were compared by *t* test with the no-arabinose diet and the CS20 diet containing the same amount of L-arabinose. Differences were considered significant at $P < 0.05$.

RESULTS

Food intake, weights of body, adipose tissue and cecum with wet contents and cecum content pH. Changes in relative body weight [g/(100 g body · d^{-1})] did not differ among the groups (data not shown). Careful attention to the food consumption of rats ensured it was similar in the dietary groups. The standard deviations of relative food consumption [g/(100 g body · d)] were 3.3% of the mean values during the experimental period, except in rats fed the CS30 plus 1 g L-arabinose/100 g diet, in which food consumption was reduced to 85 ± 1.3% of the CS30 group. However, food consumption in the CS30 plus 1 g L-arabinose/100 g group was reduced most for 3 d at the beginning of the feeding but was >90% of the CS30 group for the latter 5 d. No rats had diarrhea during the experiment.

The weights of epididymal adipose tissue were significantly ($P < 0.001$) reduced by L-arabinose in rats fed the diets containing sucrose (Table 1). The cecum weights including

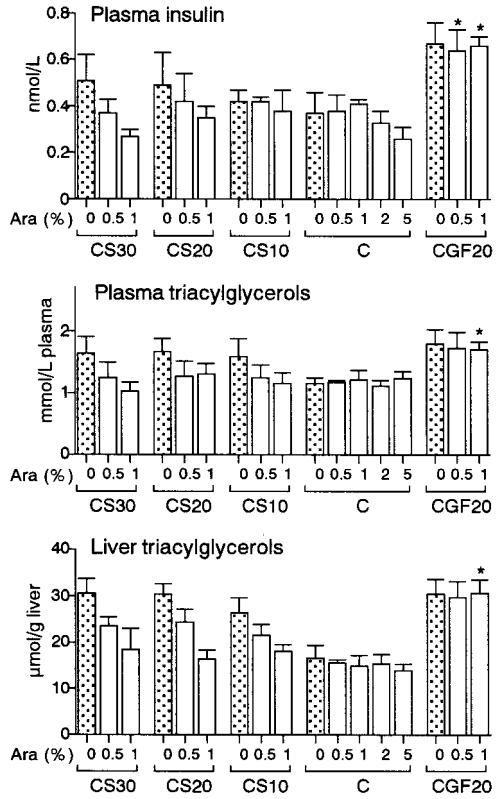

FIGURE 1 Effects of L-arabinose feeding on concentrations of plasma insulin and of plasma and liver triacylglycerols in rats fed CS30, CS20, CS10, C or CGF20 diet. CS10, CS20 and CS30 diets contained 10, 20 and 30 g/100 g sucrose, respectively. Values are means ± SD, $n = 4$. Two-way ANOVA was followed by inspection of data in each figure for C, CS10, CS20 and CS30 diets containing 0–1 g L-arabinose/100 g. ANOVA ($P < 0.05$): Suc, sucrose main effect; Ara, L-arabinose main effect; and Suc × Ara, interactions. Plasma insulin: Ara, Suc × Ara; plasma triacylglycerols: Ara; liver triacylglycerols: Suc, Ara, Suc × Ara. In C diets containing 0–5 g L-arabinose/100 g, not different from no arabinose by *t* test. In CGF20 diets containing 0–1 g L-arabinose/100 g, *different from the CS20 diet containing the same amount of L-arabinose ($P < 0.05$); not different from no arabinose by *t* test.

wet contents decreased ($P < 0.001$) with increasing dietary sucrose and increased with increasing dietary L-arabinose. The pH of the cecum contents was markedly ($P < 0.001$) lowered by L-arabinose.

Plasma glucose and insulin concentrations. The plasma glucose concentrations were slightly ($P < 0.05$) elevated by sucrose (Table 1) but were not affected by dietary L-arabinose. Plasma insulin concentrations were significantly lowered by L-arabinose feeding (Fig. 1).

Plasma and liver triacylglycerol concentrations. Liver triacylglycerol concentrations were increased ($P < 0.001$) by dietary sucrose, and L-arabinose feeding prevented the increases ($P < 0.001$) (Fig. 1). Plasma triacylglycerol concentrations were not significantly affected by dietary sucrose. L-Arabinose feeding ($P < 0.01$) reduced plasma triacylglycerol concentrations.

Liver lipogenic enzyme activities. The activities of acetyl-CoA carboxylase, fatty acid synthase and ATP citrate-lyase were significantly ($P < 0.01$) increased by dietary sucrose, and these increases were prevented by dietary L-arabinose (Fig. 2, $P < 0.001$).

Effects of extra L-arabinose feeding in rats fed the C diet. The lipogenic enzyme activities and the plasma and liver triacylglycerol concentrations of rats fed the C diet were not affected by the addition of 0.5 or 1 g L-arabinose/100 g to the diet compared with no addition of arabinose. Therefore, the results for rats fed the C diet containing a large amount (2 or 5 g/100 g) of L-arabinose are also shown in Table 1 and Figs. 1 and 2. The lipogenic enzyme activities and plasma and liver triacylglycerol levels were not reduced even by the addition of 2 or 5 g arabinose/100 g to the C diet compared with no addition of arabinose. Compared with no arabinose, however, the epididymal adipose tissue weights were reduced by feeding 5 g L-arabinose/100 g. Moreover, the cecum weights with contents were significantly increased, and the pH of the cecum content was markedly lowered by feeding 2 or 5 g L-arabinose/100 g.

Effects of L-arabinose feeding in rats fed a fructose-plus-glucose diet. Compared with not being fed arabinose, L-arabinose feeding did not affect the weights of the cecum with wet contents in the CGF20 groups but lowered the pH of the cecum contents. No effects of L-arabinose on plasma and liver triacylglycerol concentrations or on liver lipogenic enzyme activities were observed in the CGF20 groups. The weights of epididymal adipose tissue were also not affected by L-arabinose feeding. Plasma glucose and insulin concentrations were not affected by L-arabinose in the CGF20 groups.

DISCUSSION

The concentrations of liver triacylglycerols were significantly increased with dietary sucrose. The lipogenic enzyme activities in the liver were also significantly increased with dietary sucrose. Fukuda et al. (16) previously reported that the lipogenic enzyme activities were higher of rats fed diets of (in order) fructose > sucrose > α-cornstarch in both normal and

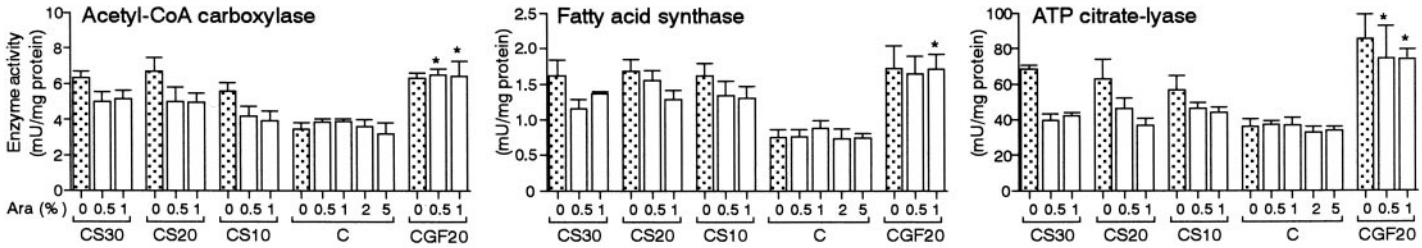

FIGURE 2 Effects of L-arabinose feeding on liver lipogenic enzyme activities in rats fed CS30, CS20, CS10, C or CGF20 diet. CS10, CS20 and CS30 diets contained 10, 20 and 30 g/100 g sucrose, respectively. Values are means ± SD, $n = 4$. Two-way ANOVA was followed by inspection of data in each figure for C, CS10, CS20 and CS30 diets containing 0–1 g L-arabinose/100 g. ANOVA ($P < 0.01$): Suc, sucrose main effect; Ara, L-arabinose main effect; Suc × Ara, interactions. Acetyl-coenzyme A carboxylase, fatty acid synthase, ATP citrate-lyase: Suc, Ara, Suc × Ara. In C diets containing 0–5 g L-arabinose/100 g: not different from no arabinose by *t* test. In CGF20 diets containing 0–1 g L-arabinose/100 g: *different from the CS20 diet containing the same amount of L-arabinose ($P < 0.05$); not different from no arabinose by *t* test.

diabetic states. The concentrations of the substrate (acetyl-CoA) and the activator (citrate) of acetyl-CoA carboxylase, a key enzyme of fatty acid synthesis in the livers, were significantly higher in that order. This may be one of the reasons that fructose stimulates lipogenic enzyme activities and lipogenesis. Thus, dietary sucrose is considered to be more lipogenic than starch.

In rats fed the C (no sucrose) diets containing the higher concentrations (2 or 5 g/100 g) of arabinose, the cecum with content weights were increased and the pH was acidified compared with no arabinose. Bacteria in the small intestine may ferment L-arabinose. Schutte et al. (17) found in a study with pigs that the presence of L-arabinose in the diet increased ileal flow of volatile fatty acids and lactic acid, suggesting the occurrence of microbial degradation of L-arabinose in the small intestine.

In rats fed sucrose, the cecum with content weights were dose-dependently increased by arabinose feeding, and the pH of the cecum contents was markedly lowered. We suggest that L-arabinose inhibited the sucrase activity of intestinal mucosa and that dietary sucrose was fermented by intestinal bacteria to generate the acidic products, in addition to arabinose degradation. Sanai et al. (6) observed the suppressive effects of L-arabinose on the increase in blood glucose after sucrose loading in rats. In the present experiment, plasma glucose concentrations were significantly increased with dietary sucrose, but the increase was not significantly suppressed by arabinose. In rats fed the CS30 diet, however, plasma glucose levels were significantly lowered by the arabinose, and plasma insulin concentrations were also lowered. The lowered insulin concentrations were possibly due to the suppression of hyperglycemia.

L-Arabinose feeding prevented the increases due to sucrose feeding in activities of lipogenic enzymes and the increases in triacylglycerol concentrations of livers. Moreover, arabinose feeding reduced the weights of adipose tissue. However, no effects of L-arabinose feeding on the increases due to fructose plus glucose were found in rats fed the CFG20 diet. Therefore, the suppression of lipogenesis could be ascribed to the reduction in sucrose utilization due to inhibition of intestinal sucrase by L-arabinose. We previously reported that the lipogenic enzyme activities were sigmoidly increased relative to the quantity of a high sucrose diet and were greatly increased by feeding >75% of ad libitum intake (18). L-Arabinose may be useful for preventing obesity due to extreme sucrose ingestion.

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L-Arabinose Selectively Inhibits Intestinal Sucrase in an Uncompetitive Manner and Suppresses Glycemic Response After Sucrose Ingestion in Animals

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The objective of this study was to investigate the effects of L-arabinose on intestinal α-glucosidase activities in vitro and to evaluate its effects on postprandial glycemic responses in vivo. L-Arabinose inhibited the sucrase activity of intestinal mucosa in an uncompetitive manner (K_i, 2 mmol/L). Neither the optical isomer D-arabinose nor the disaccharide L-arabinobiose inhibited sucrase activity, whereas D-xylose was as potent as L-arabinose in inhibiting this activity. L-Arabinose and D-xylose showed no inhibitory effect on the activities of intestinal maltase, isomaltase, trehalase, lactase, and glucoamylase, or pancreatic amylase. In contrast, a known α-glucosidase inhibitor, acarbose, competitively inhibited (K_i, 1.1 μmol/L) sucrase activity and also inhibited intestinal maltase, glucoamylase, and pancreatic amylase. L -Arabinose suppressed the increase of blood glucose after sucrose loading dose-dependently in mice (ED_{50}, 35 mg/kg), but showed no effect after starch loading. The suppressive effect of D-xylose on the increase of blood glucose after sucrose loading was 2.4 times less than that of L-arabinose, probably due to intestinal absorption of the former. Acarbose strongly suppressed glycemic responses in both sucrose loading (ED_{50}, 1.1 mg/kg) and starch loading (ED_{50}, 1.7 mg/kg) in mice. L-Arabinose suppressed the increase of plasma glucose and insulin in rats after sucrose loading, the suppression of the former being uninterruptedly observed in mice for 3 weeks. Thus, the results demonstrated that L-arabinose selectively inhibits intestinal sucrase activity in an uncompetitive manner and suppresses the glycemic response after sucrose ingestion by inhibition of sucrase activity.

IT HAS BEEN PROVEN that strict glycemic control is associated with a low incidence of microvascular and macrovascular complications in diabetes,[1] and a delay and/or inhibition of carbohydrate digestion could be helpful for avoiding postprandial hyperglycemia in diabetic patients.[2,3] Specific inhibitors of α-glucosidases have shown a definite therapeutic value in suppressing the postprandial glycemic increase by delaying carbohydrate digestion.[2-4] Acarbose[2-6] and its analog[7] are known to be competitive inhibitors of the intestinal α-glucosidases, ie, glucoamylase, sucrase, and maltase. It has also been shown that pancreatic amylase is inhibited by acarbose.[6] Although the major portion of dietary carbohydrate is starch, daily ingestion of sucrose is large in many advanced countries (60 g/person · d, 1991, Japan); however, agents that selectively inhibit sucrose digestion have been of no practical use.

In our preliminary studies, we observed that the L-arabinose–containing fraction obtained by enzymatic hydrolysis of plant gums and sugar beet suppressed the increase of blood glucose after sucrose loading in mice. L-Arabinose is known as a less absorptive pentose with a sweet taste. Although broadly present in nature, it has been little used to date, and there are no known reports of its physiological effect in vivo.

The objective of this study was to investigate the effects of L-arabinose and related pentoses on the activities of intestinal α-glucosidases and pancreatic amylase in vitro, and to evaluate the effects of L-arabinose on postprandial glycemic responses using several experimental animals in vivo.

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MATERIALS AND METHODS

Inhibitory Effect on α-Glucosidase Activities of Porcine Intestinal Mucosa and on Amylase Activity of Mice Pancreas

Small intestines of pigs were obtained immediately after death at a slaughterhouse, rinsed with ice-cold saline, and stored at −20°C until use. The small intestines were thawed and cut open, and the mucosa was gently scraped off with a glass cover slip. All the collected mucosa from three pigs was homogenized together with 5 mmol/L EDTA-phosphate buffer (pH 7.0) and centrifuged at 4°C for 60 minutes at 60,000 × *g*, and the resulting pellet was collected and stored at −20°C until use. The pellet was rehomogenized with 10 mmol/L phosphate buffer (pH 7.0) and used for assay of the activities of sucrase, maltase, isomaltase, trehalase, lactase, and glucoamylase by the methods of Caspary and Graf[5] and Dahlqvist.[8] The standard assay mixture contained 150 μL 100-mmol/L maleate buffer (pH 6.8), 25 μL substrate solution (200 mmol/L sucrose, maltose, trehalose, and lactose, 100 mmol/L isomaltose, and 20 mg/mL soluble starch, for assay of sucrase, maltase, trehalase, lactase, isomaltase, and glucoamylase activity, respectively), and 50 μL of a test substance solution (final concentration, 1 and 10 mmol/L for L-arabinose, D-arabinose, L-arabinobiose, D-xylose, L-xylose, and D-xylulose and 3 or 10 μmol/L for acarbose). The reaction was initiated by addition of 25 μL of appropriate dilutions of intestinal mucosa preparations (total assay vol, 250 μL) and performed for 15 minutes at 37°C, and then the glucose concentration of the reaction mixture was determined. Specific activity was calculated as micromoles of substrate hydrolyzed per milligram protein within 1 minute. For amylase assay, pancreases were removed from five mice and homogenized together with 10 mmol/L, phosphate buffer (pH 7.0), and the homogenate was used for amylase assay with the method of Whelan.[9]

Kinetic Analysis of Sucrase Inhibition by L-Arabinose

To learn the mode of the inhibitory effect of L-arabinose on intestinal sucrase activity, mucosal homogenate prepared from the porcine intestines mentioned earlier was incubated with increasing concentrations of sucrose in the absence and presence of two concentrations of L-arabinose (1 and 3 mmol/L) or acarbose (0.62 and 1.55 μmol/L). Doses of L-arabinose were selected based on results of the inhibitory effect on sucrase activity by 1 and 10 mmol/L L-arabinose (first experiment). Doses of acarbose were

determined according to the results of Caspary and Graf.[5] Results were plotted according to Lineweaver-Burk.

Effects of L-Arabinose, D-Xylose, and Acarbose on Blood Glucose Level After Sucrose or Starch Loading in Mice

To evaluate the potency of L-arabinose, D-xylose, and acarbose in vivo, the effects of these substances on plasma glucose after sucrose or starch loading were examined.

Five-week-old male ICR mice were purchased from Charles River Japan (Atsugi, Japan). Six mice in each group were fasted overnight for 16 hours before the experiment. L-Arabinose (0, 12.5, 25, and 50 mg/kg), D-xylose (0, 12.5, 25, and 50 mg/kg), or acarbose (0, 0.625, 1.25, and 2.5 mg/kg) was orally administered via gavage with 1 g/kg sucrose. In the preliminary study in mice, 25 mg/kg L-arabinose, 25 mg/kg D-xylose, and 1.25 mg/kg acarbose were found to be effective for suppression of the blood glucose increase after sucrose loading, and thus we selected three doses that covered 25 mg/kg L-arabinose and D-xylose and 1.25 mg/kg acarbose, respectively. In the starch loading test, L-arabinose (0, 25, 50, and 100 mg/kg) or acarbose (0, 0.625, 1.25, and 2.5 mg/kg) was orally administered via gavage with 1 g/kg soluble starch. At 0, 15, 30, 60, and 120 minutes after loading, 10 μL blood was taken from the orbital sinus for glucose determination.

ED_{50} values were obtained as follows. First, the mean increase in blood glucose at 15, 30, 60, and 120 minutes after loading versus the basal value was plotted, and the area under the curve of the blood glucose increase was calculated. Second, the inhibition ratio for each dose to the control group was calculated as follows: inhibition ratio (%) = $(1 - T/C) \times 100$, where T is the area of blood glucose increase during 120 minutes in the treated group, and C is the area of blood glucose increase during 120 minutes in the control group. The area of blood glucose increase during 120 minutes was calculated by the area surrounded by the glucose curve and the X-axis using the trapezoidal rule. Third, ED_{50} and ED_{20} values for acarbose, L-arabinose, and D-xylose were obtained by the corresponding dosage with 50% inhibition and 20% inhibition, respectively.

Effect of L-Arabinose on Plasma Glucose and Insulin Levels After Sucrose Loading in Rats

Rats were used in the experiment to evaluate the effects of L-arabinose on both plasma glucose and insulin. As in the previous experiments, 5-week-old male Wistar rats were purchased from Charles River Japan. The day before the experiment, a polyethylene catheter was inserted into the left jugular vein under ether anesthesia. The other end of the catheter was tunneled subcutaneously to exit the back of the neck. The catheter was filled with saline containing sodium heparin (200 U/mL) and plugged with stainless wire at the open end until the experiment. Rats were housed in individual cages after surgery. In the experiment, five rats in each group were fasted overnight for 16 hours. L-Arabinose (0, 50, and 100 mg/kg) was orally administered via gavage with 2.5 g/kg sucrose. L-Arabinose 50 mg/kg was found to be effective in suppressing the blood glucose increase after sucrose 2.5 g/kg had been administered to rats in the preliminary study; thus, we selected two doses including L-arabinose 50 mg/kg. At 0, 15, 30, 60, and 120 minutes after loading, 1 mL blood was taken from the catheter for determination of plasma glucose and insulin.

Influence of Consecutive Use of L-Arabinose on Glycemic Responses After Sucrose Loading in Mice

This experiment examined whether L-arabinose would be effective for plasma glucose if consecutively used. Eight-week-old male ICR mice were divided into two groups (n = 6): (1) control and (2) L-arabinose–treated. Sucrose was administered orally to the control group via gavage once per day at a dose of 1 g/kg for 3 weeks. The L-arabinose–treated group was administered 25 mg/kg L-arabinose simultaneously with 1 g/kg sucrose once per day for 3 weeks. Once per week, mice were fasted overnight for 16 hours. After administration of 1 g/kg sucrose or 25 mg/kg L-arabinose with 1 g/kg sucrose, 10 μL blood was taken from the orbital sinus at 0, 15, 30, 60, and 120 minutes to determine glucose levels. The area of the blood glucose increase during 120 minutes was calculated by the method described earlier.

Absorption of L-Arabinose in Rats

Five-week-old male Wistar rats were divided into seven groups (n = 3). Rats from six groups were orally administered via gavage a single dose of L-arabinose at 1,000 mg/kg. Blood samples were taken from each group by cardiac puncture at 0.5, 1, 2, 4, 8, and 24 hours, respectively, after the administration, and then plasma concentrations of L-arabinose were measured. L-Arabinose concentration in plasma from nontreated rats (n = 3) was designated as the basal value.

Urinary Excretion of L-Arabinose and D-Xylose in Rats

Five-week-old male Wistar rats were divided into three groups (n = 6) and orally administered L-arabinose (1,000 mg/kg) or D-xylose (1,000 mg/kg) via gavage. The control rats received water in the same manner. Immediately after administration, rats were housed individually in metabolic cages and allowed free access to tap water and diet. Urine flow during 0 to 24 hours was collected to determine L-arabinose and D-xylose concentrations. The urine of control rats was similarly collected for determination of basal excretion of L-arabinose and D-xylose.

Analytical Methods

Glucose was determined by the glucose oxidase method (Glucose-B Test; Wako Pure Chemical Industries, Osaka, Japan), and plasma insulin was measured by the enzyme immunoassay (EIA) method (Glazyme Insulin-EIA Test; Wako). Protein was determined by the method of Lowry et al.[10] Plasma L-arabinose was determined enzymatically by the method of Sturgeon.[11] Urinary concentrations of L-arabinose and D-xylose were determined simultaneously by high-performance liquid chromatography (HPLC) under the following conditions: columns, Shodex Ionpack KS-801 (8 × 300 mm; Showa Denko, Tokyo, Japan) and Shodex Sugar SH-1011 (8 × 300 mm; Showa Denko); mobile phase, H_2O; flow rate, 1.0 mL/min; column temperature, 80°C; and retractive index by the detector.

Statistics

Data are expressed as the mean ± SEM. Comparisons were made using one-way ANOVA, with means testing by Dunnett's test when appropriate. *P* values less than .05 were considered significant. When the comparison was only between two groups, Student's *t* test was used.

RESULTS

Inhibitory Effect on α-Glucosidase Activities of Porcine Intestinal Mucosa and on Amylase Activity of Mice Pancreas

L-Arabinose (10 mmol/L) potently inhibited sucrase activity but showed no inhibition of maltase, isomaltase, trehalase, lactase, or glucoamylase activities of porcine intestinal mucosa, and did not inhibit amylase activity of the mice pancreas homogenate (Table 1). However, acarbose 3

Table 1. Inhibitory Effect on α-Glucosidase Activities of Porcine Intestinal Mucosa and on Amylase Activity of Mice Pancreas

α-Glucosidase Activity	Acarbose		L-Arabinose		D-Xylose	
	Dose (μmol/L)	Inhibition (%)	Dose (mmol/L)	Inhibition (%)	Dose (mmol/L)	Inhibition (%)
Porcine intestine						
Sucrase	3	97.3 ± 1.9	10	64.9 ± 0.8	10	57.6 ± 3.9
Maltase	3	88.9 ± 4.0	10	9.6 ± 1.2	10	12.0 ± 3.7
Isomaltase	10	11.2 ± 2.0	10	3.1 ± 1.8	10	3.1 ± 2.0
Trehalase	10	1.5 ± 2.2	10	0.3 ± 1.5	10	2.1 ± 0.8
Lactase	10	0.4 ± 1.6	10	−1.1 ± 2.3	10	−2.5 ± 4.1
Glucoamylase	3	99.4 ± 0.6	10	0.4 ± 1.6	10	0.1 ± 0.5
Mouse pancreas						
Amylase	10	59.9 ± 5.8	10	−0.6 ± 1.6	10	1.6 ± 3.9

NOTE. Values are the mean ± SEM of 3 experiments.

μmol/L inhibited the activities of sucrase, maltase, and glucoamylase of the intestinal mucosa, and also inhibited pancreatic amylase activity at a concentration of 10 μmol/L. Some pentoses and L-arabinose–related disaccharide were examined for effects on the sucrase activity of porcine intestinal mucosa. Neither D-arabinose, an optical isomer of L-arabinose, nor L-arabinobiose, a disaccharide, inhibited this activity. Among the stereoisomers of L-arabinose, D-xylose was as potent as L-arabinose, whereas the optical isomer L-xylose had no inhibitory effect (Table 2).

Kinetic Analysis of Sucrase Inhibition by L-Arabinose

Lineweaver-Burk plots of the results revealed that L-arabinose inhibited sucrase activity in an uncompetitive manner, whereas acarbose inhibited it in a fully competitive manner (K_i, 2.0 mmol/L and 1.1 μmol/L, respectively; Fig 1A and B). L-Arabinose had a 12.0-fold higher affinity for sucrase than for its natural substrate, sucrose.

Effects of L-Arabinose, D-Xylose, and Acarbose on Blood Glucose Levels After Sucrose or Starch Loading in Mice

L-Arabinose suppressed the increase of blood glucose dose-dependently after sucrose loading in fasted mice (Fig 2A), but showed no effect on this increase after starch loading (Fig 2B). Acarbose, in contrast, suppressed the increase of blood glucose in both sucrose and starch

Table 2. Inhibitory Effect of L-Arabinose and Related Sugars on Porcine Intestinal Sucrase Activity

Sugar	Dose (mmol/L)	Inhibitory Ratio (%)
L-Arabinose	1	12.9 ± 0.9
	10	56.2 ± 4.3
D-Arabinose	1	0.8 ± 0.3
	10	0.8 ± 0.4
L-Arabinobiose	1	0.7 ± 0.4
	10	0.3 ± 0.3
D-Xylose	1	14.3 ± 2.7
	10	52.1 ± 1.4
L-Xylose	1	0.3 ± 0.6
	10	0.6 ± 0.3
D-Xylulose	1	0.6 ± 0.3
	10	0.5 ± 0.8

NOTE. Values are the mean ± SEM of 3 to 5 experiments.

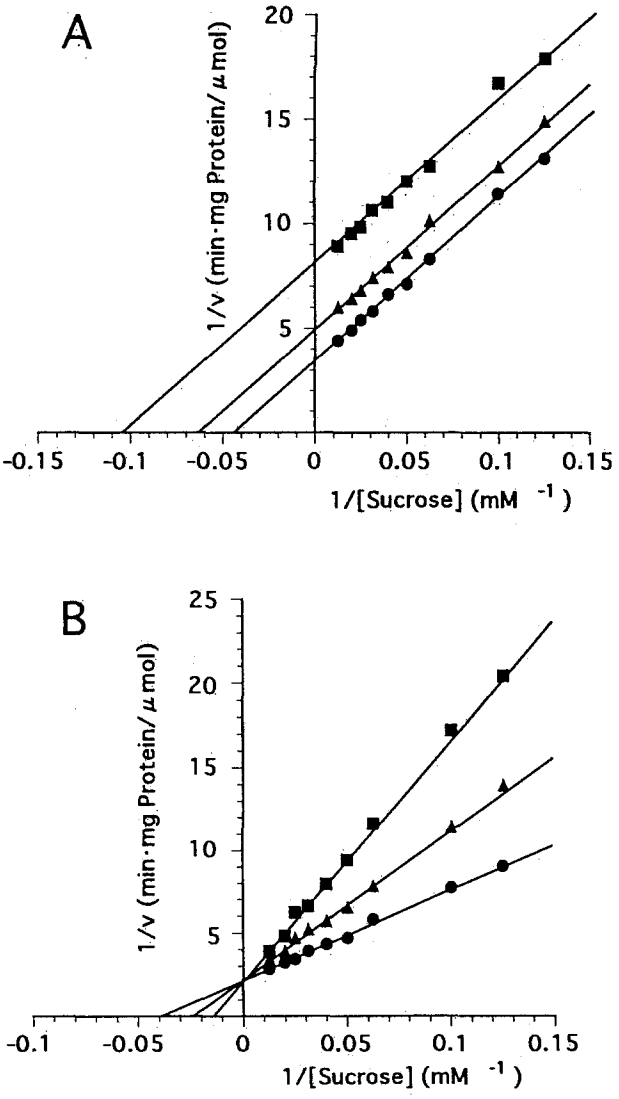

Fig 1. Kinetic analysis of sucrase inhibition by L-arabinose (A) and acarbose (B). Mucosal homogenates prepared from porcine intestine were incubated with increasing concentrations of sucrose in the absence and presence of inhibitor: (A) L-arabinose (▲) 1 mmol/L or (■) 3 mmol/L and (B) acarbose (▲) 0.62 μmol/L or (■) 1.55 μmol/L. (●) Assays without inhibitor. Results are plotted according to Lineweaver-Burk.

loading. The ED_{50} value can be estimated as around 35 mg/kg for L -arabinose and 1.1 mg/kg for acarbose in the sucrose-loading test. ED_{20} value can be estimated to be around 44 mg/kg for D-xylose and 18.5 mg/kg for L-arabinose. The former value was 2.4 times less potent than the latter (Fig 2A).

Effect of L-Arabinose on Plasma Glucose and Insulin Levels After Sucrose Loading in Rats

Basal values for plasma glucose and insulin in fasted rats were 76.2 ± 4.82 mg/dL and 2.2 ± 0.87 μU/ml, respectively. L-Arabinose (50 and 100 mg/kg) significantly suppressed the increase of plasma glucose levels after sucrose loading in fasted rats (Fig 3A): 15 minutes after ingestion, the increase was suppressed approximately 50% by both 50 mg/kg and 100 mg/kg L-arabinose. Significant suppression lasted from 15 to 60 minutes in the L-arabinose (100 mg/kg) group. L -Arabinose also significantly suppressed the increase in plasma insulin after sucrose loading (Fig 3B): 15 minutes after ingestion, the increase was suppressed 57% and 64% by 50 mg/kg and 100 mg/kg L-arabinose, respectively.

Influence of Consecutive Use of L-Arabinose on Glycemic Responses After Sucrose Loading in Mice

In the control group, the area of the blood glucose increase after sucrose loading significantly increased 2 and 3 weeks later (Fig 4); in the L-arabinose–treated group, the area of the glucose increase was significantly suppressed on the first day of the experiment. The area of the glucose increase in the L-arabinose–treated group also significantly increased after consecutive sucrose feeding; however, the suppression ratio was almost constant (28.3% ~ 32.2%).

Absorption of L-Arabinose in Rats

The basal concentration of L-arabinose in rat plasma was 5.4 ± 1.37 μg/mL. In this study, L-arabinose was determined enzymatically using galactose dehydrogenase (EC 1.1.1.48). Galactose dehydrogenase catalyzes the following two reactions: (1) L-arabinose + NAD → L-arabinonic acid + NADH + H^+; and (2) D-galactose + NAD → D-galactonic acid + NADH + H^+: The basal value of 5.4 μg/mL is thus virtually the sum of the concentrations of L-arabinose and D-galactose in rat plasma. After oral administration of L-arabinose, plasma concentrations of L-arabinose were low, and 38.6 ± 2.0 μg/mL at 30 minutes was the highest value (Fig 5).

Urinary Excretion of L-Arabinose and D-Xylose in Rats

There was a significant difference between L-arabinose–treated and D-xylose–treated ingested rats for the ratio of urinary excretion to ingested dose (L-arabinose, 3.5% ± 0.13%; D-xylose, 22.8% ± 0.63%; Fig 6). This result suggested that L-arabinose is a much less absorbable pentose than D-xylose.

DISCUSSION

We demonstrated in this study that L-arabinose selectively inhibited the sucrase activity of porcine intestinal mucosa in an uncompetitive manner. We also showed that L-arabinose suppressed the increase of blood glucose dose-dependently after ingestion of sucrose but did not suppress this increase after starch ingestion in mice. L-Arabinose also suppressed the increase of plasma glucose and insulin after sucrose ingestion in rats. Semenza and Balthazar[12] reported a similar inhibition of sucrase activity by L-arabinose in rabbit in vitro; however, they did not examine the selectivity. In addition, no known in vivo studies have been reported until this one.

Acarbose and other α-glucosidase inhibitors[2-7,13,14] are recognized as potent competitive inhibitors of the activities of intestinal glucoamylase, maltase, and sucrase, and it has also been shown that acarbose has an inhibitory effect on pancreatic amylase activity.[6] We confirmed these results in this study. In many advanced countries, starch accounts for approximately 60%, sucrose 30%, and lactose 10% of ingested carbohydrates.[5] Since the digestion of both starch and sucrose is delayed by acarbose and its analogs, these α-glucosidase inhibitors have a valuable therapeutic effect

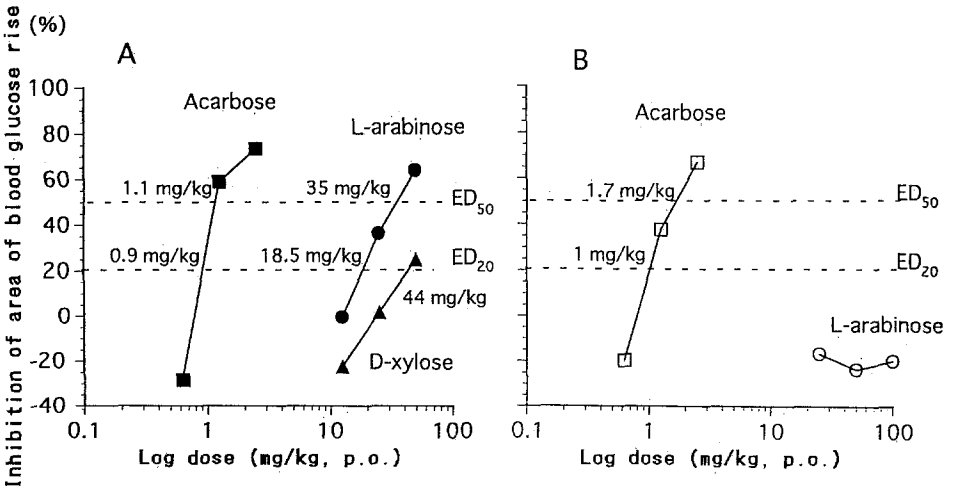

Fig 2. Effects of L-arabinose, D-xylose, and acarbose on glycemic responses after sucrose loading in normoglycemic mice. Inhibitory ratios of the area under the curve of the blood glucose increase in 1 g/kg sucrose loading (A: ●, L-arabinose; ▲, D-xylose; ■, acarbose; n = 6, respectively) and in 1 g/kg starch loading (B: ○, L-arabinose; □, acarbose; n = 6, respectively) are shown.

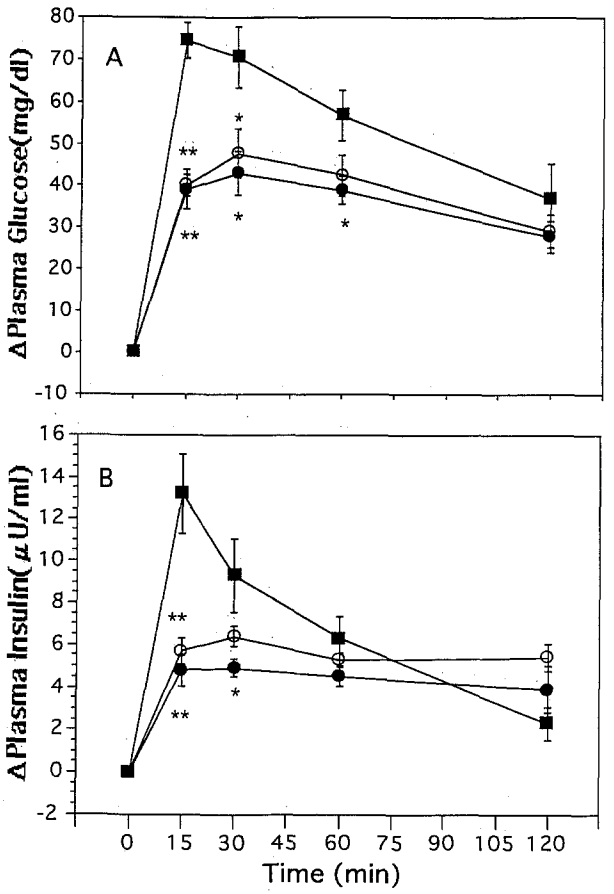

Fig 3. Effects of L-arabinose on plasma glucose (A) and insulin (B) after sucrose loading in rats. Overnight-fasted Wistar rats were given 2.5 g/kg sucrose (■), and blood samples were taken at 0, 15, 30, 60, and 120 minutes after the loading to determine plasma glucose and insulin. L-Arabinose (○, 50 mg/kg; ●, 100 mg/kg) was administered simultaneously with sucrose. Values are the mean (n = 5) ± SEM. **$P < .01$, *$P < .05$: L-arabinose–treated group *v* control group.

in reducing postprandial hyperglycemia in diabetic patients.[2,3]

The major portion of dietary carbohydrate is starch, but sucrose is used in many foods as a sweetener or other ingredient, and its daily intake is large in many advanced countries. It has been shown that Tris competitively inhibits intestinal sucrase and suppresses the increase in blood glucose after sucrose ingestion by rats and human subjects[15]; however, Tris is of no practical use, because of its unpleasant taste and the necessity of large doses. Thus, there are no known inhibitors of practical use that selectively inhibit intestinal sucrase and delay the ingestion of sucrose.

L-Arabinose is a natural pentose with a sweet taste. In this study, it suppressed the increase in blood glucose at a low dose after sucrose ingestion (ED_{50}, 35 mg/kg) but showed no suppression of the increase in blood glucose after starch loading in mice. Furthermore, in our preliminary study, we found that approximately 25 to 100 mg/kg L-arabinose showed no effect on the blood glucose increase in a glucose loading test (1 g/kg) in mice and that there was no delay of the peak, in contrast to the effects of guar gum in the same test, which suppressed the blood glucose increase and delayed the peak. These results suggest that L-arabinose does not affect the glucose absorption or gastric emptying. Among the pentoses structurally related to L-arabinose, D-xylose was equally potent in its inhibitory effect on the sucrase activity of porcine intestinal mucosa in this study. Neither D-arabinose nor L-xylose inhibited sucrase activity, nor did L-arabinobiose, a dimer of L-arabinose. These results suggest that some stereospecific interaction may exist among the intestinal sucrases, the inhibitory pentoses, and the substrate to elicit the inhibitory action of L-arabinose or D-xylose.

Although D-xylose was as potent as L-arabinose in its inhibitory effect on sucrase activity in vitro, its potency for suppression of the blood glucose increase following sucrose ingestion was 2.4 times lower. The difference in potency between the two substances in vivo might depend on the difference in their absorption ratio after oral administration from the small intestine. To explore this possibility, we compared the urinary excretion rates after oral administration, and found that the excretion ratio of D-xylose was 6.5 times greater than that of L-arabinose. Thus, we can conclude that L-arabinose is less absorable than D-xylose, and an effective L-arabinose concentration in the small intestine can be maintained while the concentration of D-xylose in the small intestine may rapidly decrease, resulting in a weaker in vivo effect of D-xylose. A similar difference in the absorption between D-xylose and L-arabinose has been demonstrated in other species.[16-18] Another interesting biological difference between them was reported by Segal and Foley,[19] who demonstrated that D-xylose was catabolized to respiratory $^{14}CO_2$ to some extent but that L-arabinose gave rise to negligible amounts of respiratory $^{14}CO_2$ in a study of the metabolic fate of injected ^{14}C-labeled pentoses in man. These findings sug-

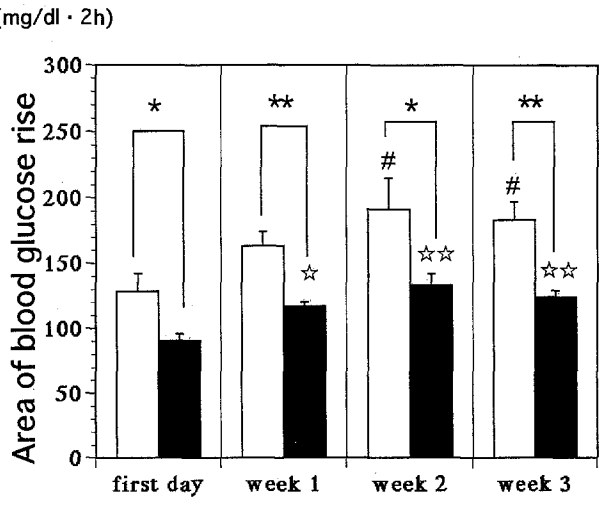

Fig 4. Influence of consecutive ingestion of sucrose on the glycemic response after sucrose loading and the effect of L-arabinose in mice. Values are the mean ± SEM (n = 6) for the area of the blood glucose increase in controls (□) and L-arabinose–treated rats (■). **$P < .01$, *$P < .05$: L-arabinose–treated group *v* control group. #$P < .05$ *v* first day in control group. ☆☆$P < .01$, ☆$P < .05$: *v* first day in L-arabinose–treated group.

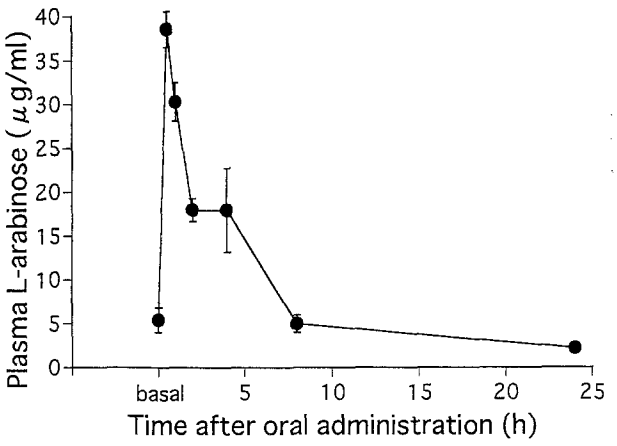

Fig 5. Plasma concentration of L-arabinose after oral administration in rats. L-Arabinose concentration in plasma from a nontreated group of animals (n = 3) is shown as the basal value. Values are the mean ± SEM.

gest that a larger quantity of D-xylose, greater than 22.8%, was excreted in the urine which had been absorbed from the intestine. The concept of good absorbability of D-xylose is consistent with the results of previous reports in which D-xylose was absorbed by an active transport process at low concentration.[20,21]

Although the in vitro inhibitory potency of L-arabinose (K_i, 2.0 mmol/L) was low compared with acarbose (K_i, 1.1 μmol/L), we found that L-arabinose possessed a more potent in vivo effect: the ED_{50} value of L-arabinose and acarbose was 35 and 1.1 mg/kg, respectively, in the sucrose loading test. One possible reason that the in vivo effect of L-arabinose is more potent than expected from in vitro experiments is its biochemical stability in the gastrointestinal tract and uncompetitive manner of sucrase inhibition, in addition to its low absorbability in the small intestine.

Consecutive sucrose feeding caused a significant increase of the areas under the curve of blood glucose. This might be due to an increase of intestinal sucrase activity, as reported in the sucrose-fed rat.[22,23] Despite the increase in the area under the blood glucose curve after consecutive sucrose feeding, L-arabinose in the present study showed a stable, significant suppression of the glucose areas for 3 weeks, probably by inhibiting sucrase activity in the small intestine.

L-Arabinose is prevalent in nature as a component of plant gums and sugar beet. It has a potent, sweet taste and low toxicity; the LD_{50} value was approximately 20 g/kg orally in mice in our preliminary test. L-Arabinose caused no diarrhea at a dose of 1 g/kg in the rat study, nor was diarrhea observed in a human study in which eight healthy volunteers ingested 2 g L-arabinose with 50 g sucrose (Yao T, et al, unpublished data, October 1993). Although a definite therapeutic value of acarbose and other known α-glucosidase inhibitors in diabetic patients has been demonstrated, unpleasant side effects associated with incomplete absorption of dietary carbohydrate, ie, flatulence, abdominal discomfort, diarrhea,[2,3] and ileus-like symptoms,[24] have been reported. These side effects may be due to the potent inhibition of amylase, maltase, and sucrase, which in turn inhibits the digestion of both sucrose and starch. As shown in this study, L-arabinose only inhibited intestinal sucrase activity and specifically suppressed the blood glucose increase after sucrose ingestion, resulting in little adverse effect on the gastrointestinal tract.

There are three types of reversible enzyme inhibition: (1) competitive, (2) uncompetitive, and (3) noncompetitive. Our kinetic study of sucrase inhibition demonstrated that this was induced by L-arabinose in an uncompetitive manner and by acarbose in a competitive manner. A competitive manner is defined as one in which an inhibitor binds to the catalytic site of the enzyme and competes with the primary substrate, so that the activity of the enzyme is inhibited. An uncompetitive inhibition is defined as one in which an inhibitor binds only to an enzyme-substrate complex and inhibits its activity. An inhibition other than these two is defined as noncompetitive. Acarbose, a widely investigated α-glucosidase inhibitor, has been reported to be a competitive inhibitor of intestinal maltase, glucoamylase, and sucrase. We confirmed in this study that acarbose inhibited sucrase in a competitive manner, and found that L-arabinose selectively inhibited sucrase activity in an uncompetitive manner. Based on the results, we speculate that L-arabinose possesses a selective high affinity for intestinal sucrase-sucrose complex and forms a triple complex with a low sucrase activity, resulting in inhibition of sucrase.

In summary, the present study demonstrated that L-arabinose selectively inhibits intestinal sucrase activity in an uncompetitive manner and suppresses the plasma glucose increase after sucrose ingestion. Thus, L-arabinose may be useful in preventing postprandial hyperglycemia in diabetic patients when foods containing sucrose are ingested. This is the first report indicating selective inhibition of sucrase activity by L-arabinose both in vitro and in vivo.

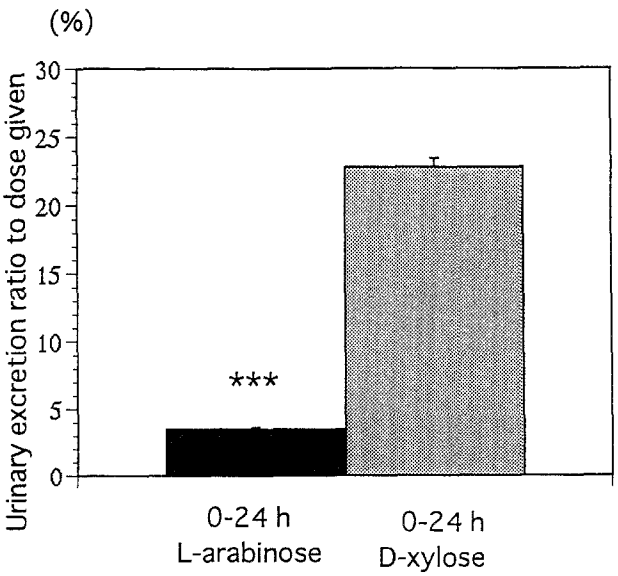

Fig 6. Urinary excretion of L-arabinose and D-xylose in rats. Values are the mean ± SEM. ***$P < .001$: L-arabinose group *v* D-xylose group.

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NUTRITION

Comparison of acute absorption of commercially available chromium supplements

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Abstract

Chromium (Cr) supplements are available as picolinate, nicotinate or chloride (the latter primarily in multivitamin–mineral supplements). The picolinate form has been reported to be the best absorbed and most efficacious, but some reports question which form has superior absorption. The present study examined acute Cr absorption, based on 24 h urinary Cr values, for picolinate, two types of nicotinate, and chloride in young adult, non-overweight females. College-aged women were given 200 μg of Cr as each of the four supplement types in random order accompanied by a small standardized meal, separated by at least a week washout. Cr picolinate produced significantly higher 24 h urinary Cr than either of two nicotinate supplements or Cr chloride given in a multivitamin–mineral supplement. This difference was seen for absolute values of the urinary Cr and for percent increases. In conclusion, based on an indirect measure of acute absorption, Cr picolinate was superior to three other Cr complexes commonly sold as supplements.

Keywords: Chromium picolinate; Chromium nicotinate; Chromium chloride; Chromium supplements

Introduction

Trivalent chromium (Cr) in trace amounts is considered essential in human nutrition [1]. Several studies have reported beneficial effects of Cr intake on glucose tolerance and/or lipid metabolism (i.e. [2–4]). Nevertheless, there are indications that dietary Cr intake world-wide can often be sub-optimal [5,6].

The most widely accepted nutritional paradigm emphasizes the procurement of vitamins and minerals through food. However, obtaining adequate Cr from diet alone can sometimes be challenging for a variety of reasons. First, a diet can consist of a variety of foods but still emphasize foods low in Cr. In fact, few food groups are rich in Cr, with best sources restricted to barley, Brewer's yeast, mushrooms, organ meats, ham, broccoli, oysters, and a few cereals [7,8]. In addition, food Cr content can vary with the soils in which they were cultivated [9], and foods can lose dietary Cr during processing and cooking [10]. Also, phytates, dietary fiber, antacids, high sugar intake, and other trace elements can reduce the absorption of Cr [11,12]. Moreover, Cr requirements may be raised by chronic illness including diabetes, aging, or stress [1,5,13–15].

For these various reasons, Cr supplementation may be useful for certain individuals. However, different Cr complexes used for supplementation are not necessarily absorbed equally (rev in [6]). Therefore, if health-care professionals decide to recommend Cr supplements in certain situations, these professionals need guidance as

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to what complex to recommend. Similarly, if researchers want to use Cr supplements to study health effects of increased Cr intake, these researchers need to know which Cr complex to use. Therefore, a study was undertaken to compare relative absorption of different Cr complexes in commercially available supplements.

Some comparisons have already been done, but have not settled the issue of what Cr complex is best absorbed. For example, in one animal study, radioactive Cr is used to show superior absorption of Cr picolinate versus other Cr complexes [16]. In contrast, a different animal study with radioactive Cr shows better tissue retention for Cr nicotinate than for Cr picolinate [17]. The latter study, though interesting, should be interpreted carefully. For example, the results vary with tissue and time point, and the total Cr intake is not overly high for Cr (which may influence relative absorption). Also, the retentions are based on fractional absorptions, not absolute labeling. Although the fractional approach has certain advantages for error corrections, there are also limits to how the data can be applied. For example, total Cr retention in a tissue for one type complex could exceed the other, but still have a lower fractional retention.

In human work based on acute urinary Cr excretion [18], Cr picolinate shows better absorption than Cr chloride, which shows better absorption than Cr nicotinate. In this study, a Cr-histidine complex shows the best absorption, though no commercially available supplement of this form currently exists. This study is very informative for comparison of different supplement types for acute absorption. However, before a conclusion can be reached about absorption differences among different Cr complexes, some issues require addressing. The following list notes such issues plus mentions how the present study will address them:

- The former study examines only three people of each gender with undisclosed characteristics; a stronger evaluation of relative absorption of different Cr complexes can be made with more people with some traits in common. The present study examined 12 people of one gender in a narrow age and BMI range.
- This last study gives Cr on an empty stomach, but most people take supplements with food. This study gave Cr with food.
- Cr chloride is the form of Cr found in most Cr-containing multivitamin–mineral studies, but this form is not generally found in stand-alone Cr supplements; the last study evaluates Cr chloride in the latter rather than in the former state. The current study examined Cr chloride as part of a multivitamin–mineral supplement.
- The last study examines one commercial version of Cr nicotinate, but two types of nicotinate Cr supplements are marketed. The current study examined both.
- The last study administers the supplement types in just one order for each subject; a random order among subjects is the more conventional approach. The current study used the random order approach.

The present study utilized urinary Cr collected for 24 h after a single 200 μg dose of various commercial Cr supplement forms. The dose choice carried some uncertainty because the optimal Cr dose for different situations remains poorly defined. The 200 μg dose was chosen because it is the lower end of what has been typically used in Cr supplementation studies (rev in [6]). A 24 h urinary Cr was selected since the majority of the absorbed Cr is excreted relatively quickly in the urine [19], and according to stable isotope studies, the acute increase in urinary Cr after a single Cr dose does not reflect tissue losses [20] nor Cr status [21]. This approach has become an accepted measure of Cr absorption and has been used in several human studies (i.e. [22–24]). On the other hand, serum or plasma measures after single Cr dosing are not overly useful. This is because the time course and magnitude for increases in values vary greatly among subjects [24].

Materials and methods

Supplements

The supplements used were as follows:

Cr chloride: as part of the multivitamin–mineral product Fortify (Kroger).
Cr polynicotinate, also called GTF Chromium (Interhealth).
Cr nicotinate-glycinate, also called Chelavite (Albion).
Cr picolinate (Nutrition 21).

All supplements used were the commercially available forms purchased from a local store or were provided directly by the manufacturer. All products were within the expiry date window during use in the study.

Subjects and treatments

This study protocol was approved by the Biomedical Sciences Human Subjects Institutional Review Board of The Ohio State University. Subjects gave informed written consent. Twelve healthy female subjects aged 19–22 yr (19.1 ± 1.1, mean $\pm$ SD) were recruited from the student population at The Ohio State University. All subjects had BMI in the normal range (18.5–24.3, 21.4 ± 1.9, mean $\pm$ SD). Each subject was given each of four different supplements with at least a 1-week

washout between successive dosings. The dose was 200 μg Cr, which was given at the research site with a serving of macaroni and cheese, a serving of canned pears without added sugar, and one can of diet soft drink. Subjects collected urine for the next 24 h. Subjects also donated a 24 h urine sample prior to any supplementation. Subjects were instructed to eat a generally consistent diet for the 24 h before each supplement ingestion. Subjects were not consumers of Cr-containing supplements at the time of study participation. The order of supplement administration was randomized for each subject who was blinded as to its identity.

Cr and statistical analysis

Cr was determined in urine samples in triplicate by ICP-MS at Trace Element Research Laboratory in the Ohio State University School of Earth Sciences directed by Dr. John Olesik. An ELAN 6100^{plus} Inductively Coupled Plasma Mass Spectrometer with Dynamic Reaction Cell (ICP-DRC-MS, Perkin-Elmer Sciex, Norwalk, CT, USA) was used to measure the urine samples using a method similar to that described by Nixon et al. [25]. Ammonia (99.999% Electronic Grade, Scott Specialty Gases, Plumsteadville, PA, USA) was introduced into the reaction cell to reduce the signals due to ArC^+, ClO^+, and $ClOH^+$. An ammonia gas flow rate of 0.5 Ar-equivalent mL/min was used. The RPq value of the DRC was set to 0.45 to prevent undesired product ions from the reactions with ammonia and other background or elemental ions at the analyte mass. Detection parameters were: 2500 ms integration time (100 ms dwell time, 5 sweeps/reading, 5 readings/replicate), 5 replicates. Standards were made in 2% v/v double-distilled nitric acid (GFS Chemical, Powell, OH, USA) by serial dilution from a Cr standard (CPI International, Santa Rosa, CA, USA). Concentrations based on ^{52}Cr were in good agreement with those based on ^{53}Cr and were normalized to the measured Sc internal standard concentration in each sample. The background equivalent of the reagent blank was 0.02 ng/mL.

Statistical analysis was done by Jump 3.1, SAS Institute, Cary, NC, USA.

Results

Among Cr supplements that are currently commercially available, Cr picolinate produced the highest urinary Cr readings (Table 1). The percent increase above baseline was very low for Cr chloride, and high for Cr picolinate compared to the other current commercially available supplements (Fig. 1). In fact, urinary Cr for Cr picolinate was almost 16 times higher than for Cr chloride, and over twice that of either of the two nicotinate complexes.

Table 1. Acute chromium (Cr) supplementation effects on 24 h urinary Cr values

Cr chloride	154 ± 21^a
Cr polynicotinate	339 ± 58^a
Cr nicotinate-glycinate	276 ± 78^a
Cr picolinate	834 ± 160^b

Cr supplements were given as a single dose of 200 μg just prior to collection of 24 h urine samples. Values are means ± SD in ng/day. Different superscripts connote statistically significant differences (ANOVA + Tukey, $p < 0.05$).

Discussion

This work reinforces the concept that Cr picolinate is the best absorbed among Cr supplements that are currently commercially available. This observation especially reinforces recent results from Anderson's group [18], though in the present study, the observation was demonstrated under some different circumstances than the previous work. The present work also suggests that Cr chloride from multivitamin–mineral supplements does not provide substantial amounts of Cr to people.

Both the present study and recent work of Anderson's group [18] do not support the concept that Cr nicotinate is better absorbed than Cr picolinate. The present study examined two versions of Cr nicotinate. As noted above, the concept of superior absorption of Cr nicotinate is based on a rat study [17] subject to multiple interpretations.

It should be noted that the present study assessed Cr uptake rather than a functional effect such as lowering blood sugar. However, the present study's relative results do resemble the relative results obtained for uptake of glucose or leucine in cultured human muscle cells pre-cultured with Cr picolinate, Cr chloride, or Cr nicotinate [26]. In that study, insulin binding and internalization is also greater with Cr picolinate.

Even with the relatively high bioavailability of Cr in Cr picolinate, long-term exposures to this complex have not generally shown adverse health effects in animals or humans. For example, Cefalu et al. [27] report that Cr given to humans at 1000 μg/d as Cr picolinate for 8 months is without adverse effects. Also, up to 15 mg/kg/d of trivalent Cr as Cr picolinate has been administered to rats for 20 weeks with no signs of toxicity, even though liver Cr levels rise 10-fold [28].

Conclusions

Based on urinary Cr, Cr picolinate was absorbed to a considerably better than either of two nicotinate-based

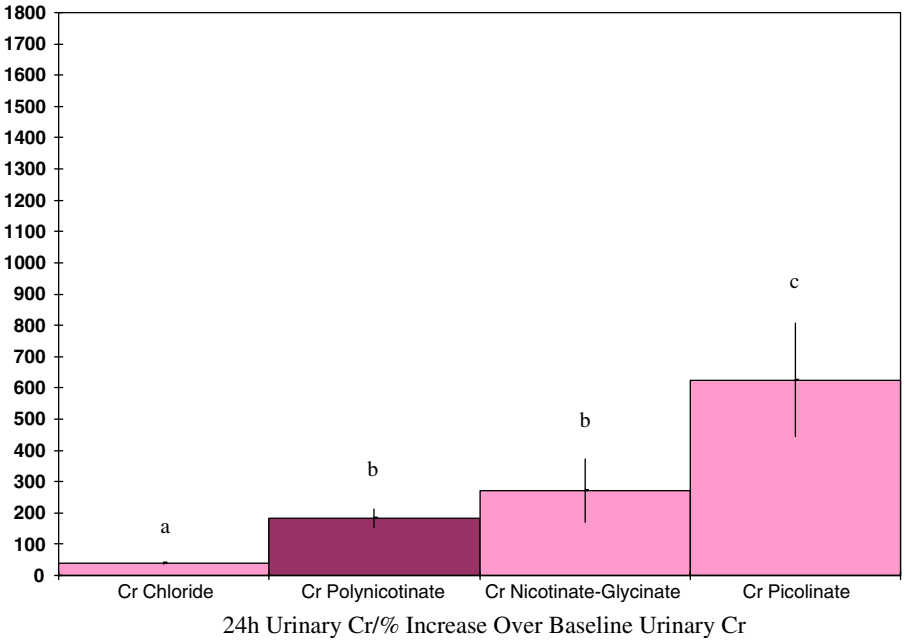

Fig. 1. Acute chromium (Cr) supplementation effects on percent increases over baseline for 24 h urinary Cr values. Cr supplements were given as a single dose of 200 μg just prior to collection of 24 h urine samples. Different superscripts connote statistically significant differences (ANOVA + Tukey, $p < 0.05$).

preparations or Cr chloride, which showed very little absorption.

Acknowledgments

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Original Research

Effects of Acute Chromium Supplementation on Postprandial Metabolism in Healthy Young Men

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Key words: chromium, glycemic index, glycemia, postprandial metabolism, glucose, insulin

Background: Chromium (Cr) potentiates the action of insulin in the cell and improves glucose tolerance after long-term supplementation.

Objective: We hypothesized that Cr may also have acute effects and might be beneficial in lowering the glycemic index of a meal.

Methods: We studied the effects of short-term Cr supplementation using a randomized crossover design. Thirteen apparently healthy, non-smoking young men of normal body mass index performed three trials each separated by one week. Test meals, providing 75 g of available carbohydrates, consisted of white bread with added Cr (400 or 800 μg as Cr picolinate) or placebo.

Results: After addition of 400 and 800 μg Cr incremental area under the curve (AUC) for capillary glucose was 23% ($p = 0.053$) and 20% ($p = 0.054$), respectively, lower than after the white bread meal. These differences reached significance if the subjects were divided into responders (n = 10) and non-responders (n = 3). For the responders AUC after 400 and 800 μg Cr was reduced by 36% and 30%, respectively (Placebo 175 $\pm$ 22, Cr400 111 $\pm$ 14 ($p < 0.01$), Cr800 122 $\pm$ 15 mmol $\cdot$ min/L ($p < 0.01$)). Glycemia was unchanged after addition of Cr in the non-responders. Responders and non-responders differed significantly in their nutrient intake and eating pattern, and total serum iron concentration tended to be lower in the responder group ($p = 0.07$).

Conclusions: Acute chromium supplementation showed an effect on postprandial glucose metabolism in most but not all subjects. The response to Cr may be influenced by dietary patterns.

INTRODUCTION

According to the 1998 World Health Report of the WHO the incidence of non-insulin dependent diabetes mellitus (NIDDM) will more than double from 143 million in 1997 to 300 million in 2025 [1]. Along with other environmental risk factors, nutrition plays an important role in the etiology of NIDDM. The type of carbohydrates and the glycemic response to a meal may be important risk factors, with growing evidence that high-glycemic diets increase the risk of developing insulin resistance and ultimately NIDDM in later life [2,3]. On the other hand, low-glycemic diets may protect against NIDDM [4,5]. Several intervention studies have indicated that low-glycemic diets may improve blood glucose control and insulin sensitivity [6–9]. Also, two large prospective studies have shown associations between low-glycemic diets and a lower risk of NIDDM for women [2] and men [3]. Lowering the glycemic response to a meal or a diet may therefore represent an important preventive approach in delaying the onset of insulin resistance and NIDDM. The trace mineral chromium (Cr) might have an effect on glycemia, since it influences carbohydrate metabolism by potentiating the action of insulin in the cell. Cr has been shown to normalize or improve glucose tolerance in hypoglycemics [10], in hyperglycemics [11], and in subjects with NIDDM [12–14].

Most studies investigating the metabolic effects of Cr used supplementation periods of several weeks or even months. There are only few data on the effects of a short-term

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supplementation on the metabolism [15,16]. Cr is rapidly absorbed and the maximal blood concentration is reached within 90 min after ingestion [17]. An acute effect of Cr may therefore be expected shortly after intake.

Cr functions as a nutrient and will only benefit those with a deficiency [18]. Subjects with normal glucose tolerance and no signs of Cr deficiency do not seem to respond to supplementation [11,19]. The Food and Nutrition Board of the U.S. National Academy of Sciences recently set the Adequate Intake for Cr at 35 μg/d for men and 25 μg/d for women [20]. These recommendations were based on estimated mean intakes, as the Board concluded that there was not enough scientific evidence to set an Estimated Average Requirement. Just one year earlier the Nutrition Societies of Germany, Austria, and Switzerland set the reference intake for adults at 30–100 μg/d [21]. These differing values reflect the existing uncertainty about the exact Cr requirements. As the estimated average Cr intake seems to be on the low side of the recommended intake, there might be individuals with marginal Cr status even in the healthy population and these could possibly benefit from supplemental Cr. We hypothesized that single doses of Cr given to young, healthy men would reduce glycemia after a high-glycemic meal.

The aim of this study was to investigate the effects of acute Cr supplementation (400 and 800 μg) on postprandial carbohydrate metabolism after a high-glycemic meal and to evaluate which amount of Cr would be more beneficial.

SUBJECTS AND METHODS

Subjects

Thirteen seemingly healthy, nonsmoking males aged 24.7 $\pm$ 0.9 years (mean $\pm$ SEM) and with normal body mass indexes (22.5 $\pm$ 0.5 kg/m^2) participated in the study. They had no family history of diabetes and did not use any medication nor take any nutritional supplements for the last two months before and until completion of the study. The subjects performed only moderate amounts of physical activity (exercise volume up to 1–2 h/wk). All participants were informed of the purpose of the study and signed an informed-consent form. The Scientific Ethics Committee of the Swiss Federal Institute of Technology in Zurich approved the study.

Study Design

The study was performed as a placebo-controlled, single-blind crossover experiment. Subjects underwent three different trials in random order. Test meals consisted of white bread with supplemental Cr (Cr400 and Cr800) or placebo (WB), each meal providing 75 g available carbohydrates. Participants were tested at least one week apart to avoid carry-over effects and all three trials were performed within four weeks. Each subject was told to maintain the same dietary habits and physical activity level until completion of the study. On the evening before each trial, subjects consumed a standardized rice meal providing approximately 3.9 MJ energy (180 g carbohydrates, 13 g fat, 23 g protein), and were told not to eat anything else until the next morning. In addition, subjects were asked not to ingest alcohol or caffeine containing drinks and foods, and were requested to avoid heavy physical exercise the day prior to each trial. Subjects were told to use local transport to get to the laboratory in order to avoid any intense physical exertion. They arrived at the laboratory after a 10–12 h overnight fast and then completed a short questionnaire assessing recent food intake and activity patterns. Three people were tested daily, beginning at 7:45, 8:00, and 8:15 a.m., respectively. Following the insertion of an indwelling catheter (Insyte-W, Becton Dickinson, Rutherford, NJ, USA) into an antecubital vein, a fasting blood sample was taken. After assessment of baseline values, test meals were given and eaten within ten minutes. Test meals consisted of commercially available white bread (140 g, 1.8 MJ) and provided 75 g of carbohydrates, 2 g of fat, and 13 g of protein. Postprandial blood samples were taken at 15, 30, 45, 60, 90, and 120 min after beginning of the meal. Finger-prick capillary blood samples for analysis of glucose were taken at the same times than the venous samples. After baseline assessment and 30 min before ingestion of the test meal, 400 or 800 μg Cr as Cr picolinate in pill form (GNC, Pittsburgh, PA, USA) was given with the Cr trials and a placebo (Hänseler AG, Herisau, Switzerland) with the WB trial. Placebo pills contained 120 mg lactose and 50 mg potato starch and could not be distinguished from the Cr pills.

Blood Sampling

Venous blood was collected in different tubes for whole blood (glycosylated hemoglobin (HbA_{1c})), plasma (glucose, insulin) and serum samples (iron, transferrin, ferritin). Tubes with blood for plasma samples were immediately placed on ice and then centrifuged at 3000 g for 15 min at 8° C. Tubes for serum samples were left at room temperature for 30 min to allow coagulation before centrifugation. Plasma and serum samples were stored at -20° C until analysis.

HbA_{1c} samples were analyzed within 24 h on a Cobas Integra 700 (Roche, Basel, Switzerland) using a Cobas Integra Hemoglobin A_{1c} kit. Plasma metabolites were analyzed enzymatically with a Cobas Mira analyzer (Roche, Basel, Switzerland) using commercial kits: glucose, iron and transferrin (Roche, Basel, Switzerland). Insulin was assessed by a standard radioimmunoassay kit (Pharmacia AB, Uppsala, Sweden). Capillary blood glucose concentrations were determined with a glucose oxidoreductase method with photometric end-point measurement using the Glucotrend® 2 system (Roche Diagnostics, Rotkreuz, Switzerland).

Diet Diary

The subjects were asked to take home and complete an open-ended estimated 5-day diet diary. A diet diary booklet containing instructions and four sets of color photographs was explained and then given to them. Each set of photographs showed three portion sizes of a common food item. They were provided to help the subjects estimate portion sizes. The instructions indicated that the participant should record the name, food brand, and amount of all foods eaten. The quantity of food eaten was estimated either in common household measures (e.g. tablespoons, cups), in whole units (e.g. number of apples, slices of bread), or in portion sizes (i.e. small, medium or large). Nutrient intake was calculated using the EBISpro software (University of Hohenheim, Hohenheim, Germany).

Insulin Sensitivity

The quantitative insulin sensitivity check index (QUICKI = 1/[log (fasting insulin) + log (fasting glucose)]) was used to assess insulin sensitivity [22].

Statistical Analysis

All results are expressed as means ± SEM and/or range. The general linear model (analysis of variance) was used to compare the pattern of the postprandial changes in blood variables between treatments. For significant overall differences between treatments, the data were further analyzed with Tukey's *post hoc* comparisons. Calculation of correlation coefficients between variables were performed by using the Pearson product-moment test. Glucose and insulin responses were calculated as incremental areas under the curve (AUC) using the trapezoidal method [23] and then compared between trials using paired *t*-tests with Bonferroni correction. The level of significance was set at $p < 0.05$. Data were analyzed by using the statistics software SYSTAT 9.01 (SPSS Inc., Chicago, IL, USA).

RESULTS

The HbA_{1c} concentration was normal for all subjects and ranged from 4.8% to 5.7% (5.4% ± 0.1%). We observed no significant differences between trials in the fasting concentration of all measured indexes and all fasting values were within the normal range for healthy people.

Glucose

There was a main effect of treatment for capillary ($p < 0.05$) but not venous ($p = 0.31$) glucose measurements (Fig. 1). Capillary glucose peak values were reached at 30 min for Cr400 and at 45 min for WB and Cr800, and were higher for WB then for Cr400 and Cr800 (7.4 ± 0.2 compared with 6.9 ± 0.2 ($p < 0.05$) and 7.0 ± 0.3 mmol/L ($p = 0.13$), respectively).

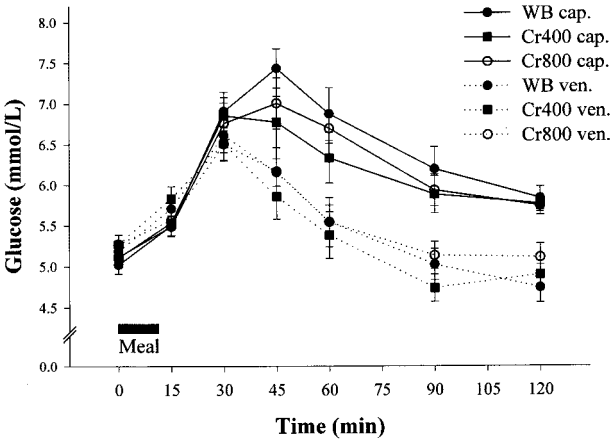

Fig. 1. Capillary and venous glucose concentrations after test meals providing 75 g available carbohydrates. Test meals were eaten within 10 min and consisted of white bread with placebo (WB, ●), white bread with 400 μg chromium (Cr400, ■) and white bread with 800 μg chromium (Cr800, ○). Values are means for thirteen subjects with standard errors of the means shown by vertical bars.

For venous glucose peak values were attained at 30 min and no differences in peak height between treatments were observed. For WB and Cr800 peak values were lower in venous compared with capillary glucose (both $p < 0.01$).

The AUC were lower for Cr400 and Cr800, respectively, than for the WB trial for capillary (23% and 20%) and venous glucose (29% and 15%). But these differences were not significant (Table 1). The differences reached significance for capillary glucose if the subjects were divided into a responder and a non-responder group. Responders were defined as subjects who showed a lower postprandial glycemia after both Cr trials compared with the WB trial, and non-responders as those who displayed no change or an increase after supplementation. Postprandial capillary glycemia and the glycemic indexes (GI) were significantly reduced after both Cr supplements for the responder group (n = 10, Cr400: $p = 0.04$, Cr800: $p = 0.03$, Table 1). The non-responders tended to show larger capillary glucose AUC after the Cr trials than after placebo, but these differences were not significant (Table 1, Cr400: $p = 0.14$, Cr800: $p = 0.15$). No differences between trials were observed for venous glucose (Table 1).

There was a positive correlation between the capillary glycemic response to WB and the extent of the glycemic response shown after supplementation with Cr400 ($r = 0.70$, $p = 0.008$) or Cr800 ($r = 0.67$, $p = 0.011$). That is, individuals with large glucose AUC after the WB trial showed large reduction in glycemia after Cr intake (Fig. 2).

Insulin

Insulin concentrations after the Cr trials were not significantly different from concentrations after the WB trial at all

Table 1. Capillary and Venous Glucose Area under the Curve (mmol · min/L), and Glycemic Index (in Parentheses) Values after Test Meals Consisting of White Bread with Placebo (WB), White Bread with 400 μg Cr (Cr400) and White Bread with 800 μg Cr (Cr800) for All Subjects, Responders and Non-Responders

	WB	Cr400	Cr800
All (n = 13)			
Capillary	163 ± 19 (100)	123 ± 14* (82)	130 ± 14* (86)
Venous	62 ± 9 (100)	44 ± 7 (84)	53 ± 8 (99)
Responders (n = 10)			
Capillary	175 ± 22 (100)	111 ± 14** (66**)	122 ± 15** (72**)
Venous	64 ± 12 (100)	39 ± 5* (79)	46 ± 5 (90)
Non-responders (n = 3)			
Capillary	121 ± 16 (100)	164 ± 26 (135)	158 ± 24 (130)
Venous	56 ± 11 (100)	61 ± 21 (101)	77 ± 24 (132)

* $p < 0.1$, ** $p < 0.01$: Cr400 and Cr800 compared with WB, value in the same row; mean ± SEM.

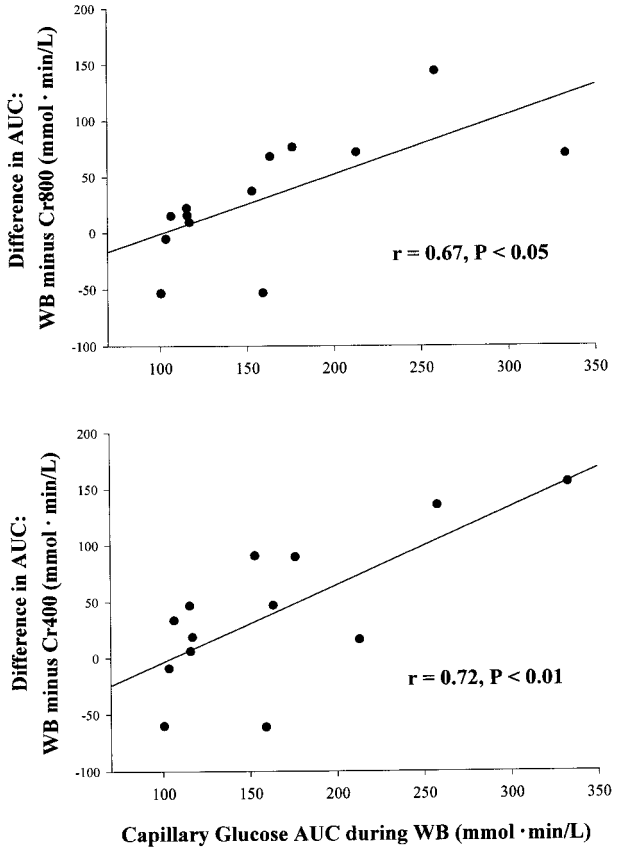

Fig. 2. Significant positive correlations were shown between the extent of the capillary glycemic response after the WB trial and the reduction in glycemia during the Cr400 and the Cr800 trial. The dots above zero (y-axis) represent the subjects having shown a decrease in glycemia after chromium supplementation (i.e. the responders), while the dots below zero symbolize the non-responders. AUC = area under the curve.

time points (Fig. 3). Accordingly, we observed no differences for the AUC (WB: 13520 ± 920, Cr400: 12840 ± 1240 (p = 0.53), and Cr800: 12600 ± 1330 (p = 0.34) pmol · min/L). Insulin sensitivity (QUICKI) was similar for responders and non-responders (0.68 ± 0.02 and 0.66 ± 0.01, p = 0.69).

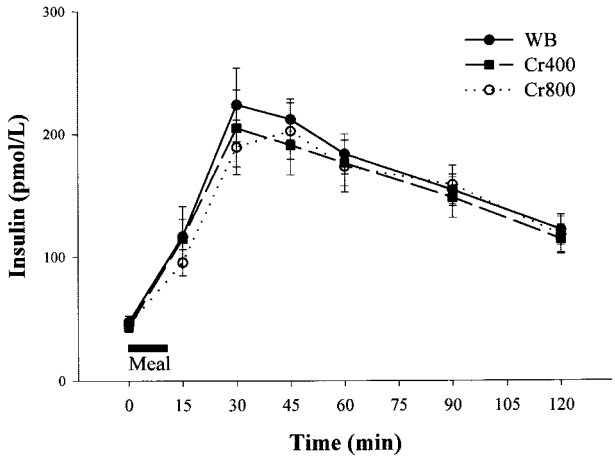

Fig. 3. Plasma insulin concentrations after test meals providing 75 g available carbohydrates. Test meals were eaten within 10 min and consisted of white bread with placebo (WB, ●), white bread with 400 μg chromium (Cr400, ■) and white bread with 800 μg chromium (Cr800, ○). Values are means for thirteen subjects with standard errors of the means shown by vertical bars.

Diet Records

Non-responders had a higher consumption of milk and meat products but tended to eat less fruit and vegetables than responders. This reflects itself in higher intakes of fat, protein, disaccharides, vitamin B_2 and B_{12} but lower intakes of fiber, folate and vitamin C for the non-responders compared with the responders (Table 2).

Iron Variables

Non-responders had significantly higher iron and transferrin concentrations in the blood compared with responders, while ferritin concentration and transferrin saturation were similar for both groups (Table 3).

Table 2. Comparison of Estimated Energy and Nutrient Intake in Responders and Non-Responders

	Responders (n = 10)		Non-Responders (n = 3)		*p*-value	DRI
	Mean	SEM	Mean	SEM		
Energy (MJ)	10.2	0.5	11.9	0.7	0.14	11.9
Protein (g)*	86 (14%)	3.2	100 (14%)	7.6	0.05	58
Fat (g)*	98 (36%)	6.3	120 (37%)	5.9	0.10	<30%
Carbohydrate (g)*	292 (49%)	16	336 (48%)	31	0.26	>55%
Monosaccharide (g)	43	8	39	15	0.86	—
Disaccharide (g)	75	5.7	114	5.0	0.01	—
Starch (g)	160	14	180	20	0.66	—
Fiber (g)	27	1.9	19	1.4	0.06	—
Vitamin B1 (mg)	1.5	0.1	1.5	0.1	0.89	1.2
Vitamin B2 (mg)	1.7	0.1	2.3	0.04	0.01	1.3
Vitamin B12 (μg)	2.5	0.3	4.7	0.9	0.02	2.4
Vitamin C (mg)	110	14	66	16	0.14	90
Folate (μg)	140	6.1	130	13	0.44	400
Vitamin E (mg)	14	1.0	12	1.9	0.42	15
Sodium (mg)	3400	310	2800	370	0.44	<2400
Potassium (mg)	3200	180	3000	150	0.58	—
Calcium (mg)	1200	110	1300	80	0.76	1000
Magnesium (mg)	420	17	360	24	0.16	400
Iron (mg)	15	0.7	13	1.5	0.30	10

* values in parentheses: percentage of energy; DRI: Dietary Reference Intake (Reference values of the German, Austrian and Swiss Nutrition Societies [21].

Table 3. Fasting Iron, Ferritin and Transferrin Concentrations and Transferrin Saturation in Responders and Non-Responders

	Responders (n = 10)		Non-Responders (n = 3)		*p*-value
	Mean	SEM	Mean	SEM	
Iron (μmol/L)	24	1.0	30	1.6	0.01
Transferrin (g/L)	2.4	0.07	2.7	0.03	0.04
Transferrin saturation (%)	37	2.2	42	3.5	0.27
Ferritin (μmol/L)	100	9.4	95	11	0.78

DISCUSSION

We tested the hypothesis that an acute single dose Cr supplementation would decrease glycemia after a high-glycemic meal in young, apparently healthy adults. A substantial reduction in postprandial glycemia was observed after addition of 400 as well as 800 μg Cr to a white bread meal compared with the white bread meal supplemented with a placebo (−23% and −20% for the incremental AUC, respectively). The reductions in glycemia were similar for both Cr trials suggesting that 400 μg are a sufficient amount to induce a beneficial effect and that there is no additional improvement when supplementing 800 μg of Cr. In a previous study performed at our laboratory using the same experimental procedure we could not detect any effects on glucose response after a high-glycemic meal and supplementation with 200 μg Cr (Frauchiger, Colombani, and Wenk, unpublished). This suggests that, when given as a single dose, 200 μg of Cr might be insufficient to influence postprandial metabolism in healthy young men and that a larger amount of Cr is needed to affect glucose metabolism acutely. To our knowledge there are no other data on the effects of an acute single dose intake of Cr on postprandial metabolism. However, there are similar findings in longer-term studies. In a review by Anderson [24] it is reported that studies showing beneficial effects of supplemental Cr in people with diabetes usually involve 400 μg or more of Cr.

The absorption of Cr seems to be quite rapid as blood concentration peak within 90 minutes after intake [17]. We expected that Cr would show its effect on the cells rapidly and gave the supplement just 30 minutes before the meal. In a recent paper by Vincent and his group [35] it was proposed that Cr picolinate enters tissues intact and is then degraded in the cells. This may suggest that even if absorption is rapid a longer time period would be needed to release Cr in its active form. Therefore, it is well possible that effects on glucose metabolism would be more pronounced if the Cr supplement were given a few hours before the test meal.

There was no significant correlation between the glucose and insulin responses in both venous and capillary blood. The smaller glycemic responses after Cr supplementation were not associated with larger insulin responses. This suggests that another mechanism than stimulation of insulin secretion was responsible for the decreased glycemia after supplemental Cr and supports the proposed mechanism of Cr action. It has been

reported that Cr potentiates the action of insulin by activating the tyrosine kinase activity of the insulin receptor and thereby amplifies insulin signaling [25], but to have no effect on insulin secretion. In our study the effects on blood glucose were more apparent in capillary than in venous blood. This possibly indicates that Cr enhances glucose uptake by peripheral tissue.

In our study fasting capillary and venous glucose concentrations were similar but postprandial values between 45 and 120 min as well as peak values were significantly higher for capillary measurements. These findings are in accordance with those of other studies that found that glucose concentrations approximate arterial values in capillary blood and that fasting concentrations are similar in venous and arterial blood [26,27]. Postprandial glucose concentrations are higher in capillary than in venous blood because of insulin-induced glucose uptake in peripheral tissues. These differences were reported to be as much as 2 mmol/L [28]. The higher concentrations reflect themselves in larger glycemic responses in capillary blood. Because of the greater differences in incremental AUC, Wolever & Bolognesi [27] suggested that using capillary rather than venous blood was a more precise way to assess glycemic responses to foods.

In our study ten out of thirteen subjects, i.e. about 80%, responded to Cr supplementation with a decrease in postprandial glycemia. Other studies [11,14] have also reported that some but not all subjects responded to longer-term supplementation. The reasons why beneficial effects are only visible in a part of the study population are not clear. Ravina *et al.* [14] found no clinical signs indicating which patient may positively respond to the addition of Cr. It has been proposed that individuals with normal glucose tolerance and who are not Cr deficient will not respond to Cr supplements [19]. But as it is still not possible to measure Cr status directly it is difficult to predict who will benefit from supplemental Cr. Offenbacher *et al.* [29] observed that subjects consuming well balanced diets did not respond to additional Cr. It has also been suggested that 30 to 40 μg of Cr per day would be adequate if balanced diets high in fruit and vegetables and low in simple sugars were consumed [24]. We estimated usual dietary intake of our subjects from 5-day diet records. The subjects responding to Cr ate more vegetables and dietary fibers but less disaccharides, meat and meat products, and milk and milk products than the others. The high consumption of vegetables and low intake of sugar for the responders seems to be in contrast to the findings of Anderson [24] and Offenbacher [29]. However, as only three subjects in our study did not respond to Cr, these differences, even if statistically significant, need verification. Another interesting observation is that the responder and non-responder group differed in parameters of iron metabolism. Non-responders tended to have higher serum iron and transferrin concentrations than responders. As Cr is probably transported in the blood by transferrin [30,31] this observation may be important and could possibly explain the differing response to Cr intake. Again, because of the low number of subjects, these results need to be confirmed before any conclusion can be drawn.

All our subjects were apparently healthy and showed normal glucose tolerance. Still, there was a correlation between the extent of postprandial glycemia after the WB trial and the glucose response observed after addition of Cr. The individuals with "poorer" glucose tolerance showed greater reductions in glycemia after supplemental Cr than those with "better" glucose tolerance. This suggests that people with impaired glucose tolerance may benefit even more from acute Cr supplementation than individuals with normal glucose tolerance. Similarly, Anderson *et al.* observed a decreased glucose response after three months of Cr supplementation only in individuals with slightly impaired glucose tolerance [32].

Low-glycemic diets may play an important role in the prevention of insulin resistance and even NIDDM. Unfortunately, lowering the GI of a diet may be difficult to achieve as many low-glycemic foods are not very popular and changing eating habits is not an easy task. Additionally, there is a lack of low-glycemic foods particularly for breakfast, as bread and ready-to-eat cereals have high GI [33]. Therefore, a substance able to lower the glycemic response to a food would be beneficial and especially useful for breakfast foods. After supplementation with 400 and 800 μg Cr the GI of white bread was reduced from 100 to 66 and 72, respectively. That is, the high-glycemic food white bread was "transformed" to a food of moderate to low GI, like oat bran (72) or parboiled rice (66) [34].

In conclusion, an acutely administered single dose of Cr (400 or 800 μg) improved glycemia after a high-glycemic meal in about 80% of young, healthy subjects, without visible effects on insulin concentration. These results seem to support the potentiating role of Cr on insulin action. However, additional studies are required to examine further the effects of acute Cr supplementation in humans.

ACKNOWLEDGMENTS

Marc Frauchiger contributed to this work in the design of the experiment, in the collection, analysis and interpretation of data, and by writing the manuscript. Paolo Colombani and Caspar Wenk assisted in the design of the experiment and the interpretation of the data, revised the manuscript and provided significant advice. We thank the Swiss Foundation for Nutrition Research for funding our research. Our thanks go also to Myrtha Arnold and Anthony Moses for technical assistance in the analysis of the samples.

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Effect of Chromium Picolinate on Insulin Sensitivity In Vivo

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This study assessed the effect of chromium (Cr) supplementation on insulin sensitivity and body composition in subjects at high risk for Type 2 diabetes because of family history and obesity. Twenty-nine subjects (14 men, 15 women) were evaluated in a double-blind, randomized, placebo-controlled trial using chromium picolinate (CrPic) (1,000 μg/day), or placebo for 8 months of study. Clinical and metabolic evaluations consisted of insulin sensitivity (S_I) and glucose effectiveness (S_g); measurement of glucose tolerance and insulin response to an oral glucose tolerance test (75 g OGTT); and 24-hour glucose and insulin profiles. Anthropometric measures and magnetic resonance imaging (MRI) assessed abdominal fat distribution. Fasting plasma glucose and insulin levels and measures of glycemia (glycated hemoglobin and fructosamine) were also assessed. The CrPic group showed a significant increase in insulin sensitivity at midpoint ($P < .05$) and end of study ($P < .005$) compared with controls, which had no significant changes. No change in S_g was seen in either group. There was no effect of CrPic on body weight, abdominal fat distribution, or body mass index. However, CrPic significantly improved insulin sensitivity in these obese subjects with a family history of Type 2 diabetes. Improvement in insulin sensitivity without a change in body fat distribution suggests that Cr may alter insulin sensitivity independent of a change in weight or body fat percentage, thereby implying a direct effect on muscle insulin action. Definitive double-blinded, placebo-controlled trials are currently being conducted to confirm this observation in Type 2 diabetic subjects and evaluate the effects of Cr supplementation on insulin action and glycemic control. J. Trace Elem. Exp. Med. 12:71–83, 1999.

Key words: chromium; insulin resistance; type 2 diabetes; insulin sensitivity

INTRODUCTION

Studies have demonstrated that inefficiency in insulin action (e.g., insulin resistance) may precede the development of diabetes by many years, as observed in

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prospective studies. Insulin resistance is one of the key parameters controlling glucose metabolism, in addition to insulin secretion and hepatic glucose production [1,2]. Before noninsulin-dependent diabetes mellitus (Type 2 diabetes mellitus) develops, an individual who is insulin resistant will compensate by producing more insulin in order to keep the blood sugar in the normal range [1,2]. When the pancreas begins to fail and is unable to compensate for the increased demand secondary to insulin resistance, the blood glucose begins to rise. Recently, a fasting blood glucose of >126 mg/dl has been recommended as the level considered diagnostic for Type 2 diabetes, as opposed to the previous value of >140 mg/dl [3].

Insulin resistance may, therefore, precede the diagnosis of Type 2 diabetes, especially in the majority of cases presenting with obesity. It is well established that nutritional intervention and exercise greatly improve insulin resistance, forming the cornerstone of prevention studies. Yet there is little success of dietary and behavioral modification in maintaining weight loss over a long period in humans. Therefore, a clinical improvement in insulin sensitivity secondary to pharmacological or nutritional means is an extremely attractive approach for human intervention trials.

CrPic has been postulated as one such nutritional intervention based on evidence in animal and human studies [4–16]. The hypothesis is that the improvement in insulin resistance from such an intervention would reduce insulin levels and improve glycemic control, as has been demonstrated in other clinical studies [17–19]. Further, central obesity (i.e., increased truncal fat) is highly related to the insulin resistance syndrome. The development of diabetes in such individuals and the associated risk factors at this stage contribute greatly to the development of cardiovascular disease [20–24]. Therefore, the goal of this study was to assess the effect of Cr supplementation on insulin sensitivity and body composition in subjects at high risk for the development of Type 2 diabetes because of their family history and obesity.

MATERIALS AND METHODS

Experimental Subjects

The efficacy of CrPic to alter metabolic and body fat parameters in obese individuals at high risk for the development of Type 2 diabetes was assessed in 29 patients (14 men, 15 women). These subjects were entered in the study based on their known medical history and a physical examination revealing no chronic disease. They were required to have a first-degree relative with Type 2 diabetes and >125% ideal body weight. They were not permitted to be taking any medication known to affect glucose metabolism. All patients had normal renal and liver function. The study was approved by the Clinical Research Practices Committee of the Wake Forest University School of Medicine (Winston-Salem, NC).

After an initial observation period, a baseline measure of insulin sensitivity and 24-hour glucose and insulin profiles were obtained from all subjects. They received nutritional guidelines designed to maintain their current weight. The subjects were then randomized to one of two treatment arms in a double-blind design: nutrition only, or the nutritional regimen plus CrPic. At specified intervals, clinical and metabolic evaluations were done. These consisted of determination of insulin sensitivity (S_I) and glucose effectiveness (S_g), as measured by the frequently sampled intravenous glucose tolerance test (FSIVGTT, Modified Minimal Model); measurement of glucose

tolerance and insulin response to an oral glucose tolerance test (75 g OGTT); and 24-hour glucose and insulin profiles. In addition, fasting insulin levels and measures of glycemia (glycated hemoglobin and fructosamine) were assessed throughout the study.

Study Design

The subjects were evaluated in a double-blind, randomized, placebo-controlled trial using CrPic (1,000 μg/day) or placebo. After an initial screening period (Week 0) and a 5-week baseline period, the subjects were randomized into one of the two groups from Weeks 6–38 of the active phase of the trial.

Screening. At screening, a patient history was taken and a physical examination was done, including vital signs. General laboratory parameters were obtained, consisting of a complete blood count, electrolytes, liver and renal function tests, urinalysis, and an electrocardiogram. In addition, fasting plasma glucose, insulin, and glycated hemoglobin/fructosamine values were measured.

Phase I: baseline. ***Week 1.*** Subjects found acceptable at the screening received instructions from a dietitian for continuing with a weight maintenance diet. Subjects were asked to avoid major changes in lifestyle for the duration of the study. Whatever exercise regimen subjects were routinely doing, they were asked to continue on a regular basis, e.g., joining a weight loss organization was discouraged until the study was completed. Periodically, subjects were monitored for changes. Instruction and forms for completing the 3-day food records on the assigned days were given. Also, vital signs including blood pressure, pulse, and weight were obtained.

Week 3. Subjects had baseline anthropometric measures and a magnetic resonance imaging (MRI) scan to assess abdominal fat distribution. Fasting plasma glucose and insulin values were obtained.

Week 4. Subjects returned to the General Clinical Research Center (GCRC) after an overnight fast, at which time a minimal model study was performed to assess peripheral insulin sensitivity.

Week 5. Subjects were admitted to the inpatient unit of the GCRC for 24-hour glucose and insulin profiles.

Phase II trial: chromium picolinate vs. placebo. At the conclusion of the Week 5 visit, patients were randomized to one of two groups. All subjects continued on the nutritional regimen plus placebo, or the nutritional regimen plus CrPic at 1,000 μg/day. Each treatment was taken before bedtime.

Weeks 6–38. Subjects returned to the GCRC at weekly and/or monthly intervals for metabolic and clinical evaluations. The metabolic evaluations were repeated at the midpoint (Weeks 20–22) and at the end of the study (Weeks 36–38). Specifically, MRI scans were obtained at Weeks 20 and 36, and anthropometric measurements were obtained in conjunction with the MRI scans. Insulin sensitivity tests were conducted at Weeks 21 and 37, and oral glucose challenge and 24-hour insulin and glucose profiles were obtained at Weeks 22 and 38.

Food records. Ten 3-day food records were collected throughout the study. Two records (6 days) during the baseline period: Monday, Wednesday, and Friday (MWF) of Week 3, and Tuesday, Thursday, and Saturday (TThS) of Week 4. Throughout the active period, 3-day records were assigned and collected monthly, alternating MWF and TThS. Subjects were asked to continue their "usual diet," record everything they

ingested, and not to vary their intake because of the recording procedure. Videotaped instructions from the Human Nutrition Research Center of Tufts University were used in conjunction with personal instruction from the dietician. The records were analyzed by Nutrition Data System of the University of Minnesota [26]. Each subject's records were entered by a single technician throughout the study for consistency of enterer bias, as the concern was with change in intake, not absolute quantity. For a measurement of intraenterer variability, one record of each subject was selected at random to be re-entered.

Meals. For 2 days before the determination of insulin sensitivity by the FSIVGTT and the 24-hour insulin profile, meals were provided for takeout from the metabolic kitchen of the GCRC. These consisted of weight maintenance Kcal: 50% carbohydrate, 20% protein, 30% fat; salt ad lib; non-nutritive beverages ad lib. Kcal level was determined by the 3-day food records and the Harris-Benedict equation. Menus were designed per the subject's preferences and usual intake. Checklists of the menus were given and collected from the subjects for a measure of compliance to the diet. For continuity, the same menus were repeated before each of the tests throughout the study.

Metabolic/Clinical Evaluations

Insulin Sensitivity Assessment (FSIVGTT): Modified Minimal Model. Insulin sensitivity studies were initiated in the morning after an overnight fast. Two 18-gauge intravenous catheters were placed in each forearm and kept patent by a controlled flow of saline infusion. Each line was equipped with a three-way stopcock. One line was used for intravenous administration of test substances and the other for blood samples. One-milliliter blood samples were collected from the venous catheter at −15, −5, and −1 minute, after which 0.3 g/kg glucose was injected over 30 seconds into the venous catheter, beginning at time 0.

After glucose injection, blood samples (1 ml) were drawn at 2, 3, 4, 5, 8, 10, 12, 14, 15, 18, and 20 minutes. Regular insulin (Humulin Regular, Eli Lilly, Indianapolis, IN) was injected as an intravenous bolus of 0.03 U/kg at 20 minutes. Blood withdrawals continued at 22, 24, 28, 32, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 minutes. Samples were centrifuged immediately and placed on ice. Glucose determinations were made immediately after centrifugation using the glucose oxidase method on a Glucose Analyzer 2 (Beckman Instruments, Brea, CA) (intra-assay coefficient of variance [cv] = 2%). Insulin was assayed from frozen plasma by radioimmunoassay (Incstar, Stillwater, MN) (intra-assay cv < 5%). At each time point, serum glucose and insulin measurements were determined in duplicate.

The data were analyzed with the minimal model of glucose disappearance, as previously described [27,28]. This model accounts for the effect of insulin and glucose on glucose disappearance during an intravenous glucose tolerance test. It provides two parameters: S_I (insulin sensitivity index, min^{-1} $\mu U^{-1}ml$), defined as the ability of insulin to enhance glucose disappearance and to inhibit hepatic glucose production, and S_g (glucose effectiveness, min^{-1}], defined as the ability of glucose per se to enhance its own disappearance and to inhibit glucose production at basal insulin levels. Parameters were estimated on each individual glucose and insulin concentration data set together with a measure of their precision expressed as percent coefficient of variance.

Abdominal Fat Distribution

Specific body fat content in the abdominal area was assessed by obtaining MRI scans at the umbilicus, as previously described [27,28]. MRI examinations were performed on a Picker Vista HPQ (Picker International, Cleveland, OH) MRI scanner operating at a field strength of 1.5T. The inversion recovery protocol used was first described by Seidell et al. [29] and subsequently validated on our system [30]. Total abdominal, intra-abdominal, and subcutaneous abdominal fat areas were measured. In addition, anthropometric measurements were taken at Weeks 5, 26, and 37, always by the same measurer (JES), using the techniques of Lohman et al. [31]. Skinfolds were taken at tricep, bicep, subscapular, supra-iliac, and abdomina; circumferences were taken at tricep, waist, and hip. All measurements were taken in duplicate. A third skinfold was taken if the two were not within 2 mm of each other; a third circumference was taken if the two were not within 2 cm of each other.

Glycemic Parameters

Total glycated hemoglobin was determined by automated affinity high-pressure liquid chromatography, with an intra-assay coefficient of variance of 1.2%, as previously described [32]. Serum glycated protein was determined by measuring serum fructosamine (interassay cv = 2.2, intra-assay cv = 2.4) on a Cobas Mira Chemistry Analyzer using Roche reagents (Roche Diagnostic Systems, Nutley, NJ) [33].

Twenty-Four-Hour Insulin/Glucose Profiles and 75 g OGTT

At specified times (e.g., end of baseline, Week 5; midpoint, Week 22, and end of study, Week 38), subjects were admitted to the inpatient unit of the GCRC for 24-hour glucose and insulin profiles. In an effort to obtain average daily insulin and glucose profiles representative of outpatient levels, meals during the inpatient stay (with the exception of the 75 g OGTT that replaced breakfast) were based on the caloric intake and composition established during the baseline period from the 3-day food records.

For 2 days before the inpatient 24-hour insulin study, meals were provided from the metabolic kitchen of the GCRC as described above. During the 24-hour admission, the breakfast meal consisted of 75 g glucose. The noon and 6:00 p.m. meals each consisted of two-fifths weight maintenance Kcal: 50% carbohydrate, 20% protein, 30% fat. Subjects were required to ingest the entire meal within 30 minutes. The same menus were repeated for each of the three admissions. No caffeine and no snacks, except noncaloric caffeine-free beverages, were allowed for the duration of the 24-hour admission.

On the morning of admission, a heparin lock was placed in an arm vein for withdrawing blood. At 0800 after an overnight fast, all subjects had a 75 g OGTT. Blood for glucose and insulin levels was obtained at 30, 60, 120, 180 minutes after the OGTT. At 1200 hours, subjects had the lunch meal, and blood was taken again at 30, 60, 120, and 180 minutes postprandially. Blood was then taken at 1600, 1700, and 1800 hours, after which the dinner meal was served. Blood for glucose and insulin levels postprandially was taken at 30, 60, 120, and 180 minutes after the evening meal. Additional blood was taken at 2200 and 2400 hours and at 0200 and 0800 hours of the following morning. In total, 21 glucose and insulin assessments were taken over 24 hours. Glucose and insulin response to meals were assessed by calculating the

areas under the curve (AUC) from values taken up to 3 hours after the meal and by assessing the overall summed glucose and insulin levels over the 24-hour period.

Statistical Analysis

Clinical evaluations (e.g., insulin sensitivity measures, insulin and glucose response to 75 g OGTT, and 24-hour profiles, intra-abdominal fat MRI scans) were measured at baseline and at 4 months and 8 months after randomization. The effect of treatment (CrPic) was estimated and tested for statistical significance by repeated measures analysis of covariance, using the baseline, prerandomization level of the outcome measure as the covariate. Analyses were conducted using the SAS PROC MIXED for maximum likelihood estimation for repeated measures analysis of covariance.

RESULTS

Twenty-nine subjects were randomized to participate in this study. Table I outlines the demographics for subjects entering either the placebo or CrPic treatment arm. There was no difference in age or body mass index in the two groups. There was no difference in body weight or total abdominal fat mass at baseline, and no significant change was observed in either group over the course of study (data not shown). Control subjects were noted to have a 6% change in intra-abdominal fat mass, whereas the subjects randomized to Cr had a 1% change, but these changes were not considered statistically significant. Anthropometric measures and the waist-hip ratio did not change in either the control or Cr treatment groups over the duration of the study. No change in caloric intake was observed from baseline in either group.

Figure 1 shows the insulin sensitivity (S_I) measures on all subjects. At baseline, there was no difference in insulin sensitivity between the control and Cr groups. The CrPic group showed a significant increase in insulin sensitivity at the midpoint ($P < .05$) and end of study ($P < .005$) compared with the control group, which had no significant changes at either midpoint or the end of study. No change was seen in S_g in either the control or Cr groups. (data not shown).

Total 24-hour insulin/glucose profiles did not change in the control or Cr groups over the course of study (Fig. 2). The insulin response (AUC) to the OGTT breakfast, lunch, and dinner meals over the 24-hour inpatient stay did not differ from baseline at either the 4- or 8-month time points for the control group (Fig. 3A). For the CrPic group (Fig. 3B), a trend toward a reduction in insulin was seen over the course of study, but it was not statistically significant. Similar results for the fasting insulin levels (Fig. 4) were seen in both treatment groups. Again, a trend toward a reduction in insulin was seen in the Cr Pic group over the course of study, but it was not

TABLE I. Baseline Characteristics for Subjects in Chromium and Placebo Treatment Groups*

Group	Number	Gender (M/F)	Age (yr)	Body mass index
Placebo	14	6/8	49 ± 4	33 ± 2
Chromium	15	5/10	45 ± 3	34 ± 2

*Data are mean ± SD.

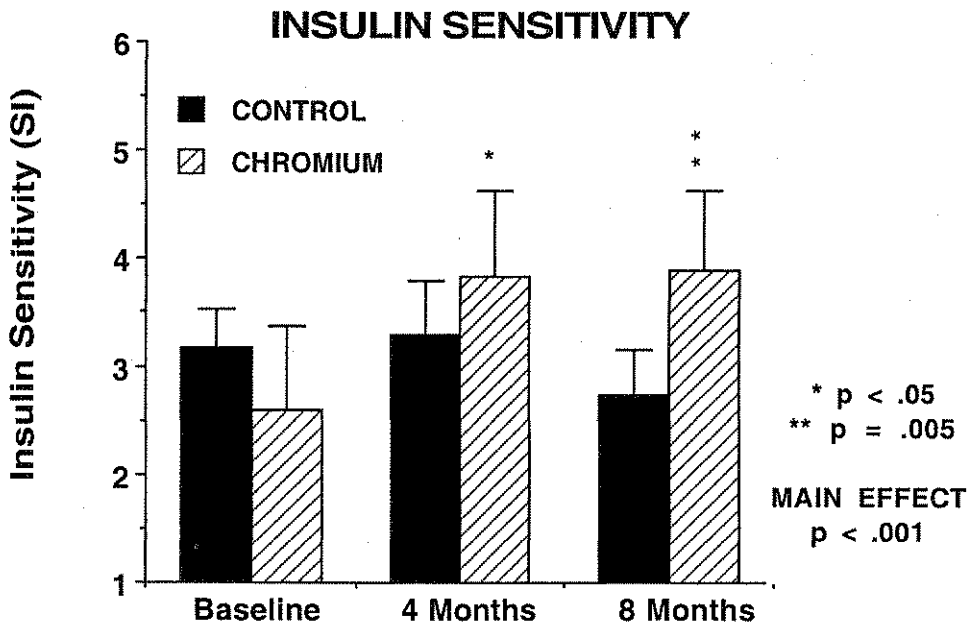

Fig. 1. Insulin sensitivity in chromium and placebo groups at baseline and at the 4- and 8-month assessment after randomization. SI units = min^{-1} $\mu U^{-1}ml$.

statistically significant. Glycated hemoglobin and fructosamine levels did not differ between groups (data not shown). There was no difference in complete blood counts, electrolytes, liver or renal function in either group at the end of the study when compared to baseline (data not shown).

DISCUSSION

CrPic was shown to significantly improve insulin sensitivity in this cohort of obese subjects with a family history of Type 2 diabetes. There was no effect of Cr Pic on body weight or on abdominal fat distribution as assessed with MRI scans. The improvement in insulin sensitivity without a change in body fat distribution suggests that Cr may alter insulin sensitivity independent of a change in weight or body fat percentage, thereby implying a direct effect on muscle insulin action.

Early studies of total parenteral nutrition (TPN), in which Cr deficiency was well documented [30,31], have provided evidence for an essential role for Cr in carbohydrate metabolism. Clinical observations and biochemical measurements of subjects on TPN demonstrated that Cr deficiency was associated with severe hyperglycemia and increased insulin demand. As a result, Cr is now routinely added to TPN solutions. With specific reference to diabetes, diabetic subjects may have altered Cr metabolism compared with nondiabetic subjects, as both absorption and excretion of Cr may be higher [17]. Further, hair and tissue levels are reported to be lower in diabetic subjects.

It appears clear, therefore, that Cr is an essential element for human nutrition. Cr deficiency is associated with hyperglycemia, and correction of the deficiency allevi-

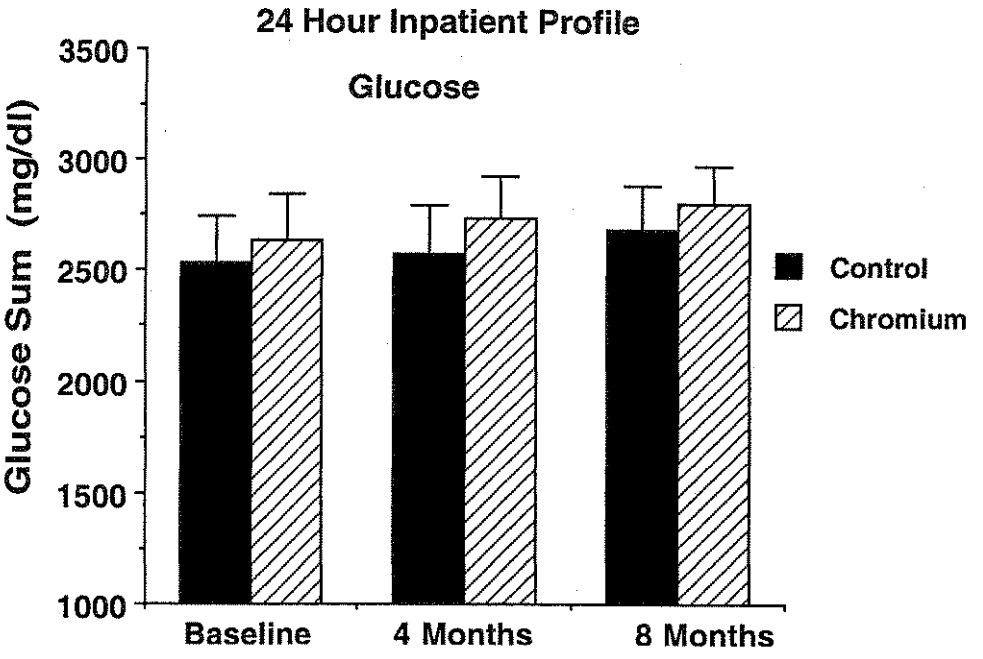

Fig. 2. Twenty-four-hour summed inpatient glucose profiles in chromium and placebo groups at baseline and 4- and 8-month time points.

ates this condition. However, it is a different question entirely to show how such findings relate to "supplemental" Cr ingestion in subjects who are not Cr deficient. Cr supplementation in clinical diabetic states to improve glucose levels and insulin sensitivity is a greatly debated and controversial issue in clinical diabetes practice, i.e., does Cr supplementation allow for a reduction in either exogenous insulin administration or a reduction in oral agents given to control the clinical hyperglycemia? In this regard, additional evidence for a role for Cr in human nutrition has demonstrated improvements in glucose and lipid levels in both Type 1 and 2 diabetes and impaired glucose tolerant subjects [7–9,18]. However, even in studies that have demonstrated some improvement in carbohydrate parameters, the effect was not seen in all subjects.

The issue is further confounded by differences in the dose of Cr used and in the source of Cr. In many of the early studies cited, lower doses of Cr were given than in the present study, and Cr chloride, not CrPic, was evaluated. Recently, it was demonstrated that supplemental Cr Pic at 1,000 μg/day improved both insulin and glucose levels in a study of Chinese diabetics, whereas treatment with 400 μg/day failed to significantly lower fasting or 2-hour glucose levels, suggesting a dose response [17]. This finding in the Chinese study agrees with the effectiveness of the 1,000 μg dosage observed in the present study.

The 1,000 μg dose, much higher than the recommended daily intake of 200 μg/day, raises the issue of the relative toxicity of Cr. As recently discussed by Anderson et al. [17], the subcommittee on the Tenth Edition of the Recommended Dietary Allowances (RDAs) of the National Research Council [32] reported that the

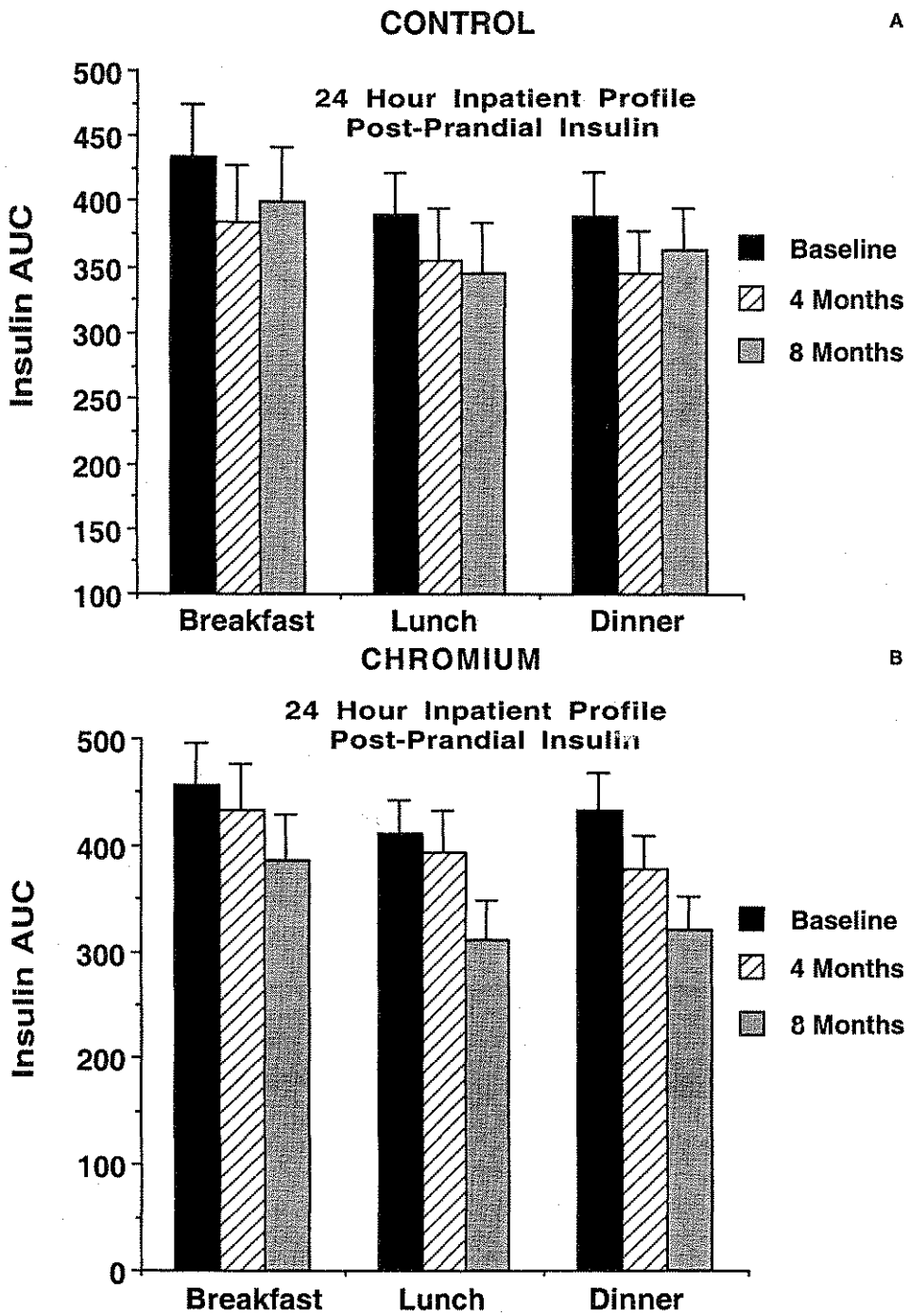

Fig. 3. Insulin response in placebo (**A**) and chromium (**B**) groups for the OGTT (breakfast), lunch and evening meals during 24-hour stay for three inpatient stays.

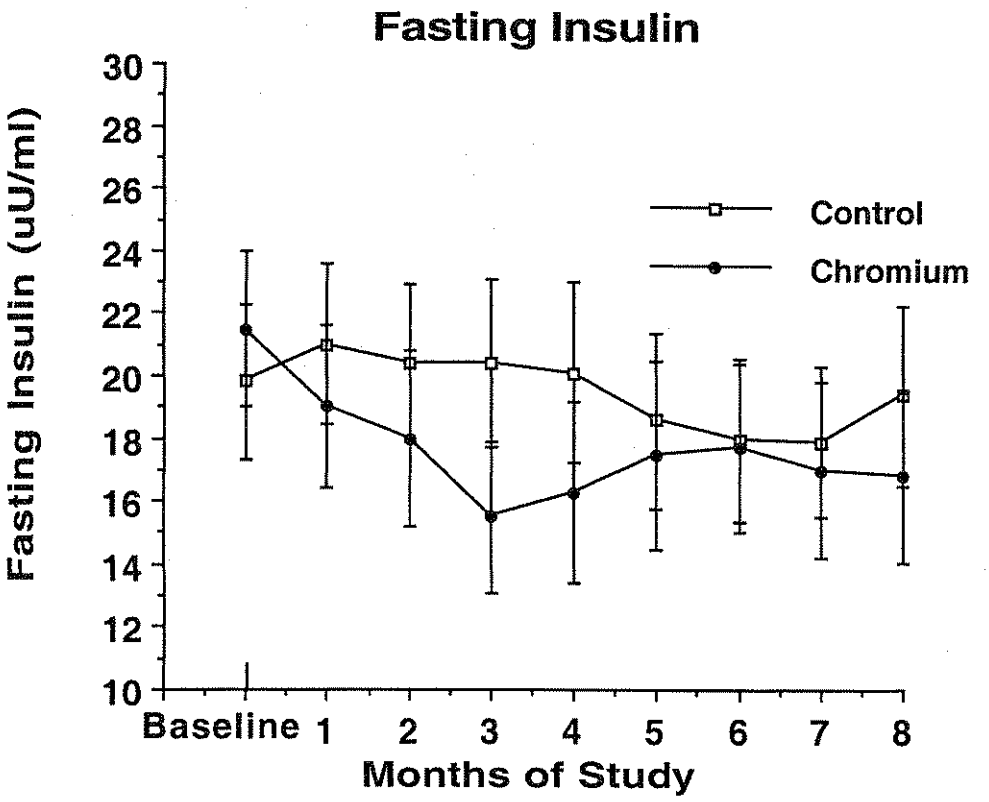

Fig. 4. Fasting insulin levels in chromium and placebo groups over the entire course of study.

toxicity of trivalent Cr, the chemical form that occurs in diets, is so low that there is a substantial margin of safety between the amounts normally consumed and those considered to have harmful effects. No adverse effect occurred in rats and mice consuming 5 mg/l in drinking water throughout their lifetimes, and no toxicity was observed in rats exposed to 100 mg/kg in the diet [36]. The reference dose established by the U.S. Environmental Protection Agency for Cr is 350 times the upper limit of the estimated safe and adequate daily dietary intake of 200 μg/day. This reference dose compares to more than two times the upper limit for zinc, two times the upper limit for manganese, and 5–7 times the upper limit for selenium [17,37]. No toxicity was reported at a 1,000 μg/day dose in the human diabetes study conducted by Anderson et al. [17], and no toxicity was seen at that dose in the present study reported here.

Finally, the ability of Cr to improve insulin sensitivity with no effect on body fat suggests a direct effect to improve muscle insulin action. Although we did not assess total body fat, as would be done with DEXA scans, we did assess body weight and a specific measure of abdominal fat, e.g., intra-abdominal fat with MRI scans. An increase in intra-abdominal fat mass has been postulated to be highly related to lipid, blood pressure, and carbohydrate abnormalities in addition to being an independent risk factor for cardiovascular disease [25,38,39]. We have shown previously that

assessment of intra-abdominal fat by MRI scans is highly related to insulin resistance in a nondiabetic population [27,28]. Further, we have shown in a multivariate analysis that accumulation of intra-abdominal fat as one ages appears to be a major contributor to the insulin resistance observed with aging [27]. Such observations, in addition to the studies suggesting a role for Cr in body composition, form the basis for studying intra-abdominal fat in this trial. Recently, in a randomized, double-blind, placebo-controlled study, Kaats et al. [40] presented data suggesting that Cr supplementation can lead to significant reductions in percent body fat and fat mass without any loss in fat-free mass.

Additional findings from our trial showed that although improved insulin sensitivity was observed, there was no significant difference in fasting insulin levels, glycated protein levels, and 24-hour insulin profiles between the control or Cr treatment groups. Several reasons may explain this observation. First, as the subjects were nondiabetic, blood glucose and glycated hemoglobin were normal at baseline. Unlike the hyperglycemia seen in the diabetic state, glucose levels in the normal range may not be expected to decrease below the normal range. Second, although there were trends toward lower fasting and postprandial insulin, the study may not have had enough subjects to demonstrate differences, as these parameters are not as sensitive as the minimal model technique in assessing insulin action.

CONCLUSIONS

Based on the findings in the trial described in this report, there appears to be evidence to support the beneficial effects of CrPic on insulin action. Yet considerable controversy remains in this area. Definitive studies using sensitive techniques to measure insulin action and glucose control and employing double-blinded studies for a Western population have yet to be completed. Therefore, definitive double-blinded, placebo-controlled trials in humans, specifically in diabetic subjects, are currently being conducted. These trials are evaluating Type 2 diabetic subjects with in vivo techniques considered the "gold standard" for assessing insulin action, i.e., euglycemic clamp studies. The findings from these studies should provide convincing and conclusive recommendations regarding Cr supplementation in diabetic states. If Cr supplementation can be demonstrated consistently to improve glycemic control and insulin action in Type 2 diabetes, recommendations for supplemental CrPic with caloric restriction and weight loss regimens and/or pharmacologic therapy to sustain acceptable blood glucose levels may then become standard clinical practice.

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A RANDOMIZED, DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY OF THE EFFECTS OF CHROMIUM PICOLINATE SUPPLEMENTATION ON BODY COMPOSITION: A REPLICATION AND EXTENSION OF A PREVIOUS STUDY

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A RANDOMIZED, DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY OF THE EFFECTS OF CHROMIUM PICOLINATE SUPPLEMENTATION ON BODY COMPOSITION: A REPLICATION AND EXTENSION OF A PREVIOUS STUDY

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ABSTRACT

A previous study using a randomized, double-masked, placebo-controlled design found that supplementation with a minimum of 200 µg of chromium (in the form of chromium picolinate [CrP]) per day can lead to significant improvement in body composition (as measured by underwater testing using the displacement method). The present study used a similar design in which 122 subjects were randomized to receive either CrP 400 µg (n = 62) or placebo (n = 60). To control caloric intake and expenditure (which was not done in the first study), participants were required to monitor and maintain a log of their daily physical activity and caloric intake. Dual energy x-ray absorptiometry measurements were taken before and after the 90-day period. Analysis of the prestudy data for the two groups revealed no significant differences in any of the initial body composition variables studied. After controlling for differences in caloric intake and expenditure, as compared with the placebo group, subjects in the active treatment group lost significantly more weight (7.79 kg vs 1.81 kg, respectively) and fat mass (7.71 kg vs 1.53 kg, respectively), and had a greater reduction in percent body fat (6.30% vs 1.20%, respectively) without any loss of fat-free mass. A more conservative analysis of covariance revealed similar and statistically significant reductions in percent body fat and fat mass without any loss of fat-free mass. It was concluded that this study replicated earlier findings that supplementation with CrP can lead to significant improvements in body composition. *Key words:* chromium picolinate, body composition, fat mass, fat-free mass, dual energy x-ray absorptiometry.

INTRODUCTION

In a previous publication,[1] the authors summarized their research on dietary chromium, an essential nutrient, reporting that its value in human nutrition has been documented conclusively.[2] They suggested that com-

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bining chromium with picolinic acid in the form of chromium picolinate (CrP) could increase the bioavailability of CrP[3–7] and, therefore, improve insulin use. Because the deposition of body fat appears to be regulated to some extent by insulin,[8] the authors reasoned that improvements in insulin use could lead to reductions in fat deposition. Enhancing the effects of insulin can also have positive effects on muscle tissue, because insulin directs amino acids into muscle cells where they are assembled into proteins through the effect of insulin on the cell's genetic material. Insulin also slows the breakdown or catabolism of body protein, with a net effect of increasing the protein available for building tissue. Because chromium is a cofactor to insulin, supplemental chromium offers the potential of facilitating the maintenance or addition of fat-free mass (FFM).[9] Hence, if CrP can lower insulin resistance, it can improve body composition, because insulin resistance or deficiency results in impaired entry of glucose and amino acids into muscle cells, increased catabolism of muscle protein, and the potential acceleration of lipid deposition.[10,11]

To test these hypotheses, in the previous study[1] the authors used a randomized, double-masked, placebo-controlled protocol in which participants completed underwater testing (displacement method) at the beginning and end of a 72-day study. During the study, subjects consumed either 0 μg, 200 μg, or 400 μg of CrP per day. Results of that study showed a significant improvement in body composition with CrP supplementation, with a specific reduction in excess body fat.

In addition to determining whether the body composition changes observed in the initial study could be replicated in this study, we sought to answer three methodologic issues raised by the reviewers of the previous manuscript: (1) Because supplementation with CrP affects appetite, metabolism, and daily activity levels, would the same results be achieved if differences in caloric intake and energy expenditure were controlled or factored out? (2) Would the results be replicated with other measures of body composition, such as dual energy x-ray absorptiometry (DEXA), which are at least as precise as underwater testing but less dependent on the subject's performance and practice effects on the unusual task of exhaling before going underwater? and (3) Because the relatively high dropout rate in the first study (29.7%) could have biased the findings through selective attrition, would these same results occur if methods were used to decrease the dropout rate?

To answer these questions, we controlled for differences in physical activity and caloric intake, used DEXA testing to determine body composition, and used a methodologic technique to reduce the dropout rate.

SUBJECTS AND METHODS

Subjects

A total of 130 subjects were enrolled in the study, 122 (93.8%; 17 men

and 105 women; mean age, 42.3 years) of whom completed the testing. Subjects were recruited from a variety of fitness and athletic clubs in San Antonio and Houston, Texas, by fitness instructors and sales personnel who provided information about the study to club members who either participated themselves or recruited friends or relatives to participate. In most cases, the fitness instructors were paid to monitor the subjects as they progressed through the study to ensure that the subjects reported their physical activity levels and caloric intake (tracked the data) and completed the testing. All subjects were asked to consult with their personal physician before giving written informed consent.

Testing Equipment: Dual Energy X-Ray Absorptiometry

A number of studies have shown that DEXA can accurately measure fat and lean content in meat samples and animal carcasses[12–15] and that DEXA measurements of actual skeletal mass and total body calcium correlate highly with those taken by neutron activation analysis,[16] with a typical precision error for total body bone mineral content <1%.[17] DEXA has also been shown to be a precise method for assessing body composition in obese and nonobese subjects.[18,19] DEXA correlates highly with underwater weighing,[20] deuterium dilution,[21] and total body potassium.[22] The reliability of DEXA makes it possible to monitor the effects of relatively short-term dietary restrictions and exercise on both regional and total body composition.[23,24] A recent review of the research on DEXA has led one reviewer to conclude that DEXA is among the most accurate instruments available today for critically analyzing body composition.[25]

DEXA provides a three-compartment model of body composition: fat, lean tissue mass, and bone mineral content. Measurements are made using a constant potential energy source at 78 kVp and a K-edge filter (cerium) to achieve a congruent, stable, dual-energy beam with effective energies of 40 and 70 keV. The unit performs a series of transverse scans moving from head to toe at 1-cm intervals; the area being scanned is approximately 60 × 200 cm. Data are collected for about 120 pixel elements per transverse, with each pixel approximately 5 × 10 mm. Total body measurements are completed in 10 to 20 minutes with a scan speed of 16 cm/s, or in 20 minutes with a scan speed of 8 cm/s. The R value (ratio of low- to high-energy attenuation in soft tissue) ranges from 1.2 to 1.4.[26]

Procedure

To minimize the dropout rate, subjects were asked before signing the informed consent form, to provide a $100 deposit by check or credit card, which would not be processed unless the subject failed to complete the last DEXA test and end-of-study questionnaire. Participants were advised that return of their deposit was based solely on their completing the last tests

no matter how well or poorly they adhered to the research protocol, as long as they reported candidly on how much or how little they complied.

After completing an initial DEXA test, subjects were provided with a report of their test results and randomly assigned a number from 1 to 130, which corresponded to a bottle containing capsules with 400 μg of CrP or placebo. None of the investigators, research technicians dispensing the product, or participants knew which subject number corresponded to the placebo or active product. An independent local pharmacist acted as trustee for the study and randomly assigned subject numbers to bottles that had been prelabeled with either an "X" or "Y" to correspond with either active product or placebo.

Participants were provided with a workbook outlining the general procedures for estimating caloric intake, nutritional information for common foods, and a log for calculating and recording daily calorie balances. To monitor and adjust for differences in energy expenditure through physical activity throughout their waking hours, all subjects wore a pedometer (same method as used in previous studies[27–29]) that reflected the number of steps they took during each day or the step equivalents for activities in which it was impractical to wear the unit. Subjects recorded the total number of steps taken each day in the same daily log used to record their caloric intake, which was subsequently used to adjust the subject's net change in body fat by using the following formula: ±3500 calories = a change of 1 lb of body fat. Subjects checked in at the research center on a weekly basis to obtain a scale weight and to report their weekly physical activity levels, estimated caloric intake, and any adverse effects (none were reported).

On completion of the study and when all data were gathered and entered in the computer system, the trustee opened an envelope supplied by the manufacturer indicating which product was active and subsequently notified the senior investigator (GRK). All information was analyzed by the Department of Computing Resources at the University of Texas Health Sciences Center at San Antonio, San Antonio, Texas, under the supervision of the second author (KB). At the conclusion of the test period, subjects completed the last body composition test, were provided with their test results and deposit checks, and were asked to report how many of the capsules were consumed each day as a cross-check of the amount of product used. A subsequent analysis of these data revealed that among participants receiving CrP, the average amount consumed was 357 μg/d.

Statistical Analysis

Comparisons were made between body composition variables for the two groups at baseline using a two-tailed Student's *t* test and between

baseline and post tests for both groups using paired t-test analyses. Comparisons of changes in body composition variables from baseline to post study were made using analysis of covariance (ANCOVA), which allows differences in body composition changes between the two groups to be adjusted statistically for individual differences in caloric intake and expenditure. Both caloric intake and expenditure were used as covariants irrespective of whether or not they were significant. A final statistical analysis was conducted using a direct adjustment of the data for caloric intake and expenditure and using Student's t test between the two groups. Finally, comparisons were made between the changes occurring in the two groups without making any adjustments for caloric intake or expenditure. All data analyses were conducted at the University of Texas Health Sciences Center's Department of Computing Resources.

RESULTS

Of the 130 subjects who were recruited for this study, only 8 failed to complete the final test: 1 subject became pregnant and was asked to withdraw from the study, 3 moved from the area, 1 was ill during the posttesting period, and 3 were lost to follow-up. A comparison of the 122 subjects who completed the study with the 8 subjects who did not revealed no significant differences in any of the body composition variables.

Baseline characteristics for the 122 subjects who completed the study are provided in Table I. No statistically significant differences in baseline characteristics were observed between the active treatment and placebo groups, suggesting that the randomization process was successful in providing two equivalent groups of subjects. Table II presents a comparison of the within (baseline-ending) and between-group changes that occurred in body composition variables in both the active treatment and placebo groups over the test period. Both groups experienced significant within-group reductions in scale weight ($P < 0.001$), percent fat ($P < 0.001$), and fat mass ($P < 0.001$), although no statistically significant changes occurred in fat-free mass in either group. A comparison of the between-group changes revealed that, although the active treatment group achieved greater improvement in all body composition variables, the differences in fat-mass reduction was the only change that reached statistical significance ($P = 0.023$).

Using an ANCOVA to equate the groups for caloric intake and energy expenditure, supplementation with CrP had an even greater significant and positive effect on percent body fat ($P = 0.03$) and fat mass ($P = 0.01$), although the differences in scale weight and FFM did not reach statistical significance. ANCOVA's statistical adjustment of the data is based on calculated relationships between the variables and is a conservative statistic that is insensitive to small changes. An alternative analysis is to apply the

Table I. Mean (±SD) baseline demographic data for 122 subjects randomized to receive either chromium picolinate (CrP) (n = 62) or placebo (n = 60).

	Age (y)	Weight (kg)	Body Fat (%)	Body-Mass Index (kg/m^2)
CrP (400 µg/d)	41.1 ± 10.5	85.5 ± 23.0	42.4 ± 8.3	30.2 ± 7.1
Placebo	43.5 ± 7.6	79.9 ± 20.4	41.8 ± 6.7	28.4 ± 5.4

corrections for caloric intake and energy expenditure directly to the data and then use Student's *t* test to examine the differences between the two groups. These analyses are presented in Table III. Using this approach revealed even greater differences between the two groups, suggesting that, as compared with the placebo group, the group receiving the active treatment had a significant reduction in scale weight (7.79 kg; $P < 0.001$), percent body fat (6.30%; $P < 0.001$), and fat mass (7.71 kg; $P < 0.001$). As with ANCOVA, no statistically significant differences in FFM were observed in either group. Thus, regardless of the statistical approach used, the findings from this study are highly consistent with, and provide a replication of, the findings from our previous study as well as a recent study of the effects of CrP supplementation in swimmers.[30]

DISCUSSION

It has been proposed that the positive effect of CrP on body composition is through its ability to improve insulin use, thereby reducing fat deposition and improving entry of glucose and amino acids into muscle cells. Although the present study did not attempt to test this assertion, the findings are consistent with this hypothesis, as are the findings of a recent study[31] of the lipogenic and antilipolytic effects of insulin in human adipocytes.

Table II. Within- and between-group comparisons of mean changes (±SD) in baseline and end-of-study body composition variables for subjects receiving either chromium picolinate (CrP) (n = 62) or placebo (n = 60) during a 90-day test period.

	Weight (kg)	Body fat (%)	Fat Mass (kg)	Fat-Free Mass (kg)
CrP (400 µg/d)	−2.88 ± 3.50	−2.07 ± 3.20	−2.81 ± 3.20	−0.07 ± 2.20
*P**	<0.001	<0.001	<0.001	=0.793
Placebo	−1.81 ± 2.99	−1.20 ± 2.90	−1.53 ± 2.80	−0.29 ± 2.00
*P**	<0.001	=0.002	<0.001	=0.265
CrP versus Placebo *P*†	=0.240	=0.120	=0.023	=0.568

* Student's *t* test for repeated measures.
† Student's *t* test for independent samples.

Table III. Within- and between-group comparisons of mean changes (±SD) in baseline and end-of-study body composition variables for subjects receiving either chromium picolinate (CrP) (n = 62) or placebo (n = 60) during a 90-day test period. All data are adjusted statistically for differences in caloric intake and expenditure.

	Weight (kg)	Body Fat (%)	Fat Mass (kg)	Fat-Free Mass (kg)
CrP (400 µg/d)	−7.79 ± 9.70	−6.30 ± 9.7	−7.71 ± 9.50	−0.07 ± 2.20
*P**	<0.001	<0.001	<0.001	=0.568
Placebo	−1.81 ± 2.99	−1.20 ± 2.9	−1.53 ± 2.80	−0.29 ± 2.00
*P**	<0.001	=0.002	<0.001	=0.265
CrP versus Placebo				
P†	<0.001	<0.001	<0.001	=0.568

* Student's *t* test for repeated measures.
† Student's *t* test for independent samples.

These researchers found that CrP completely reversed insulin stimulation of fatty acid synthase activity. They concluded that, "Since fatty acid synthase is a key enzyme in de novo lipogenesis, this reflects a coordinated activation of lipolysis and inhibition of lipogenesis with CrP treatment . . . thereby inhibiting insulin-mediated triglyceride storage."[31]

In the present study, the greatest changes in body composition were the result of reductions in body fat as revealed through DEXA. DEXA testing is one of the few technologies for measuring body composition that provides a direct physical measurement of adipose tissue. Hydrostatic testing, as well as most other measures of body composition, rely on estimating a person's body fat on the assumption that body density reflects the same percentage of fat as found in cadaver studies used to validate densitometry.[32] Furthermore, even hydrostatic testing does not actually measure a person's body volume to calculate body density—it estimates body volume from scale weights obtained in and out of water. Thus, even with hydrostatic weighing, body fat is derived from two different estimates, not from a physical measurement of adipose tissue. Of course, estimates derived from hydrostatic testing can be affected by the person's ability to exhale air consistently while under water as well as variations in lung volume over time, even when exhalation is consistent.

DEXA testing resolves these difficulties because obtaining the measurement requires that the person lie still on an open testing table for 15 to 20 minutes while the body is scanned. DEXA would seem to be the preferred technology to use, because it is critical to reduce the variability in testing when attempting to measure the efficacy of products or programs that produce relatively small changes in body composition.

In the present study, no dropouts biased the results. The requirement for subjects to provide a conditionally refundable deposit appears to have made a dramatic difference in the number of subjects who completed the

final testing, negating the need to use statistical controls, such as intention to treat. Poststudy critique revealed that subjects viewed the requirement to provide a deposit as reasonable, and such a requirement may have eliminated subjects whose motivation to complete the final tests was minimal. Although the data are not definitive, the deposit requirement appears to be an effective technique for obtaining final test data and is worthy of further study.

The requirement for subjects to provide a conditionally refundable deposit was based on the subject completing the study and an end-of-study questionnaire and had nothing to do with how little or how much the participant complied with the protocol. An equal number of subjects failing to take the product in the placebo and active treatment groups does not, of course, balance the effects across the groups. For example, a subject who fails to take a product in the placebo group would have no effect on the outcome measures because a placebo does not contain the active ingredient. However, failure of a subject to take a product in the active treatment group would attenuate the effects that the active product could be having. In fact, a completely noncompliant subject in an active treatment group would actually be a placebo subject. Thus lack of compliance would, by its very nature, attenuate differences between the two groups, stressing the need to obtain accurate data on how much of a product a subject consumed. The use of weekly check-ins and personal monitoring appears to have provided more comprehensive data and reduced the amount of bias that a lack of compliance could have on the outcome measures.

CONCLUSION

The findings of the present study suggest that supplementation with CrP each day can lead to significant improvements in body composition, particularly when the changes are corrected for differences in caloric intake and expenditure. In addition, the results of this study replicate the findings of a previous study, which suggest that the improvements observed are evident with both underwater and DEXA testing technologies. Finally, because an unusually high number of subjects (93.8%) completed the final testing, requiring research subjects to provide a conditionally refundable deposit (to be returned on completion of final testing) is a technique worthy of further study.

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Review

Clinical Studies on Chromium Picolinate Supplementation in Diabetes Mellitus—A Review

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ABSTRACT

Chromium (Cr) picolinate (CrPic) is a widely used nutritional supplement for optimal insulin function. A relationship among Cr status, diabetes, and associated pathologies has been established. Virtually all trials using CrPic supplementation for subjects with diabetes have demonstrated beneficial effects. Thirteen of 15 clinical studies (including 11 randomized, controlled studies) involving a total of 1,690 subjects (1,505 in CrPic group) reported significant improvement in at least one outcome of glycemic control. All 15 studies showed salutary effects in at least one parameter of diabetes management, including dyslipidemia. Positive outcomes from CrPic supplementation included reduced blood glucose, insulin, cholesterol, and triglyceride levels and reduced requirements for hypoglycemic medication. The greater bioavailability of CrPic compared with other forms of Cr (e.g., niacin-bound Cr or $CrCl_3$) may explain its comparatively superior efficacy in glycemic and lipidemic control. The pooled data from studies using CrPic supplementation for type 2 diabetes mellitus subjects show substantial reductions in hyperglycemia and hyperinsulinemia, which equate to a reduced risk for disease complications. Collectively, the data support the safety and therapeutic value of CrPic for the management of cholesterolemia and hyperglycemia in subjects with diabetes.

THE DIABETES EPIDEMIC

DIABETES IS A GROUP of chronic diseases marked by high levels of blood glucose that result from defects in insulin production and/or function. Type 1 diabetes mellitus (T1DM) is an insulin deficiency disease resulting from autoimmune destruction of pancreatic beta cells. It accounts for 5–10% of all diagnosed cases of diabetes. Type 2 diabetes mellitus (T2DM) begins with insulin resistance followed by reduced insulin production as the disease progresses, and makes up 90–95% of all diagnosed cases. Type 2 diabetes is associated with older age and obesity. A small percentage of diabetes (1–5%) occurs during pregnancy (gestational diabetes), following corticosteroid and other drug use, or following surgery or illness. Diabetes is the sixth leading cause of death in the United States, mostly from associated cardiovascular complications. Diabetes is also one of the leading causes of blindness, kidney failure, dental disease, lower-limb amputation, and complications of pregnancy. The es-

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timated cost of diabetes in the United States is $132 billion.[1]

Diabetes is a progressive disorder that affects an estimated 20.8 million Americans,[1–3] with over 200 million cases worldwide.[4] Since the vast majority of these cases are T2DM, managing their disease can involve a number of options. Most can control their blood glucose with diet and exercise, though some may require medications for hyperglycemia or concomitant cardiovascular disease. The question arises as to whether dietary supplements can provide nutritional support, in conjunction with other modalities, to improve glycemic and lipidemic control in diabetes.

THE CHROMIUM (CR) CONNECTION

Cr is a trace element essential in carbohydrate, lipid, and protein metabolism.[5,6] Cr is a cofactor for insulin function that increases insulin binding,[7] the number of insulin receptors,[8,9] and insulin receptor phosphorylation,[10] resulting in enhanced glucose transport into liver, muscle, and adipose tissue.[6] Since Cr is required for normal glucose and lipid metabolism, low Cr status can adversely affect blood glucose, insulin, total cholesterol, triglycerides, and high-density lipoprotein cholesterol.[6,9,11–15]

Although the minimum estimated safe and adequate daily dietary intake for Cr is 50–200 μg/day for persons 7 years and older, typical Western diets do not meet these requirements.[16–18] Anderson and Kozlovsky[16] reported that 90% of the U.S. population does not meet the estimated safe and adequate daily dietary intake. Similar studies have been documented in Canada,[19] Britain,[20] and Finland.[21] A more recent report found that U.S. adults are consuming less than the established adequate intakes of 25–35 μg of Cr/day.[22]

Dietary sources of Cr include brewer's yeast, beer, whole grains, cheese, liver, and meat; however, Cr content in foods varies widely.[18,23] In addition, the refining of grains and sugars and the processing of foods remove most of the absorbable Cr.[24] Much of the Cr measured in foods may originate from contamination from food-processing equipment and thus is not bioavailable.[23]

Both reduced Cr status[13,24] and overconsumption of refined carbohydrates[24,25] have been positively correlated with an increased prevalence of T2DM. High-sugar diets have been shown to increase urinary Cr losses 10–300%.[24] Relative Cr deficiency is further exacerbated with age,[26,27] illness,[28] pregnancy,[29] burns,[30] and stress.[31] One epidemiological study based on hair analysis showed low Cr status in over 50% of >2,000 Canadian subjects.[19]

In subjects with T2DM, Cr metabolism is altered by inadequate intake, decreased absorption, and increased loss, which is exemplified by abnormal blood, tissue, and urine Cr levels.[14,15,32] Current data strongly suggest that low levels of Cr in serum,[26,33] hair,[34] and toenail tissues[13] are significantly correlated with diabetes. However, people with diabetes show high urine Cr levels, which indicates that mobilized Cr was not reabsorbed by the kidneys.[13,35] For these reasons, Cr supplementation on the order of 1,000 μg/day has been recommended to provide significant clinical benefit in T2DM.[36]

GLYCEMIC RESPONSES TO CR PICOLINATE (CRPIC)

Methodology

Fifteen clinical studies on CrPic supplementation for diabetes mellitus were identified from a number of sources, including a recent meta-analysis,[37] a review of Cr effects on glycemic control,[38] literature searches retrieved from PubMed, Embase, *Current Contents*, *Ingenta*, *Science Direct*, journals, and abstracts from proceedings.

The study designs are summarized in Table 1. A total of 1,690 subjects, including 1,505 receiving CrPic, completed the trials. Twelve of the 15 studies were randomized, controlled trials. Three were open-label trials. Fourteen studies focused on T2DM, and one each on T1DM, corticosteroid-induced, and gestational diabetes. CrPic dosages ranged from 200 to 1,000 μg of Cr/day, and the duration of supplementation ranged from 1 week to 10 months.

Although measures of glycemic control varied, all 15 trials shared one or more measure-

Table 1. Clinical Studies Evaluating CrPic in Subjects with Diabetes

Investigator	*RCT*	*Form of diabetes*	*Number of subjects (number with CrPic)*	*CrPic (μg of Cr)*	*Study duration*	*Concomitant medication*
Anderson et al.[15]	Yes	Type 2	155 (105)	200, 1,000	4 months	Glibenclamide or glipizide
Bahadori et al.[39] (abstract)	No	Type 2	16 (16)	1,000	4 months	Sulfonylurea and metformin
Cheng et al.[40]	No	Type 2	833 (833)	500	9 months	Hypoglycemic medications
Evans[5]	Yes	Type 2	11 (6)	200	1.5 months	Hypoglycemic medications
Feng et al.[41] (abstract)	Yes	Type 2	136 (104)	500	3 months	Insulin
Ghosh et al.[42]	Yes	Type 2	43 (43)	400	3 months	Hypoglycemic medications
Kleefstra et al.[43]	Yes	Type 2	46 (29)	500, 1,000	6 months	Insulin >50 U/day
Lee and Reasner[44]	Yes	Type 2	28 (28)	200	2 months	Insulin, oral medications, diet
Martin et al.[45]	Yes	Type 2	27 (16)	1,000	6 months	Sulfonylurea
Morris et al.[46]	No	Type 2	5 (5)	400	3 months	None
Jovanovic et al.[47]	Yes	Gestational	30 (20)	300–800	2 months	Insulin or none
Rabinovitz et al.[48]	Yes	Type 2	78 (39)	400	3 weeks	Hypoglycemic medications, insulin
Ravina et al.[49]	No	Steroid-induced	54 (44)	600	1–2 weeks	Glibenclamide, metformin, or insulin
Ravina et al.[50]	Yes	Types 1 and 2	172 (162)	200	3 months	Sulfonylurea, metformin, or insulin
Vrtovec et al.[51]	Yes	Type 2	56 (56)	1,000	24 weeks	None
Totals	11		1,690 (1,505)	200–1,000	3 weeks–9 months	

ments of glycemic control, including fasting glucose (FG), postprandial glucose (PPG), fasting insulin (FI), postprandial insulin (PPI), glycated hemoglobin (HbA1c), or insulin sensitivity. Mean differences from baseline are summarized in Table 2. Some studies measured other aspects of metabolic dysfunction (i.e., blood lipids, microalbuminuria, apolipoprotein A1, or C-reactive protein) or body composition (i.e., body mass index, body fat, lean body mass). One study measured QTc interval prolongation on a standard electrocardiogram, which is a powerful predictor of mortality, cardiac death, and stroke in patients with T2DM.[52]

T2DM: summary of responses

Anderson et al.[15] conducted a landmark, randomized controlled trial (RCT) evaluating CrPic in subjects with T2DM. Sixty Chinese subjects received 200 μg/day, and 60 subjects received 1,000 μg of Cr/day as CrPic for 4 months. Supplemental CrPic led to significant improvements in FG, PPG, FI, PPI ($P < 0.05$), and HbA1c ($P < 0.01$) levels. Significance was achieved as early as 2 months, especially at the higher dose. Subjects receiving the 1,000 μg of Cr dose showed near 30% reductions in FG, PPG, FI, and HbA1c (Table 2).

A follow-up, open-label study was conducted in 833 Chinese subjects with T2DM taking insulin or hypoglycemic drugs.[40] All patients received 500 μg of Cr/day as CrPic for 10 months. Again, FG and PPG were significantly lowered ($P < 0.05$) after the first month of therapy and remained so in the following 9 months (Table 2). Close to 90% of subjects experienced marked relief from fatigue, thirst, and frequent urination. No confirmed side effects were reported.

Another RCT study[41] involving 136 Chinese subjects on insulin therapy taking 500 μg of Cr/d as CrPic for 3 months showed significant reductions in FG and PPG ($P < 0.01$). Three other studies, supplementing with 200–1,000 μg of Cr/day as CrPic from 3 weeks to 6 months (Table 1), reported significant improvement in both FG and HbA1c levels.[5,45,48] In two of those studies, the mean reduction in FG was highly significant ($P < 0.001$) (Table 2).

An RCT study on elderly subjects with T2DM recovering from stroke or hip fracture involved supplementation with 400 μg of Cr as CrPic over 3 weeks in addition to their normal

Table 2. Effect of CrPic on Glycemic Parameters in Subjects with T2DM

	FG (mmol/L)		*PPG (mmol/L)*		*FI (pmol/L)*		*PPI (pmol/L)*		*HbA1c (%)*	
Study	Δ	%	Δ	%	Δ	%	Δ	%	Δ	%
Anderson et al.[15]										
200 μg Cr/day	−1.1	−10.8	−2.3	−13.9	−35.0[a]	−25.5	−117.0[a]	−18.2	−2.3[a]	−24.5
1,000 μg Cr/day	−2.7[a]	−27.6	−4.5[a]	−30.4	−48.0[a]	−33.1	−103.0[a]	−15.6	−3.2[b]	−34.0
Bahadori et al.[39]	—	—	−0.7	−5.0	−60.4[a]	−38.5	−75.0	−13.6	−0.2	−2.4
Cheng et al.[40]	−2.0[a]	−20.0	−2.1[a]	−17.5	—	—	—	—	—	—
Evans[5]	−2.5[a]	−24.3	—	—	—	—	—	—	−1.9[a]	−16.0
Feng et al.[41]	−1.6[b]	−16.2	−3.2[b]	−20.8	—	—	—	—	—	—
Ghosh et al.[42]	−0.5[c]	−7.2	−2.0[c]	−16.4	−50.0[a]	−19.5	—	—	−0.01[d]	−0.1
Kleefstra et al.[43]	—	—	—	—	—	—	—	—	−0.4	−4.2
Martin et al.[45]	−1.7[c]	—	—	—	—	—	—	—	−1.16[b]	−11.9
Morris et al.[46]	−0.1	−1.3	—	—	—	—	—	—	0.1	1.5
Rabinovitz et al.[48]	−2.2[c]	−21.0	—	—	—	—	—	—	−0.6[b]	−7.3
Vrtovec et al.[51]	−0.2	−1.7	—	—	−27.8[a]	−28.4	—	—	0.2	2.9
Mean ± SD	−1.5 ± 0.9	−15.3 ± 9.7	−2.7 ± 1.4	−18.9 ± 8.4	−45.2 ± 12.2	−29.8 ± 7.0	−92.7 ± 16.6	−15.0 ± 1.5	−0.95 ± 1.1	−9.6 ± 12.1

A dash indicates no data are available. Δ, change from baseline; %, percent from baseline.
[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ from baseline; [d]$P < 0.05$ compared with control.

hypoglycemic and/or insulin medication.[48] These rehabilitating patients showed a significant decrease in FG and HbA1c levels. Blood glucose levels decreased from 10.5 mmol/L (190 mg/dL) at baseline to 8.3 mmol/L (150 mg/dL) at the end of the study ($P < 0.001$), and HbA1c improved from 8.2% to 7.6% ($P < 0.01$) (Table 2).

An RCT study on Asian Indian subjects taking CrPic (400 μg of Cr/day for 12 weeks) showed highly significant improvements in most measures of glycemic control (FG, PPG, and FI). Though the mean change in HbA1c was not different from baseline, it was significantly better than placebo ($P < 0.05$) (Table 2).[42]

Two studies on Caucasian subjects with T2DM either diet-treated[51] or on hypoglycemic drugs[39] showed that 1,000 μg of Cr/day as CrPic for 3 or 4 months, respectively, reduced FI significantly ($P < 0.05$, mean reduction between 28.4% and 38.5%) (Table 2). In the diet-treated study, a significant decrease in FI was associated with a shortened QTc interval in 62% of subjects, especially those with high body mass index.[51]

Insulin sensitivity was also significantly increased in three studies with CrPic[45,46,50] after 3–6 months of supplementation, with improvements as great as 72.5%.[50]

Two of the 15 studies did not show significant benefit on glycemic markers with CrPic intervention. One 6-month study examined obese patients, who exhibited poorly controlled T2DM (mean HbA1c >9.4%) despite receiving oral antidiabetic medications and high-dose insulin (mean >75 IU/day).[43] Thus, subject selection did not favor a positive outcome, particularly with single-nutrient intervention. The other negative study[44] employed 200 μg of CrPic for 2 months, which may have been an insufficient dose and duration to see positive results. Nevertheless, both studies reported a significant impact on blood lipid risk factors (see Nonglycemic parameters).

Other types of diabetes

CrPic may also improve insulin function in T1DM. Supplementation of 200 μg of Cr/day as CrPic to 48 patients with T1DM led to a 30% decrease in circulating insulin and improved blood sugar stabilization. The number of hypoglycemic episodes was also reduced. Over 70% of T1DM patients responded to CrPic therapy ($P < 0.05$).[50]

Supplementation with CrPic may be considered a safe and inexpensive way to improve glucose intolerance in gestational diabetes, the most common medical complication of pregnancy.[47] Gestational diabetes requires insulin therapy when diet does not prove effective. In a study involving 30 patients with gestational diabetes, those taking CrPic (4 or 8 μg of Cr/kg of body weight/day; 300–800 μg of Cr/day) for 8 weeks showed significantly improved glucose tolerance and reduced hyperinsulinemia compared with controls (Table 2). The 8 μg/kg/day group exhibited the lowest postprandial glucose levels.[47]

Diabetes can also result from corticosteroid treatment. Corticosteroid therapy is known to increase urinary Cr loss. Corticosteroid-induced diabetes is characterized by insulin resistance, ketosis, and acidosis—also symptoms of Cr deficiency.[49] Supplementation with CrPic has been shown to reverse corticosteroid-induced diabetes. Within 1 week, administration of 600 μg of Cr/day as CrPic significantly decreased FG values from 13.9 to 8.3 mmol/L (from 250 to 150 mg/dL, respectively) in one patient, while a maintenance dose of 200 μg of Cr/day kept glucose in the normal range. Corticosteroid-induced diabetes was ameliorated in 41 of 44 patients treated with CrPic. Hypoglycemic drugs were also reduced 50% in all patients who received CrPic supplementation.

Reducing drug requirements

CrPic supplementation reliably reduced antihyperglycemic medication requirements in several trials. In a 3-month study involving 136 patients, 81% of those in the CrPic (500 μg of Cr/day) group reduced their exogenous insulin dosage by an average of 19.4% ($P < 0.001$).[41] In a 3-week study of elderly patients with diabetes rehabilitating from stroke or hip fracture, CrPic (400 μg of Cr/day) decreased and often eliminated their need for antihyperglycemic medication.[48] Supplementation with CrPic (200 μg of Cr/day for 3 months) in 114

patients with T2DM and in 48 patients with T1DM led to a significant decrease in the insulin, sulfonylurea, or metformin requirements in >70% of patients as a result of significantly enhanced insulin sensitivity.[50]

Prediabetes

From a regulatory perspective, treatment claims for a disease like diabetes are not permissible with a dietary supplement. Thus, none of the above-cited clinical papers on diabetes and CrPic intervention can support a petition for a qualified health claim (QHC). A QHC for diabetes prevention was nevertheless issued by the Food and Drug Administration.[53] This QHC is the first for insulin resistance and was specific for CrPic. The QHC was based largely on one study by Cefalu et al.,[54] which showed that a dose of 1,000 μg of Cr as CrPic for 8 months had a significant impact on insulin resistance in obese subjects. In contrast, a recent 3-month study by Gunton et al.[55] did not show efficacy in insulin-resistant subjects. These researchers had reported using a daily dose of 800 μg of Cr, but it was later determined that only 100 μg of Cr/day (800 μg of CrPic) was provided,[56] suggesting the need for higher CrPic doses.

POOLED ANALYSES OF GLYCEMIC CONTROL

Given that virtually all studies employing CrPic supplementation for T2DM subjects showed improved glucose or insulin control, an attempt was made to express the combined effects of these changes. This analysis was conducted despite the diversity of demographics, doses, and durations in these studies. Pooled mean differences, pooled percent changes, and their standard deviations were determined for FG, PPG, FI, PPI, and HbA1c and are presented in Table 2.

FG and PPG

Six of 10 evaluable studies reported significant improvement in FG from baseline, with a mean reduction of 1.5 mmol/L (27.0 mg/dL), or 15.3%. Several studies have suggested that it is possible to decrease FG by 1.7–2.2 mmol/L (30–40 mg/dL). This change is comparable to that seen with intensive control using sulfonylureas or insulin.[57] Four of six studies measuring PPG showed significant results compared with baseline, with a mean reduction of 2.7 mmol/L (48.6 mg/dL), or 18.9%. In a study using two CrPic doses, the lower dose (200 μg of Cr/day) was ineffective, but the higher dose (1,000 μg of Cr/day) dose showed significant reductions in FG and PPG (Table 2).[15]

FI and PPI

There were four studies that evaluated FI after CrPic supplementation, one of which employed two different doses of CrPic. All trials reported significant improvements in FI from baseline regardless of CrPic dose, with an average reduction of 45.2 pmol/L (6.5 mU/L), or 29.8%. Two of three evaluable studies reported improvements in PPI from baseline, with an average reduction of 92.7 pmol/L (13.3 mU/L), or 15.0% (Table 2).

HbA1c

Of nine studies that measured HbA1c, four were significant with respect to change from baseline, and one was significant compared with placebo. One study[50] reported no difference in HbA1c between experimental and placebo groups, but did not disclose baseline data. This study also used a relatively low dose of CrPic (200 μg of Cr/day) for a short duration (2 months). In the study comparing two doses (200 vs. 1,000 μg of Cr/day), a greater reduction in HbA1c occurred with the higher dose.[15] The average reduction in HbA1c for all nine studies was -0.95, which was a 9.6% reduction from baseline (Table 2). A chart of these mean differences in ascending order shows the trends for HbA1c with CrPic intervention (Fig. 1).

NONGLYCEMIC PARAMETERS

Hyperlipidemia

CrPic supplementation also improves lipid profiles in subjects with diabetes. In a study T2DM patients, 200 μg/day Cr as CrPic for 1.5

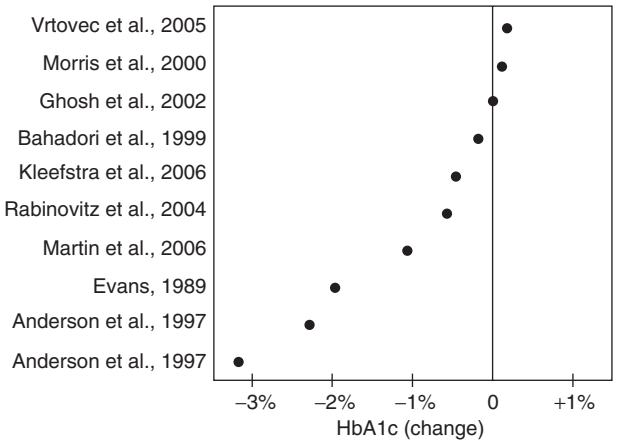

FIG. 1. Mean differences in HbA1c. The mean difference in HbA1c from baseline for the CrPic arm in each clinical study (nine studies total) is shown in ascending order: Anderson et al.,[15] Evans,[5] Martin et al.,[45] Rabinovitz et al.,[48] Kleefstra et al.,[43] Bahadori et al.,[39] Ghosh et al.,[42] Morris et al.,[46] and Vrtovec et al.[51] A negative number represents an average decrease in HbA1c.

months decreased total cholesterol and low-density lipoprotein cholesterol by 13% and 11%, respectively.[5] CrPic supplementation significantly improved total cholesterol, high-density lipoprotein cholesterol, and triglycerides in subjects with insulin-treated T2DM.[15,40,58] Rehabilitating, elderly patients with diabetes showed significant improvement in total cholesterol ($P < 0.02$) and a trend toward reduction in triglycerides.[48] Lee et al.[44] demonstrated a significant (17.4%) reduction in triglycerides in Hispanic subjects with diabetes after 2 months of CrPic supplementation (200 μg of Cr/day). Martin et al.[45] reported significant reductions in plasma free fatty acids after 6 months for T2DM subjects taking sulfonylurea and 1,000 μg of Cr/day as CrPic. Kleefstra et al.[43] showed a trend toward improvement in blood lipid profile with increasing blood Cr concentration, which became significant after 6 months for low-density lipoprotein, total cholesterol, and total-to-high-density lipoprotein cholesterol ratio.

Body composition

Improved insulin sensitivity and glucose control often result in improved body composition. This was supported in a recent study in which 27 subjects with diabetes on sulfonylurea received CrPic supplementation (1,000 μg of Cr/day) or placebo for 6 months. Those on placebo showed a significant increase in body weight, percent body fat, and total abdominal fat. Subjects randomized to sulfonylurea + CrPic experienced significant improvements in insulin sensitivity, HbA1c, and free fatty acids, which resulted in significantly attenuated body weight gain and visceral fat accumulation compared with placebo.[45]

CONCLUSIONS

The data indicate that CrPic supplementation represents a uniquely efficacious modality for glycemic control in subjects with diabetes. Indeed, 13 of 15 clinical studies reported significant improvement in at least one outcome of glycemic control. All 15 studies showed significant benefits in a least one parameter of diabetes management, including blood lipid control. Other positive outcomes linked to CrPic therapy included improved electrocardiograms, reduced need for hypoglycemic medications, and no reported adverse effects.

In contrast, a recent review[38] and meta-analysis[37] were less than positive about the effects of dietary Cr on glucose and insulin responses, in either T2DM or normoglycemic subjects. There are several reasons why these earlier reviews failed to support Cr supplementation for T2DM. First, distinguishing among the different forms of Cr appears crucial to the analysis. Other Cr complexes do not show the same consistent benefits.[59–63] Second, subjects with T2DM may require much higher Cr intakes than normal subjects to demonstrate significant benefits.[15,64] Third, in earlier reviews,[37,38] arbitrary dismissal of important CrPic clinical studies weakened the analysis. In this review, all trials using CrPic were considered, and most of those were RCTs.

A review that pools all Cr complexes fails to account for differences in their bioavailability. Several studies have shown CrPic to be significantly better absorbed than other Cr complexes.[15,65–68] In animal studies, CrPic reached significantly higher tissue concentrations in muscle, liver, and heart than Cr chloride ($CrCl_3$), Cr polynicotinate, or Cr histidinate.[66]

CrPic has also demonstrated higher absorption and insulin internalization rates compared with Cr polynicotinate.[65,68] Only one animal study reported greater bioavailability of Cr polynicotinate over CrPic, but based their analysis on a relative (percent Cr retained) rather than absolute (total Cr absorbed) scale.[69] The available data indicate that Cr polynicotinate is poorly absorbed.[65,68] Inorganic forms of Cr (e.g., $CrCl_3$) have never demonstrated consistent efficacy because of both limited intestinal absorption and intracellular uptake.[42,65,68] The addition of starch can further inhibit $CrCl_3$ but not CrPic absorption, suggesting that certain foods can interfere with bioavailability of inorganic Cr.[65]

Anderson et al.[65] have developed another Cr complex, Cr histidinate, which shows enhanced Cr bioavailability. However, this supplement does not yet have clinical or preclinical data supporting its efficacy or safety, and is not readily available in the marketplace.

There are several lines of evidence suggesting that CrPic supplementation reduces risk factors for diabetes and cardiovascular disease. According to the landmark Diabetes Control and Complications Trial[70] and UK Prospective Diabetes Study[71] trials, the risk for chronic disease complications of diabetes is closely related to the degree of glycemic control, as measured by HbA1c. In the current review, a pooled mean HbA1c change of −0.95% from 10 trials may represent substantial risk reduction, since a 1% drop in HbA1c equates to a 37% reduction in risk of microvascular complications and a 21% reduction in risk for diabetes-related mortality.[72] Cr deficiency is also associated with lipid abnormalities and an increased risk of atherosclerotic disease.[73] Given the known predisposition for coronary heart disease in diabetes, improving glycemic and lipidemic control with CrPic may translate to reduced risk. However, to substantiate real reductions in morbidity and mortality using CrPic supplementation, prevention trials will be required.

Several CrPic clinical trials in this review reported significant reductions in blood lipids.[15,44,58,74] Supplementation with CrPic may also reduce side effects (e.g., weight gain, elevated liver enzymes) associated with high sulfonylurea intake[75] by reducing the requirement for this medication.[49,50] Further risk reduction from CrPic supplementation is suggested by shortening of QTc intervals. QTc prolongation is a powerful predictor of total mortality, cardiac death, and future stroke in patients with T2DM.[51,52] Prolonged QTc in T2DM is related directly to impaired glucose tolerance and FI levels, and inversely with insulin sensitivity.[76]

Insulin resistance is an important risk factor for the development of diabetes and cardiovascular disease.[77] Up to 80% of Americans with T2DM are insulin-resistant.[78] Insulin resistance can affect a host of metabolic and mitogenic processes.[79] Chronic hyperinsulinemia is associated with hypertriglyceridemia, which is an atherogenic risk factor.[80,81] Hyperinsulinemia is also associated with an altered, proinflammatory fatty acid pattern in plasma.[82] High insulin levels also inhibit fatty acid oxidation and the regulation of body fat distribution, which can promote obesity.[83] Lowering the FI is associated with decreased risk of obesity, diabetes, and heart failure.[84]

The marked and consistent reduction in FI seen with CrPic supplementation in T2DM subjects (mean −29.8%) indicates improvements in insulin sensitivity. Insulin sensitivity was also shown directly in three other studies using CrPic supplementation. Since Cr helps improve insulin function and stabilizes blood glucose levels, less insulin is required.[41] Cr has been shown to reduce plasma triglycerides in T2DM patients.[44] Furthermore, body weight, body fat, and fat distribution may be positively impacted with CrPic supplementation.[45]

In conclusion, a significant body of clinical evidence supports the use of CrPic supplementation for treating hyperglycemia, hyperinsulinemia, and dyslipidemia in diabetes. Supplementation with CrPic, particularly at higher doses, may improve insulin sensitivity and glucose metabolism in gestational diabetes, corticosteroid-induced diabetes, and T1DM and T2DM patients. This review also underscores the importance of distinguishing CrPic from other forms of Cr based on bioavailability. Considering its compelling safety profile, as recently affirmed by the Food and Drug Administration,[53] CrPic is an inexpensive and efficacious modality with which to control

the high costs associated with diabetes treatment.[85] It could also prove useful as a nutritional adjunct to existing pharmacotherapies, corticosteroid use, and hypoglycemic drugs,[45] and may help reduce the requirement for these medications. Though the data supporting the benefits of supplemental CrPic for subjects with diabetes are strong, future studies may require a more careful selection of subjects to pinpoint its usefulness.

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SUMMARY AND CONCLUSION OF THE EXPERT PANEL REGARDING THE GENERALLY RECOGNIZED AS SAFE STATUS OF CHROMAX® CHROMIUM PICOLINATE AS A NUTRIENT SUPPLEMENT IN FOOD

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June 2002

SUMMARY AND CONCLUSION OF THE EXPERT PANEL REGARDING THE GENERALLY RECOGNIZED AS SAFE STATUS OF CHROMAX® CHROMIUM PICOLINATE AS A NUTRIENT SUPPLEMENT IN FOOD

We, the undersigned members of the Expert Panel, have, individually and collectively, critically evaluated the available information regarding Nutrition 21's chromium tripicolinate product, Chromax® Chromium Picolinate, summarized in the accompanying dossier, and other materials deemed appropriate and necessary. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- The substance that is the subject of this GRAS determination is a product to be marketed and sold by Nutrition 21 under the brand name, Chromax® Chromium Picolinate (chemical name: chromium tripicolinate). Chromax® Chromium Picolinate is a stable complex of trivalent chromium (Cr(III)) and picolinic acid, containing 12.4 percent by weight trivalent chromium. The two primary raw materials used in its manufacture are 2-cyanopyridine and potassium chromium sulfate.

- Appropriate product specifications have been established by Nutrition 21 to ensure that the final product, Chromax® Chromium Picolinate, is food grade, and compositional analysis of the product supports that there are no toxicological concerns from any product impurities. The safety of consumption of Chromax® Chromium Picolinate when used as an ingredient in food is based on scientific procedures by comparing the estimated daily intake (EDI) of trivalent chromium under the intended conditions of use of Chromax® Chromium Picolinate with the acceptable daily intake (ADI) of trivalent chromium derived from animal and/or human toxicity data.

- Trivalent chromium is currently ingested as part of a normal diet, as it is widely distributed throughout the food supply, although many foods contribute less than 1 to 2 mcg per serving. Trivalent chromium is recognized as an essential nutrient, although the Institute of Medicine (IOM) was recently unable to establish an Estimated Average Requirement (EAR). Instead, the IOM set Adequate Intakes (AIs) for trivalent chromium based on estimated daily intakes of trivalent chromium in the U.S. population; AIs for adults ranged from 25 to 35 mcg/day. The approach to evaluating the safety of the increased intake of trivalent chromium resulting from consumption of foods containing Chromax® Chromium Picolinate is based on an evaluation of the incremental increase this ingestion will produce compared to current background exposures to trivalent chromium. A reasonable assurance of safety is established when the intake of trivalent chromium resulting from the proposed uses of Chromax® Chromium Picolinate in food, added to current background exposures, results in a cumulative intake of trivalent chromium that is less than an intake that has been determined to be safe.

- The estimated intake of trivalent chromium from foods by the U.S. adult population currently averages about 30 mcg/day. Additionally, trivalent chromium is ingested from both single-ingredient dietary supplements and multivitamin (and mineral) formulations; these products are consumed by about 10 percent of the U.S. adult population with a modal intake of 25 mcg/day. Thus, the total background intake of trivalent chromium from these two sources is estimated to be as high as 55 μg/day for adults in the U.S.

- Chromax® Chromium Picolinate will be added to nutritional ready-to-drink beverages, beverage mixes, and bars at a maximum level of use of 2.4 mg Chromax® Chromium Picolinate per serving, which is equivalent to 300 mcg trivalent chromium per serving. The estimated mean and 90th percentile intakes of trivalent chromium resulting from these proposed uses of Chromax® Chromium Picolinate by consumers age 2 years and older is 304 and 545 mcg/person/day, respectively. The total cumulative intake of trivalent chromium from all food and dietary supplement sources of trivalent chromium by this same population, consisting of the 90th percentile trivalent chromium intake resulting from the proposed uses of Chromax® Chromium Picolinate and the 55 mcg/person/day contribution from other dietary sources, is estimated to be 600 mcg/person/day.

- Based on a review of the publicly available toxicity data on trivalent chromium, an estimated ADI for trivalent chromium of greater than or equal to 900 mcg/person/day (when administered as chromium tripicolinate) was derived. The primary basis for this ADI was a subchronic animal study in which a no-observed-adverse-effect level (NOAEL) for chromium tripicolinate via ingestion was established at a trivalent chromium dose of 15 mg/kg/day, the highest dose administered in the study. Applying a safety factor of 1,000 to this NOAEL, an ADI for trivalent chromium (when administered as chromium tripicolinate) of 15 mcg/kg/day was derived, or 900 mcg/day for a 60-kg person. In addition, an evaluation of the available clinical efficacy studies employing chromium tripicolinate suggests that this compound has a long history of safe use in humans as a nutritional supplement and, other than isolated case reports, there is no consistent evidence of adverse effects following its use in humans at doses as high as 1,000 mcg per day trivalent chromium. This upper safe limit in humans of 1,000 mcg per day trivalent chromium agrees quite favorably with the 900 mcg/day ADI for trivalent chromium derived from a subchronic animal study, lending further support to the validity of this ADI. Furthermore, published chronic animal studies of other trivalent chromium compounds provide corroborating evidence regarding the safety of long-term intakes of trivalent chromium at comparable doses.

- The cumulative EDI of trivalent chromium of 600 mcg/person/day, resulting from the proposed uses of Chromax® Chromium Picolinate and the 55 mcg/person/day contribution from other dietary sources, is less than the ADI established for trivalent chromium of greater than or equal to 900 mcg/person/day (when administered as chromium tripicolinate). Therefore, Chromax® Chromium Picolinate is considered safe under its intended conditions of use.

Conclusion

We, the undersigned members of the Expert Panel, have, individually and collectively, concluded the following:

> Chromax® Chromium Picolinate to be marketed and sold by Nutrition 21 has been sufficiently characterized to ensure consistent production of a food-grade product that yields no toxicity concerns from impurities. Ingestion of Chromax® Chromium Picolinate from the proposed uses and at the maximum use level results in a total cumulative intake of trivalent chromium that remains within safe limits established by published animal and human studies. Therefore, Nutrition 21's Chromax® Chromium Picolinate, meeting the specifications described in the accompanying dossier, to be used as a food ingredient in nutritional ready-to-drink beverages, beverage mixes, and bars at a maximum level of use providing 300 mcg trivalent chromium per serving, and resulting—when added to existing dietary intakes—in a cumulative daily intake of no more than 600 mcg trivalent chromium, is judged safe, and GRAS, by scientific procedures.

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(This expert opinion is being expressed by Dr. Anderson in his private capacity as an independent scientist, and is not to be regarded as a policy statement of the U.S. Department of Agriculture or the Federal Government.)

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Let's Juice! The Glycemic Index of Carrot Juice and Controlling Blood Glucose Levels

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Abstract

Carrot juice is an integral part of the Hallelujah Diet[sm]. The effect of carrot juice on blood sugar was tested. Through this study we measured the glycemic index of carrot juice to be 86, on a scale where the glycemic index of bread is 100. The glycemic response of carrot juice was lowered to 66 by consuming oil along with the juice. Chromium was also found to be beneficial for 4 of 6 people who participated in a 1-week supplement test. Carrot juice is likely to cause fewer problems to individuals struggling to lower their blood sugar than animal fats, refined sugar, bread, and flour products.

Introduction

The Hallelujah Diet[sm] is a pure vegetarian diet emphasizing raw fruits and vegetables along with the use of freshly extracted vegetable juices and Barleygreen. An integral part of the Hallelujah Diet[sm] is carrot juice. The carrot juice might include small amounts of leafy greens and other vegetables, but the principle ingredient in the juice is carrot.

The carrot is a root vegetable that contains a substantial amount of sugar. A 100-gram serving of raw carrots contains a total of 6.6 grams of sugar (1). It is estimated that a cup (~230 ml) of carrot juice contains 14 grams of sugar (1).

Blood sugar control. Sugar is a very metabolically active component of food. The intake of sugar (whether natural, in foods, or in a refined, concentrated form) causes a rise in blood glucose, as depicted in Figure 1. This rise in blood glucose stimulates the secretion of insulin by the pancreas. Insulin causes the cells of the body to take up glucose and store it as glycogen to be released at a later time. Often, if the rise in blood glucose is rapid, the pancreas will secrete too much insulin. Too much of the glucose will be sent to storage, leaving a deficit in the blood stream—a reactive hypoglycemic effect. So, the high blood glucose peak is followed by a low glucose valley. It can be a real roller coaster ride for the body, causing metabolic disaster over a long period of time. This is one type of loss of blood glucose control. Type I diabetics (insulin-dependent) and people consuming high-sugar, high-fat diets are susceptible to this type of hypoglycemic effect.

Another way to lose control of blood sugar occurs as people age, increase in body fat, and become less conditioned. In this state the body becomes less sensitive to the effects of insulin. So, more insulin is required to do the same amount of work that just a small amount of insulin was able to do previously. The body produces more and more insulin in its attempt to lower blood sugar concentrations to healthy levels. Over time this person develops what is called adult-onset, or type II, diabetes. In this diabetic state, when a person consumes carbohydrates their blood sugar rises quickly, but returns to normal very slowly (see Figure 1). Their body is resistant to the action of insulin and blood sugar levels always run high. Sugar will spill over into the urine as the body desperately tries to get rid of the sugar.

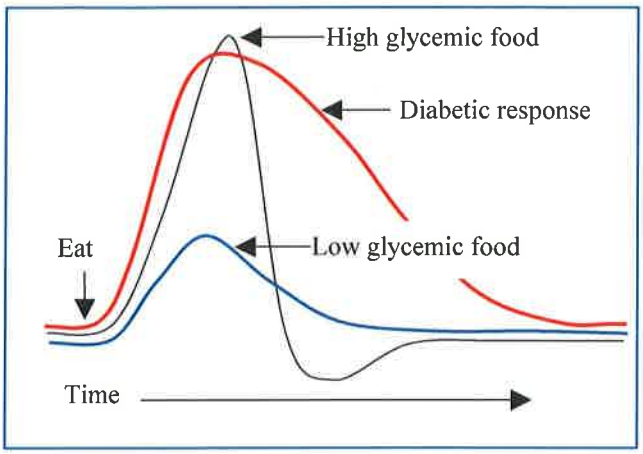

Figure 1. Blood sugar response. Depicted are a normal response to a high glycemic load and to a low glycemic load, and a Type II diabetic's response to a high glycemic load.

Elevated blood glucose is not near as detrimental in itself as is the accompanying elevated insulin levels. Elevated insulin levels cause many of the side effects of diabetes—high blood pressure, weight gain, retinal degeneration and blindness, peripheral neuropathies and amputation, kidney damage and failure, and heart disease. By controlling blood glucose in a normal range (and preferably in the low-normal range) these side effects are dampened, even eliminated. So, it is important to understand how carrot juice affects the control of blood glucose.

Glycemic index. Complex carbohydrates are converted into sugars in the body, through digestion. The rate of starch digestion depends on the amount of carbohydrate, the type of monosaccharides present (glucose, galactose, fructose), the nature of the starch (straight or branched chain, resistant), form of the food,

starch particle size, and degree of food processing. All of these factors affect how much and how quickly a food will cause a rise in blood sugar. These factors are all taken into account intrinsically with the glycemic index. The glycemic index of some common foods is given in Table 1.

The glycemic index is a normalized measurement of blood glucose response to food. The official definition of the glycemic index is "the incremental area under the blood response curve of a 50g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject." (2) The standard is either pure glucose or white bread. Because the glycemic index is normalized, the variation between individuals' responses to carbohydrate is removed, and the results are more easily applied to other individuals.

Table 1. Glycemic Index of some common foods (3).

Food	GI	Food	GI
Baked potato	121	Yam	73
Bread	100	Oatmeal	70
Dry beans	40	Beans, canned	60-75
Banana	77	Dates	146
Apple	54	Apple juice	58
Pasta	53-65	Nuts	16-32
Sucrose	92	Fructose	32

The question we will address in this study is this: what is the effect of carrot juice on blood glucose levels? What is the Glycemic index of carrot juice? This question is a concern for people, especially people who already know they are sensitive to large sugar intakes. This question is particularly important for diabetics who wish to regain their health. Is it best for them to stay away from carrot juice and its health benefits, or can they consume it safely? This question is a concern for others, too, who desire their blood sugar to stay in the low normal range, which is correlated with high resistance to infection.

Along with this question of carrot juice and blood sugar, we will explore ways to decrease the rate of blood sugar rise when drinking carrot juice. It is well known that fat will decrease the rate of gastric emptying. Large effects are seen when 0.5 – 1.0 g fat / g carbohydrate are added to a test meal (4). Consuming Udo's Oil along with the carrot juice will likely slow down the rate of blood glucose increase. We tested whether or not including Udo's oil along with the carrot juice would alter the area under the glucose response curve (glycemic index).

A second method of reducing the glycemic index of carrot juice will also be examined—using chromium to boost the effectiveness of insulin to respond quickly to a carbohydrate load. Chromium, as part of the Glucose Tolerance Factor, is a trace mineral that is a necessary co-factor for insulin's action. Chromium makes the body more sensitive to the action of insulin, possibly by several mechanisms. Individuals who have impaired glucose tolerance can often improve their glucose tolerance by supplementation with chromium (5-9). A review of controlled interventions with subjects who had impaired glucose tolerance found that in 12 of 15 studies chromium had a beneficial effect on insulin sensitivity or blood lipid profile (10). Many diabetics have low levels of chromium possibly due to a higher requirement for chromium in the diabetic state (7).

Methods

Subjects

6 volunteers (2 females, 4 males) were recruited from Hallelujah Acres to participate in this study. An oral presentation of the study was given, along with a written informed consent document, and written informed consent was received from each volunteer before participating in the study.

Physical characteristics (age, sex, height, weight, % body fat) were gathered for each subject. A short food-screener was administered to determine usual food intake patterns of the volunteers.

Test Protocol

Juice Preparation and analysis. Carrot juice was made using a commercial juicer (Model X-1, Goodnature Products, Inc., Buffalo, NY) on Monday and Wednesday mornings in the kitchen at Hallelujah Acres, in Shelby, NC. Large commercial-grade California juicing carrots were used. Sufficient juice was made so that all volunteers consumed the same juice. Tests were conducted on Tuesday, Wednesday, and Friday mornings of 3 consecutive weeks. There were possibly slight differences in sugar and mineral content of different batches of carrot juice. 4 samples of carrot juice were analyzed for total sugar by an independent laboratory (Southern Testing & Research Laboratory). Two samples of carrot juice were tested by STRL for total carbohydrate as well. Estimates of the total carbohydrate of all samples used were based on these analyses. Total carbohydrate of a sample is determined by what is left after everything else is accounted for. So, the moisture, fiber, fat, protein, and mineral (ash) content of the samples had to be determined to find total carbohydrate content. Protein was determined by the Kjeldahl method, fat by methanol extraction, fiber by a crude fiber test, and moisture in a forced draft oven. Total sugars were determined by an HPLC separation method.

GI Testing protocol. Tests were conducted on Tuesday, Wednesday, and Friday mornings of 3 consecutive weeks. Blood glucose concentrations (whole blood values) were measured using a finger prick device and a blood glucose monitoring system (OneTouch Ultra, LifeScan, Inc., Milpitas, CA). This device allowed rapid testing (5 seconds to get the result) with a small sample of blood, minimizing pain from multiple samples.

After an overnight fast, before eating anything in the morning (drinking water OK), subjects had their blood glucose checked (fasting) and then consumed the test food (served in a randomized order). Subjects were then allowed to carry out their normal duties (light office work) while their blood glucose level was checked over the next 2 hours (0, 15, 30, 60, 90 and 120 minutes).

In order to determine the glycemic index of carrot juice a standard carbohydrate source was tested. The usual standard is a 50g carbohydrate portion of white bread (note that dietary fiber is not included in this carbohydrate count). In this study we used a 50g carbohydrate portion of whole wheat bread. The glycemic index of whole wheat bread is identical to the glycemic index of white bread. (Note that this demonstrates that there are many more factors than just fiber intrinsically accounted for in the glycemic index.) Since the glycemic indexes of white bread and whole wheat bread are identical, and our subjects and investigators preferred whole wheat bread, we used the whole wheat bread as our standard.

This testing was repeated once for each sample. Two samples were tested: (1) 14.5 oz of pure carrot juice, and (2) 14.5 oz of carrot juice with 30g of Udo's oil. Along with these 2 samples we tested the standard food 3 times.

Chromium. About 1 month after these tests were completed, a follow-up sub-study was carried out. Subjects in the previous study, along with 2 new participants participated in the chromium study. Two tests using 14.5 oz of carrot juice were done on consecutive days to establish a baseline blood glucose response of the participants. For 7 days, subjects then took 200 µg of chromium daily, supplied as chromium picolinate (Soloray). Subjects were then retested twice on consecutive days to determine if there was any change in their blood glucose response to 14.5 oz of carrot juice.

Analysis

The blood glucose responses to carrot juice were analyzed by calculating the area under the curve (AUC) (11). AUC is a measure of the amount of blood glucose greater than the fasting level integrated over time. Only the area greater than the fasting value was included in the AUC calculation. Responses were normalized to each individual's response to the test food, whole wheat bread. This yielded that individual's glycemic index for carrot juice. The 6 individual glycemic indices were averaged to obtain an overall glycemic index rating for carrot juice.

Since the carbohydrate load of 14.5 oz of carrot juice was not 50 g, a formula was used to evaluate the glycemic index for a 50g carbohydrate portion of carrot juice. The formula, from Wolever & Bolognesi (4) is:

$$GR = 1.5 \cdot GI\,(1 - e^{-0.018 \cdot D}) + 13 \qquad (1)$$

GR is the glucose relative response (compared to wheat bread), GI is the glycemic index of the food, and D is the amount of carbohydrate in the serving of food. By rearrangement of the equation, knowing GR and D, the GI of carrot juice was determined.

Results

The physical characteristics of the volunteers are given in Table 2. There was a wide range in ages and body sizes within this group.

Table 2. Characteristics of Volunteers.

Subject	% body fat	Age	Sex	BMI, kg/m^2
1	32.5	36	F	21.1
2	39.2	68	F	38.8
3	25.6	38	M	27.6
4	12.4	53	M	20.9
5	18.9	68	M	23.6
6	15.6	31	M	22.1
Mean	24.0	49		25.7

Total carbohydrate and total sugar content of carrot juice was analyzed by STRL, an independent laboratory. Results are shown in Table 3. All of the numbers are averages of 2 samples, except for total sugar, which is the average of 4 samples—3 from California carrots and one sample of carrots only marked as grown in the USA. The total sugar of just the 3 samples of California carrots is slightly higher, at 5.3 g/100g, compared to the average of 4.9 g/100g of all 4 samples. The sugar profiles are the average of 2 samples of California carrots. The concentrations of sucrose, maltose, and lactose were less than 0.1 g/100g.

In an 8 ounce glass of carrot juice (~230 g), there would be about 76 calories, 11.3 g sugar, lots of vitamins and minerals, and very little fat or protein. By comparison, an 8-ounce serving of soda pop would have between 25 and 31 g of sugar, along with caffeine and phosphoric acid.

Table 3. Food analysis of carrot juice.

	Carrot juice, this study	Carrot juice, USDA (1)
	Units are g/100g of juice	
Calories	33 Cal	40 Cal
Moisture	91	89
Protein	<0.2	0.95
Fat	0.3	0.15
Ash	0.76	0.75
Fiber, crude	0.3	0.8
Total Carbohydrates	8.0	9.3
Total Sugar*	4.9	6.0
Fructose	0.7	1.0
Glucose	4.25	1.0
Sucrose	<0.1	3.6

*Average of 4 samples.

Figure 2 shows what the average glycemic response was for the test foods. The beginning blood glucose is set to zero, in order to normalize all of the data to the same starting point. Fasting glucose values varied from 70 to 115 mg/dl in this study group. The mean fasting glucose value was 88 mg/dl. So, the average peak in blood glucose after consuming 14.5 oz of carrot juice is only 116 mg/dl, still in the range of normal blood glucose values.

In fact, there were very few abnormal readings throughout this study. Out of 66 blood glucose trials, there were only 8 tests with peaks higher than 140 mg/dl, and 3 of these were when bread was consumed. Also, there were only 4 trials with a nadir lower than 70 mg/dl, with none lower than 65 mg/dl. So, even with a large serving of carrot juice, 14.5 oz, blood glucose values did not soar or plunge in this study group.

The glycemic index for carrot juice determined here is 86, and the glycemic index for carrot juice + Udo's oil is 66, with standard deviations of 33 and 26, respectively. There was a lot of variation within and between individuals. Responses by an individual on different days to the same test food varied as much as two-fold.

In order to determine what factors affected individual's response to the same carbohydrate load, a supplementation test using chromium was performed. 6 individuals completed the chromium supplementation study, following the protocol above in the Methods section.

As shown in Table 4, chromium was beneficial to 4 of the 6 subjects. Overall, a paired t-test showed that

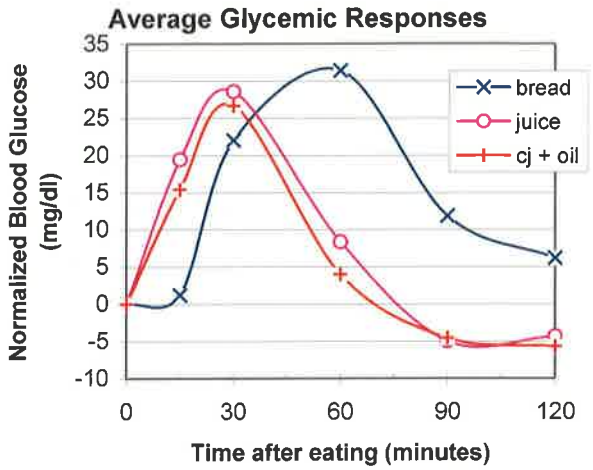

Figure 2. Average Glycemic Responses. Blood sugar values are normalized to fasting level for clarity of presentation. Values for carrot juice and carrot juice + oil are not corrected for lower carbohydrate load (30 g, see text). Bread (x) is a 50g carbohydrate portion. Juice (o) is a 14.5 oz serving of carrot juice. cj + oil (+) is 14.5 oz of carrot juice with 30 g of Udo's Oil.

the difference for this small test group was almost significant (p=0.064). In a slightly larger group the measured change in glucose response would be statistically different. Note that changes greater than 50 percent reduction were possible after just a week of supplementation. Also, a higher blood glucose response did not predict that chromium would be beneficial (see subject #2 data). Finally, this is not a definitive report proving that chromium is beneficial. To show this conclusively, it is helpful also to monitor glycated hemoglobin (Hgb A_{1c}), C peptide excretion, and fasting insulin levels also. However, this does give an indication that chromium is beneficial for lowering blood glucose response to a carbohydrate load.

Table 4. Response to chromium supplementation. Data shown is the calculated area under the blood glucose response curve. Data are the average of 4 tests before and 2 tests after chromium supplementation.

Subject	AUC, Before	AUC, After	% Change
1	1,109	1,114	0.5%
2	1,416	1,457	2.9%
3	1,531	710	-54%
4	1,021	548	-46%
5	1,765	1,209	-32%
6	859	678	-21%

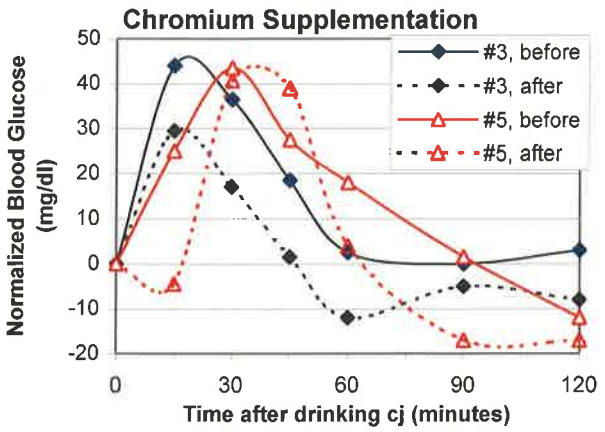

Figure 3. Effect of chromium supplementation on 2 volunteers. The normalized blood glucose response to 14.5 oz of carrot juice is shown, both before and after daily supplementation with 200 µg of chromium as chromium picolinate for 7 days. Data are the average of 2 samples each before and after supplementation.

Figure 3 shows the blood glucose profile of 2 volunteers before and after chromium supplementation. The figure adds some information not seen in Table 4 in the AUC data. First, the blood glucose concentration at 15 minutes after consuming carrot juice is much lower for these two subjects. The lower peak glucose concentration contributed to the smaller AUC for subject #3, while a slower rise and quicker fall in elevated blood glucose contributed to the smaller AUC for subject #5, without reducing the peak glucose concentration at all. So, the response to chromium in different individuals will vary even in the way that the AUC is reduced.

It is of note that subjects could not predict what their blood glucose level would be based on how they felt. Even at the peak or after a sharp drop in the blood glucose concentration there was generally no change in the way the individual felt. None of the blood glucose measurements were below 65 mg/dl, and only 4 tests fell below 70 mg/dl. At lower concentrations of glucose there would be symptoms of low blood glucose, but none of the subjects here felt any symptoms associated with hypoglycemia.

Because of the small size of our study group there were no conclusive correlations between anthropomorphic measurements and response to bread, carrot juice, or fasting glucose concentrations. Correlations between body composition and fasting glucose levels and insulin resistance do exist (12), but they could not be confirmed here in our study.

Discussion

Carrot juice composition. Though there is some composition information on carrot juice in the USDA database, a look at the information reveals that much of data is inferred from the composition of carrots without any direct measurement (see number of measurements of each nutrient on the Nutrient Data Laboratory website (1)). Therefore, it was felt that a direct measurement of the nutrients most pertinent to this study would be important, since they might vary from the estimates in the database. The main difference was the lower measurement of protein in our study, and the slightly lower carbohydrate content of the carrots we tested, compared to the values given in the USDA database. These differences could easily be accounted for by variations in the carrots, our smaller sample, and perhaps differences in varieties and growing conditions of the carrots.

The sugar profile that was measured by STRL is remarkably different from the profile reported for carrots in the USDA database. The main sugar in the carrots analyzed here was glucose, while sucrose was found to be the main storage sugar in other reports (1, 13). It is unclear the reason for this disparity. STRL used a HPLC method and detected no peaks for sucrose, despite having a clean chromatograph. It is possible that there was an invertase enzyme present in the juice, which would convert sucrose into its two sugar components, glucose and fructose. However, the amount of detected fructose was low, indicating that any invertase activity, if present, could not have changed the profile very much.

There was one other difference in methodology that should be noted. Total sugar reported here only includes the five sugars fructose, glucose, sucrose, maltose, and lactose, as required by food labeling guidelines. The total sugar data in the USDA database also includes other sugars, usually present in small amounts, about 15 percent of the total sugar. This may account for the slightly higher numbers reported by the USDA compared to our numbers reported here.

Some assumptions made in the analysis in this report. First, it was not feasible for subjects to consume a 50 g carbohydrate load of carrot juice, approximately 24 ounces. The serving given in the study, 14.5 ounces was already almost twice the usual serving. This fact alone points out how difficult it is to get a truly large carbohydrate load from drinking carrot juice, compared to eating a typical serving of complex carbohydrates.

However, in serving a smaller amount of carbohydrates, we had to approximate the glycemic response to a full 50 g carbohydrate load. To do this we used an equation derived from servings of complex carbohydrates. It is not known whether this equation is

truly valid for foods made mostly of sugars rather than complex carbohydrates.

We also had to approximate the total amount of carbohydrate in each serving of carrot juice. It was not feasible to get a laboratory analysis of each juice before using it, since the laboratory analysis took a week to complete. We assumed that the carbohydrate content, and total sugar content of the juice did not vary dramatically from batch to batch. An attempt was made to correlate Brix measurements of total sugars with total sugar assays from STRL laboratory, but no correlation could be obtained using the 4 samples submitted. Furthermore, the only published correlation between Brix and total sugar (13) also reported a much higher content of total sugar for California carrots (12g/ 100g), making their correlation irrelevant to our study.

Glycemic Index of Carrot Juice. The glycemic index of carrot juice was found to be 86, on a scale where bread has a glycemic index of 100. The other published reports for the glycemic index value of carrot juice list it as 85 (14), and 64 (3), which are in agreement with our number. If anything, our estimate appears to be on the high side. This places carrot juice in the medium range of foods in terms of the sugar response generated by the carbohydrates in the food.

Glycemic Response. The response of individuals to carrot juice is characteristically different from the response to a complex carbohydrate. By 15 minutes the blood glucose had risen significantly after drinking carrot juice, while there was no rise in blood glucose 15 minutes after eating bread. Note that if blood glucose rise is part of the signal to the brain to tell us that we are getting full, then this would indicate that it is much easier to overeat on complex carbohydrates than on simple sugars as are found in fruits and carrot juice. However, stomach distention and peptide hormone production in the small intestine are also part of the complex signal to stop eating (15), so this theory is incomplete. Note that when the carrot juice is used with the oil, the shape of the curve does not change. Only the height is different. But this difference in height is significant when the area under the curve is calculated, as done when determining the glycemic index. The difference is 20 points on the GI scale, which can make a difference in long-term use.

Variation in glycemic response between individuals. Our study group was not large enough to make any correlations between glycemic response, fasting glucose levels, and anthropomorphic measures. However, the onset of diabetes is strongly correlated with obesity, indicating that these measures of body composition can be used to evaluate somebody's risk of becoming diabetic.

Variation in glycemic response within individuals. Throughout this study it was noted that day-to-day variations in an individual's glycemic response to a food could vary two-fold. This result means that there are factors that vary daily in individuals that have a dramatic effect on how the body responds to the same glycemic load. Two known factors are the overall glycemic index of a person's last meal (16) and the amount and quality of sleep the night before a glycemic test (17, 18). These factors were not recorded in this study. It is possible that other factors also are important. Possibly the chromium content of the food eaten the day before a glycemic test would also be important along with glycemic index of the diet, especially for people with low body stores of chromium.

Chromium. The chromium supplementation study showed that additional chromium is beneficial even among healthy individuals eating a diet low in refined sugar. This test was very short; many studies of chromium supplementation have been done for 8 weeks or longer. Even so, dramatic results were seen in 4 of the 6 individuals in the study. An average of 4 tests of carrot juice before supplementation and 2 tests after supplementation were used to help minimize the daily variation in glycemic response.

Chromium taken by diabetics and others has increased insulin sensitivity, reduced fasting insulin, decreased fasting glucose levels, decreased cholesterol and triglyceride levels, and stimulated weight loss (10). However, not all diabetics or normal people respond to chromium supplementation. Supplemental chromium is only helpful for people who have low body stores of chromium. This was true in our study as well, since 2 of the 6 volunteers had no change in glycemic response after supplementing with chromium.

Application

Carrot juice—too much sugar? Carrot juice is not a low-glycemic food. The intention is not to suggest that it is a low-glycemic food, but rather to understand the role of carrot juice in the context of other foods. First of all, the serving size of carrot juice generally recommended is about 8 oz, which only contains about 12g of sugar, 18 g of carbohydrate (see Table 5). This is a not a large sugar load. However, if an individual is intolerant of fruit, then carrot juice would have to be considered carefully as well.

In Table 5 a comparison is given between carrot juice, a few common fruits, and three common carbohydrate foods. The glycemic response expected from a serving of these foods, compared to a 50 g carbohydrate serving of bread, is given in the last column, labeled as the serving size GI. This calculation is based on equation (1), given above.

As you can see, even though the GI of carrot juice is 86, the typical serving size would yield only about half as much of a glycemic response as 2 large slices of

Table 5. Context for carrot juice. Serving size GI is the predicted glycemic response for this amount of each food, compared to a 50g carbohydrate serving of bread.

Food Item	Weight (g)	Carb (g)	Sugar (g)	Svg size GI
Carrot Juice, 1 C	230	18	11	46
Medium Apple	138	21	17	35
Medium Orange	131	15	12	32
Medium Banana	118	28	22	55
Medium Sweet Potato	114	28	11	52
Medium Baked Potato	122	31	2	84
Whole Wheat Bread-Homemade, 2 slices	92	47	4	92

bread (46 vs 92). From this analysis it appears that a diabetic individual, or others who have poor blood sugar control, would be able to drink carrot juice and eat fruit in moderation. Emphasizing grains and complex carbohydrates over fruits and carrot juice is not sound advice for the sugar-sensitive individual.

Juice with oil. The glycemic response of carrot juice can be reduced by drinking it with a spoonful of oil. About 1 tablespoon of oil with an 8-ounce serving would get the results seen in this report. The oil has an added benefit of increasing the absorption of the fat-soluble carotenoids, such as beta-carotene, alpha-carotene, and lutein. Any oil or fat will produce this effect. This is a great way to get in beneficial oils and carrot juice at the same time.

Diabetics and carrot juice. Hallelujah Acres has recommended that diabetics start with smaller servings of carrot juice. Diluting 4 oz of carrot juice with 4 oz of distilled water has been the recommendation. Since less carrot juice is consumed, the glycemic response will be less, and the sugar-sensitive individual will tolerate the juice better. Many people with diabetes do not have problems with drinking an 8-ounce serving of carrot juice right from the beginning. So, a person needs to monitor their blood sugar and be very careful when making effective dietary changes, such as adopting the Hallelujah Diet[sm].

As time passes and a person with diabetes becomes a person who *used* to have diabetes they should be able to consume more carrot juice at one time without harmful effects.

Chromium. Chromium intake is sub-optimal in most individuals. High sugar diets promote the excretion of chromium in the urine (19). A two-week trial with daily chromium supplementation would be adequate to see if a person receives any benefit. Up to 1000 µg/day of chromium has been used with no negative side-effects. There is some evidence that more than 200 µg/day of chromium is necessary to see a positive effect in diabetics (7, 20). Fasting blood glucose concentration may be reduced along with fasting insulin, which is harder to measure. Also, a morning challenge with carrot juice such as done in this study would easily reveal any benefit from the chromium supplement (plot results as shown in Figure 3). Diabetic symptoms, such as frequency of urination, may also be alleviated, along with weight loss and lean tissue gain.

Chromium is found in nuts, seeds, whole grains, and in brewer's yeast. Increasing nut and seed intake will provide more chromium and more of the beneficial plant oils associated with successful raw food diets (14). It will also reduce the amount of sugar in the diet, thus lowering the requirement for chromium in the body.

Conclusion

Carrot juice has a glycemic index of 86, while carrot juice and oil has a glycemic index of 66. While this is a moderately high value, all grain flour products have higher glycemic index values. Since an 8-oz serving of carrot juice only contains 18 g of carbohydrates, most people can consume it without causing any sugar imbalances. Loss of blood sugar control is more likely due to over consumption of animal fat, refined sugar, and grain products rather than moderate consumption of carrot juice and fruit.

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DM

Letters

Chromium supplementation improves insulin resistance in patients with Type 2 diabetes mellitus

Trivalent chromium (Cr) is an essential nutrient thought to have a role in lipid and carbohydrate metabolism. Recently, chromium picolinate was found to improve markers of diabetic control in patients with Type 2 diabetes mellitus [1] and reverse corticosteroid-induced diabetes [2]. In the study by Anderson *et al.* [1] insulin sensitivity was not measured. We have previously reported that supplementation with 200 μg/day chromium picolinate for 10 weeks improved insulin sensitivity (as assessed by a low-dose short insulin tolerance test (ITT)) in a small group of healthy volunteers [3]. Type 2 diabetic patients have been shown to have low levels of plasma chromium and high levels of urine chromium [4] and we have now examined the effect of chromium supplementation on insulin sensitivity in a small group of patients with diet-controlled Type 2 diabetes.

Following approval by the local ethics committee, five patients (three male, two female, age range 43–76 years) newly diagnosed with Type 2 diabetes and maintained on diet alone, received 400 μg/day chromium picolinate for 12 weeks. Patients' insulin sensitivity was assessed by short ITT using an intravenous bolus of human soluble insulin (Actrapid, Novo Laboratories, Copenhagen, Denmark) 0.1 units/kg body weight prior to chromium supplementation, after 12 weeks of chromium and then again 4 weeks post-cessation of chromium. At 1,6,12,14 and 16 weeks, patients provided fasting blood samples for plasma chromium, glucose, HbA_{1c} and insulin and a second morning void urine sample for chromium and creatinine analysis.

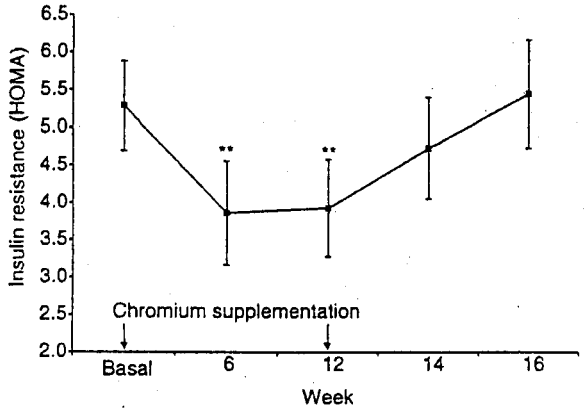

Figure 1 Changes in insulin resistance in Type 2 diabetic patients following 12 weeks once daily supplementation with 400 μg chromium picolinate. (n = 5, $^{**}P < 0.01$ – difference from basal (pre-supplementation) values).

All patients showed significantly increased glucose utilization (derived from the linear slope of the glucose concentration during the short ITT from t = 3 mins to t = 15 mins) when taking chromium with a mean increase of 60% (range 16–100%) which returned to pre-supplementation levels when chromium was withdrawn. Insulin resistance (IR) calculated using a HOMA technique [5] from fasting insulin and glucose concentrations improved significantly after 6 weeks of chromium supplementation (P = < 0.01) remaining so until supplementation ceased after which IR returned towards pre-supplementation values (Fig. 1). There were no significant changes in mean values for weight (pre-Cr 84.9 kg, post-Cr 84.6 kg), fasting glucose (pre-Cr 8.0 mmol/l, post-Cr 7.9 mmol/l), HbA_{1c} (pre-Cr 6.8%, post-Cr 6.9%) or urine creatinine (pre-Cr 12 388 μmol/l, post-Cr 13290 μmol/l). Fasting levels of plasma and urine chromium were measured by Zeeman furnace electrothermal atomic spectrophotometry. Plasma levels increased from a pre-supplementation mean ± SEM of 0.11 ± 0.02 μg/l (current laboratory reference range for healthy population 0.12–0.53 μg/l), to peak at 2.29 ± 0.57 μg/l at 12 weeks supplementation and declined to 0.25 ± 0.03 μg/l 4 weeks after cessation, significantly higher than pre-supplementation values (P = 0.008). Urine chromium results followed a similar pattern returning to pre-supplementation levels 2 weeks after cessation.

In this short-term study, the insulin-sparing effect of chromium supplementation did not result in any improvement in glycaemic control. It is possible that this might have been observed with a longer period or higher level of chromium supplementation. However, the results of this pilot study indicate that chromium supplementation improves insulin sensitivity in patients with diet-controlled Type 2 diabetes comparable to that seen during treatment with thiazolidinediones [6]. In the absence of a change in weight the likeliest explanation is a direct effect of chromium on insulin action in line with previous *in vitro* studies reported from our laboratory [7].

While the mechanism of chromium enhancement of insulin sensitivity remains to be determined, this study suggests that this trace element may be beneficial to patients with Type 2 diabetes by improving their sensitivity to their own hormone. This may extend the period during which their diabetes may be managed without exogenous insulin administration. This study strongly supports the earlier larger study [1] in which insulin sensitivity was not assessed. Serious consideration should now be given to

large-scale placebo-controlled studies of chromium supplementation for Type 2 diabetic patients in the UK.

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Heterogeneity in the clinical course of patients with Type 2 diabetes on dialysis – the need for different preventative strategies

In the last decade there has been a significant increase in the number of patients with Type 2 diabetes mellitus who commence dialysis for end-stage renal failure (ESRF) [1]. Diabetic nephropathy, predominantly caused by Type 2 diabetes mellitus, is now the commonest cause of dialysis-dependent renal failure in the USA and much of western Europe [2,3]. This is attributable to three factors: first, the increased incidence of Type 2 diabetes in ageing, obese Western societies; second, the increased willingness of nephrologists to dialyse patients who have major diabetic complications outside the kidney; and third, improved cardiovascular survival resulting in more patients progressing to ESRF.

The clinical course of the development and progression of diabetic nephropathy in Type 2 diabetes is less well defined than in Type 1 diabetes. Although Type 2 diabetes is a major cause of ESRF, less than 1% of patients in the UKPDS developed renal failure in 10 years' follow-up after diagnosis [4]. It is uncertain how to identify those subjects at high risk. The detection of microalbuminuria will have a low predictive power for the development of renal failure, as this was found in over 28% of patients in the UKPDS at diagnosis [5].

We have reviewed our dialysis patients with Type 2 diabetes with the aim of identifying whether there had been missed opportunities to detect and treat diabetes and its complications before the development of progressive renal disease.

We examined all the available hospital and primary care (general practitioner) records for the Type 2 diabetic patients currently on haemodialysis or peritoneal dialysis at our main and satellite renal units. There were 12 Type 2 diabetic patients out of a total of 223 dialysis patients. Eleven of these patients gave signed consent for their primary care records to be examined. Five of the patients were on haemodialysis and seven on peritoneal dialysis.

For each patient the date of diagnosis of diabetes was recorded, age at diagnosis and the number of years from diagnosis to the commencement of dialysis. The date of the first recorded hypertension (> 140/90) and the number of years from diagnosis to the first recorded proteinuria (> 0.3 g/l on dip-stick testing) and abnormal creatinine (> 120 μmol/l) were documented. Specialized renal investigations, diabetic complications and attendance at a hospital diabetes clinic were noted.

We identified two distinct groups of patients with Type 2 diabetes on dialysis as defined by the serum creatinine being elevated within 5 years of the diagnosis of diabetes (Table 1). Group 1 (elevated creatinine within 5 years of diagnosis) required earlier dialysis support (5.8 vs. 13.9 years $P = 0.005$). Consistent with group 1 having pre-existent renal disease at the time of diagnosis of diabetes, four out of the five patients in this group had longstanding hypertension of at least 10 years duration prior to the onset of diabetes and they all had proteinuria and a raised serum creatinine at the time, or within 5 years, of diagnosis of diabetes. In two patients, urinalysis was not recorded at diagnosis.

Group 2 (elevated creatinine greater than 5 years after diagnosis) consisted of young-onset Type 2 diabetic patients, mean age 47.6 years, with a longer time course to the commencement of dialysis (mean 13.9 years). One of these patients was hypertensive prior to the diagnosis of diabetes, two were diagnosed at the same time as being diagnosed diabetic, three after the diagnosis of diabetes

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Follow-up Survey of People in China With Type 2 Diabetes Mellitus Consuming Supplemental Chromium

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In a recent double-blind, placebo-controlled study involving 180 people with Type 2 diabetes mellitus, supplemental chromium (Cr) was shown to improve fasting glucose, postprandial glucose, insulin, hemoglobin A1c, and cholesterol. In a follow-up survey, the fasting glucose, postprandial glucose, and diabetic symptoms of 833 people with Type 2 diabetes mellitus were monitored for up to 10 months following Cr supplementation (500 μg/d Cr as chromium picolinate). All subjects were on hypoglycemic medication and/or insulin. Fasting and postprandial glucose improved in >90% of the subjects, and similar improvements occurred after 1–10 months. Mean fasting glucose values before consuming additional Cr were 10.0 ± 0.14 mmol/L (mean ± SEM); they decreased to 8.0 ± 0.15 after 1 month and remained significantly lower during the ensuing 9 months. Values for postprandial glucose decreased from 12.0 ± 0.21 to 9.9 ± 0.40 mmol/L in 1 month and also remained significantly lower in the following 9 months. Symptoms of diabetes including fatigue decreased from 443 people who reported feeling fatigued before Cr to 52 people after supplementation. Subjects reporting symptoms of thirst decreased from 334 to 47; frequency of urination episodes dropped from 322 to 40 people after Cr supplementation for 1 month or longer. Similar effects were observed in women and men. There were no confirmed negative side effects of supplemental Cr. These data confirm the safety and beneficial effects of supplemental Cr and demonstrate that beneficial effects of supplemental Cr observed in a few months are also present after 10 months. J. Trace Elem. Exp. Med. 12:55–60, 1999. Published 1999 Wiley-Liss, Inc.†

Key words: chromium; glucose tolerance; diabetes; insulin

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This work was presented at the International Symposium on the Health Effects of Dietary Chromium, Tufts University School of Medicine, U.S. Department of Agriculture, and Chromium Information Bureau, Dedham, MA, 1 May 1998.

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INTRODUCTION

The incidence of noninsulin-dependent, or Type 2, diabetes mellitus (Type 2 DM) is widespread, affecting ~3% of the population, or roughly 100 million people [1]. It is predicted that the number of people with Type 2 DM will double in the next 10 years [2]. In addition to enormous financial burdens (more than $45 billion annually alone in the U.S. Medicare system), there are also immeasurable effects on the quality of life. Among the Pima Indians and Nauruans, the prevalence of Type 2 DM approaches 40% [2]. In the Chinese population, the incidence of diabetes ranges from <1% in some rural areas of mainland China to 6–12% among Chinese living in Hong Kong, Singapore, and Taiwan, and to 16% in a small group of Chinese living in Mauritius [3]. It has been predicted that by the year 2010, the number of people with diabetes will exceed 200 million, the majority of whom will live in Asia [3,4].

In Beijing, China, we completed a double-blind, placebo-controlled study involving 180 people with Type 2 DM to determine if supplemental Cr was effective in reversing the signs and symptoms of Type 2 DM [5]. Chromium (Cr) as Cr picolinate (CrPic) (200 or 1,000 μg/Cr/d) was given for 4 months while subjects remained on their normal medications. Cr was shown to improve glucose, insulin, cholesterol, and hemoglobin A1c (HbA_{1c}) in these patients in a dose-dependent manner. Cr effects were greater after 4 months than after 2 months and greater in the group receiving 1,000 μg/Cr/d than in the 200 μg/Cr/d group [5].

As a follow-up to this study, we monitored the fasting and postprandial blood glucose and select symptoms of diabetes including fatigue, thirst, and frequency of urination in a cohort of 833 people with Type 2 DM. Subjects were selected at random from a pool of people with Type 2 DM known to be consuming supplemental Cr.

MATERIALS AND METHODS

Subjects

Subjects were recruited at random from those who were known to be consuming supplemental Cr based upon those who purchased supplemental Cr. Subjects being treated for Type 2 DM were invited to an informational seminar and given information regarding the possible potential benefits of Cr. After this initial seminar, many of the participants purchased Cr supplements, 500 μg/Cr/d as CrPic. Glucose measurements were made at individual hospitals or clinics, and glucose values before and during supplementation were made at the same facilities. Compliance was monitored by personal communication with the participants.

Statistical Analysis

Fasting and postprandial glucose were each analyzed as a one-factor general linear repeated measures model using PROC MIXED (SAS Institute, Cary, NC) with time as the fixed effect. Correlation in the measurement of glucose across time within a subject was taken into account in the model. Means were completed using pairwise contrasts. Symptoms of diabetes before and during Cr supplementation were analyzed using the student's t-test.

RESULTS

There was a significant reduction in the fasting blood glucose of patients consuming Cr in the first month, and values remained lower in the ensuing 9 months (Fig. 1). We are continuing to monitor many of these subjects. Values have remained reduced for 1 year or more. Similar results were observed in the postprandial blood glucose values (Fig. 2).

The reduction in the number of patients reporting excessive thirst, urination, and fatigue is shown in Table I. Subjects completed a questionnaire before beginning consumption of supplemental Cr and monthly after Cr supplementation (500 μg/d as CrPic). There was more than an 85% reduction in the number of people who experienced excessive thirst, urination, or fatigue during the Cr supplementation period. There were no confirmed negative side effects from consuming Cr for up to 1 year.

DISCUSSION

This study is a follow-up survey of people before and after self-initiation of supplemental Cr. It should not be confused with our previous study, which was double-blind and placebo-controlled [5]. The effects of supplemental Cr on people

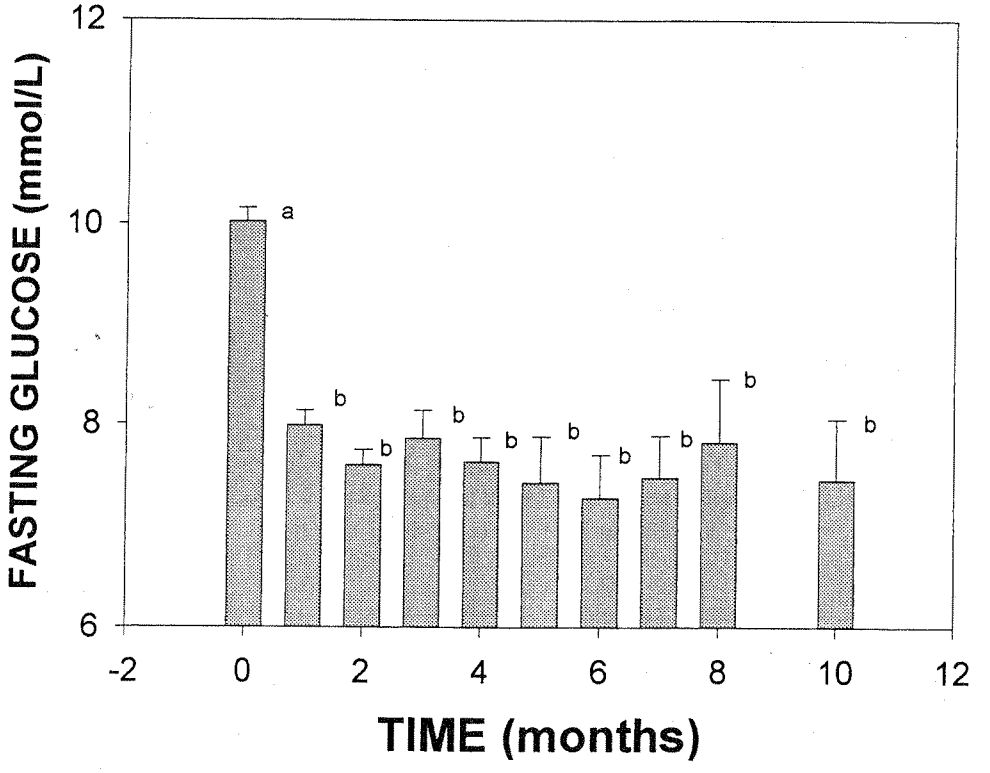

Fig. 1. Supplemental Cr (500 μg/d of Cr as Cr picolinate) on fasting blood glucose. There were 833 people with type 2 DM participating in the study. Bars with different superscripts are significantly different at $P < .05$.

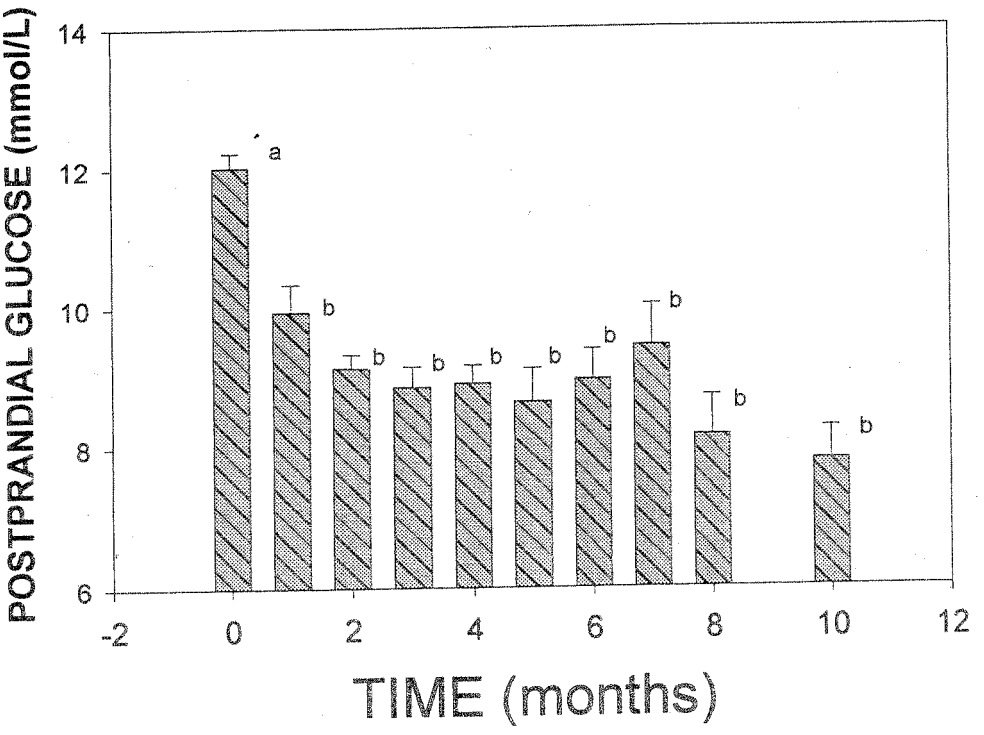

Fig. 2. Supplemental chromium effects on postprandial blood glucose. Conditions as described in Figure 1.

with varying degrees of glucose intolerance ranging from hypoglycemic to Type 2 DM have been reviewed recently [6]. Like this follow-up survey, the controlled studies generally support beneficial effects of supplemental Cr. There have been no confirmed negative side effects. Similarly, in this study there were no confirmed negative side effects of supplemental Cr at 500 μg/d for up to 1 year. More than 80% of the subjects reported improved fasting and postprandial glucose as well as improvements in symptoms of excessive thirst, urination, and fatigue.

It should be noted that not all of the controlled studies have reported beneficial effects of supplemental Cr. This finding is likely due to a number of factors including type and amount of supplemental Cr as well as the Cr intake and status of the patients. There is presently no measure to determine Cr status other than to monitor glucose,

TABLE I. Chromium Effects on Symptoms of Excessive Thirst, Urination, and Fatigue[1]

Symptom	Before	After 1 month	Percent with improvement
Excessive thirst	334	47	86*
Excessive urination	322	40	88*
Excessive fatigue	443	52	88*

[1]Numbers in the before and after 1 month columns denote number of people with specific complaint of the total of 833 patients.
*Significant at $P < 0.001$.

insulin, blood lipids, and related variables before and after Cr supplementation. The amount of Cr is critical. Whereas 200 μg/Cr/d or less appears to be sufficient for subjects with varying degrees of glucose intolerance, subjects with Type 2 DM require more than 200 μg/Cr/d [6]. In the study of Anderson et al. [5], 200 μg/Cr/d was less effective than 1,000 μg/Cr/d. Based on previous studies, it was not anticipated that the 200 μg Cr group would show any effects of supplemental Cr, but Cr was given as 100 μg twice a day rather than 200 μg once a day. Also, Chinese patients with Type 2 DM are likely to weigh less than Type 2 DM patients in the United States.

Mossop [7] reported a reduction in fasting glucose in patients with diabetes from 14.4–6.6 mmol/L following 16–32 weeks of supplementation with 600 μg Cr as Cr chloride. Similar results were reported by Nath et al. [8] following 500 μg/d of supplemental Cr. Positive effects in people with diabetes also have been observed with up to 1,000 μg/Cr/d as chromium chloride [9] and with 250 μg/Cr/d for blood lipids of patients with diabetes and atherosclerotic disease [10]. Studies reporting no effects of supplemental Cr on people with diabetes usually employed 200 μg of supplemental Cr or less and did not use Cr as CrPic [11–13]. The effects of 8 μg of Cr as CrPic per kg/body weight were also more effective than 4 μg/kg body weight in patients with gestational diabetes [14]. It is also obvious that Type 2 DM is due to a number of causes, only one of which is Cr. Patients whose diabetes is due to causes other than Cr deficiency would not be anticipated to respond to supplemental Cr.

CONCLUSIONS

This follow-up survey documented beneficial effects of supplemental Cr in people with Type 2 DM without any negative side effects. Chromium is considered one of the least toxic nutrients and has one of the largest safety factors of all nutrients when the nutrient levels associated with toxicity are compared with nutritional levels [15].

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Elevated Intakes of Supplemental Chromium Improve Glucose and Insulin Variables in Individuals With Type 2 Diabetes

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Chromium is an essential nutrient involved in normal carbohydrate and lipid metabolism. The chromium requirement is postulated to increase with increased glucose intolerance and diabetes. The objective of this study was to test the hypothesis that the elevated intake of supplemental chromium is involved in the control of type 2 diabetes. Individuals being treated for type 2 diabetes (180 men and women) were divided randomly into three groups and supplemented with: *1*) placebo, *2*) 1.92 μmol (100 μg) Cr as chromium picolinate two times per day, or *3*) 9.6 μmol (500 μg) Cr two times per day. Subjects continued to take their normal medications and were instructed not to change their normal eating and living habits. HbA_{1c} values improved significantly after 2 months in the group receiving 19.2 μmol (1,000 μg) Cr per day and was lower in both chromium groups after 4 months (placebo, 8.5 ± 0.2%; 3.85 μmol Cr, 7.5 ± 0.2%; 19.2 μmol Cr, 6.6 ± 0.1%). Fasting glucose was lower in the 19.2-μmol group after 2 and 4 months (4-month values: placebo, 8.8 ± 0.3 mmol/l; 19.2 μmol Cr, 7.1 ± 0.2 mmol/l). Two-hour glucose values were also significantly lower for the subjects consuming 19.2 μmol supplemental Cr after both 2 and 4 months (4-month values: placebo, 12.3 ± 0.4 mmol/l; 19.2 μmol Cr, 10.5 ± 0.2 mmol/l). Fasting and 2-h insulin values decreased significantly in both groups receiving supplemental chromium after 2 and 4 months. Plasma total cholesterol also decreased after 4 months in the subjects receiving 19.2 μmol/day Cr. These data demonstrate that supplemental chromium had significant beneficial effects on HbA_{1c}, glucose, insulin, and cholesterol variables in subjects with type 2 diabetes. The beneficial effects of chromium in individuals with diabetes were observed at levels higher than the upper limit of the Estimated Safe and Adequate Daily Dietary Intake. *Diabetes* 46:1786–1791, 1997

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Conclusive evidence of the role of trivalent chromium in human nutrition was reported in 1977 (1) when the severe diabetic symptoms of a female patient on total parenteral nutrition were alleviated by supplemental chromium. Diabetic symptoms, in addition to elevated blood glucose, included unexpected weight loss, impaired nerve conduction, and abnormal respiratory quotient that were refractory to exogenous insulin. Upon the daily addition of 4.81 μmol supplemental Cr to her total parenteral nutrition solution for 2 weeks, the diabetic symptoms were alleviated and the exogenous insulin requirement dropped from 45 U/day to zero. This work has been verified on many occasions and documented in the scientific literature on three occasions (2–4). Chromium is now routinely added to total parenteral nutrition solutions (5). However, the chromium concentrations in total parenteral nutrition solutions may not be adequate, since the normalization of nerve conduction occurred in a patient on home parenteral nutrition after the administration of supplemental chromium (6).

Signs of chromium deficiency in humans are not limited to subjects on total parenteral nutrition. Improvements in glucose and/or lipid concentrations have been reported in children with protein calorie malnutrition (7,8); the elderly (9); and individuals with type 1 and type 2 diabetes (10–13), hypoglycemia (14,15), and marginally impaired glucose tolerance (16,17).

Individuals with diabetes have altered chromium metabolism, compared with nondiabetic control subjects, with higher chromium absorption but also greater chromium excretion (18). Hair and tissue chromium levels of individuals with diabetes are lower than those of nondiabetic control subjects. Depending on the stage of diabetes, individuals with diabetes tend to lose the ability to convert chromium to a useable form (18). Diabetic mice also lose the ability to convert inorganic chromium to a useable form that potentiates insulin (19).

We conducted a double-blind placebo-controlled study involving 180 people with type 2 diabetes to determine the role of supplemental chromium in the control of diabetes. Our hypothesis was that the elevated intake of supplemental chromium is involved in the control of type 2 diabetes. The study was conducted in China to obtain a relatively homogeneous study group free of nutrient supplementation.

RESEARCH DESIGN AND METHODS

Subjects. A total of 303 individuals being treated for diabetes at two hospitals in Beijing, China, were screened to obtain 180 subjects meeting the selection criteria. To be eligible for the study, subjects had to be free of disease other than type

2 diabetes and 35–65 years of age and have a fasting blood glucose concentration of 7.2–15.5 mmol/l, a 2-h blood glucose concentration of 9.4–16.7 mmol/l, and an HbA_{1c} level of 8.0–12.0%. Subjects were informed of the purpose of the study, that there were no known risks associated with the study other than the minimal risks associated with blood drawing, and that they were free to drop out of the study with no effect on their present health care. Subjects were not reimbursed for their participation. Subjects were motivated to participate because of the possible benefits of the study. Compliance appeared to be very good and was assessed by personal communication and pill count. The study was approved by the Beijing Medical Review Committee with concurrence from the U.S. Department of Agriculture Human Studies Review Board.

A total of 180 individuals with diabetes were randomly divided into three groups. Sixty subjects received placebo, 60 received 1.92 µmol Cr as chromium picolinate (furnished by Nutrition 21, San Diego, CA) 2 times per day, and the remainder received 9.6 µmol Cr as chromium picolinate twice per day. Subjects were instructed to take one tablet in the morning and one in the evening between meals. Subjects were also urged to maintain their normal eating and exercise habits. Subjects continued their normal visits to monitor their diabetes. A fasting blood sample and a blood sample after a 2-h glucose challenge (75 g glucose) were obtained at the beginning of the study and after 2 and 4 months. Subjects were middle-aged healthy subjects of normal height, weight, and BMI with diabetes for <10 years (Table 1). Nineteen subjects did not complete all three testing dates, and six subjects had missing values for at least one variable; their results were not included in the final analyses. Data from these subjects were omitted to maintain a complete homogeneous data set with all subjects represented during each study period. Data for all subjects who completed all phases of the study were included in all of the respective analyses, and there were no samples omitted. Of the 155 subjects who were included in the final analyses, most of the subjects (92) were taking sulfonylurea drugs (i.e., glibenclamide, glinclazid, glipizide). Sixty-nine were on phenformin, 38 were on traditional Chinese medicines, 22 were on no medication, and nine were on insulin. Several subjects were taking more than one medication. Medications were constant during the study.

Study design was double blind and placebo controlled. Placebo tablets were indistinguishable from those containing either level of chromium. Measured chromium content of the placebo capsules was 0.01 ± 0.001 µmol and was 2.04 ± 0.16 and 11.0 ± 1.2 µmol for the 1.92- and 9.6-µmol capsules, respectively. Data are means ± SD for six capsules from each batch. A crossover study design was discarded because of the possible carryover effects of 1,000 µg Cr/day.

Glucose was analyzed by glucose oxidase method (20), and insulin was analyzed by radioimmunoassay (21). HbA_{1c} values were measured using BioRad HbA_{1c} columns (BioRad, Richmond, CA). Total cholesterol was determined by chemical hydrolysis (22), HDL cholesterol by phosphotungstate-Mg precipitation (23), and triglycerides by direct enzymic measurement (24). Blood urea nitrogen was determined by a direct method (25). Analyses presented were completed in China. Several dozen samples were exchanged between the U.S. and China laboratories to ensure accuracy and reproducibility of the data.

The variables HbA_{1c}, total cholesterol, blood urea nitrogen, HDL cholesterol, triglycerides, fasting and 2-h glucose, and insulin were analyzed as three-factor repeated-measures mixed linear models, using PROC MIXED (SAS Institute, Cary, NC). Since the variables were measured at 0, 2, and 4 months for each subject, repeated measures analyses were used. Several covariance structures were modeled, and the unstructured model was found to fit best, except for triglycerides and total cholesterol, where the compound symmetry model was best. For HbA_{1c}, cholesterol, and triglycerides, the $\log_{10}$ transformed values fit the model better and were used in the analyses. Data in the table and figures are means ± SE for the nontransformed data.

RESULTS

Fasting blood glucose concentrations were significantly lower in the group receiving 19.2 µmol Cr daily after both 2 and 4 months (Fig. 1). Similar results were observed for blood glucose concentrations 2 h after the ingestion of 75 g glucose (Fig. 2). Fasting and 2-h glucose concentrations of the subjects in the placebo group also decreased, but the decreases in the subjects receiving 19.2 µmol supplemental Cr were much larger. The chromium × time interaction was significant at $P < 0.0001$.

Fasting insulin concentrations were significantly lower in the group receiving 3.85 µmol Cr daily with a mean fasting insulin concentration of 95 ± 2 pmol/l after 4 months, which was identical to that of the group receiving the higher level of chromium, compared with 118 ± 3 pmol/l in the placebo group (Fig. 3). Fasting insulin concentrations were also significantly lower after 2 months in both of the groups receiving supplemental chromium. Similar results were observed for the insulin 2 h after a glucose challenge (Fig. 4). The fasting

TABLE 1
Characteristics of control and chromium-supplemented subjects at the beginning of the study

	Supplemental chromium (µmol/day)		
	0	3.85	19.2
Height (meters)			
All	1.67 ± 0.01 (50)	1.67 ± 0.01 (53)	1.65 ± 0.01 (52)
Women	1.61 ± 0.01 (17)	1.60 ± 0.01 (20)	1.59 ± 0.01 (26)
Men	1.70 ± 0.01 (33)	1.71 ± 0.01 (33)	1.70 ± 0.01 (26)
Weight (kg)			
All	69.1 ± 1.3	69.0 ± 1.5	67.8 ± 1.4
Women	66.4 ± 2.5	63.4 ± 2.5	63.4 ± 1.6
Men	70.5 ± 1.4	72.6 ± 1.5	72.0 ± 1.8
BMI (kg/m^2)			
All	24.8 ± 0.5	25.0 ± 0.5	24.8 ± 0.4
Women	25.8 ± 1.1	25.0 ± 0.9	25.0 ± 0.6
Men	24.3 ± 0.5	25.0 ± 0.5	24.6 ± 0.6
Duration of diabetes (years)			
All	5.4 ± 0.7†	8.0 ± 1.0*	5.3 ± 0.7†
Women	5.6 ± 1.0*	8.4 ± 1.6*	6.8 ± 1.1*
Men	5.2 ± 0.9*†	7.8 ± 1.2*	3.7 ± 0.7†
Age (years)			
All	55.5 ± 1.2	55.7 ± 1.2	54.6 ± 1.4
Women	56.4 ± 1.8	53.8 ± 1.8	54.1 ± 2.3
Men	55.1 ± 1.5	56.8 ± 1.7	55.2 ± 1.8

Number in parentheses denotes number of subjects who completed all phases of the study and had no missing experimental analyses. *†Values in the same row with different superscripts are significantly different at $P < 0.05$.

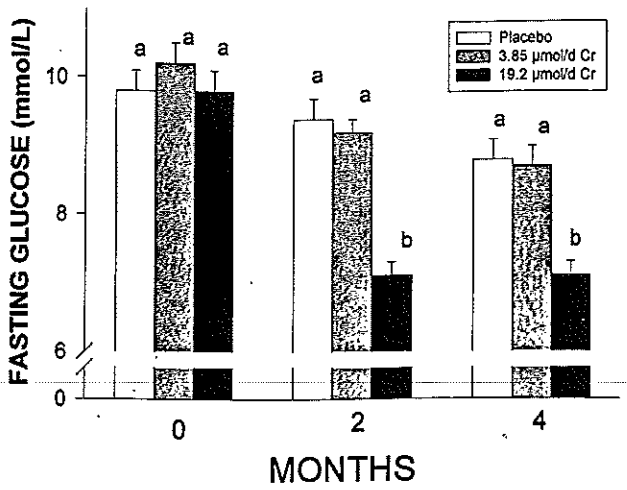

FIG. 1. Supplemental chromium effects on fasting serum glucose. Chromium was taken in two doses between meals. There were 50 subjects in placebo group, 53 in the 3.85-µmol group, and 52 in the 19.6-µmol group. Bars with different letters are significantly different from other groups for the same time period at $P < 0.05$.

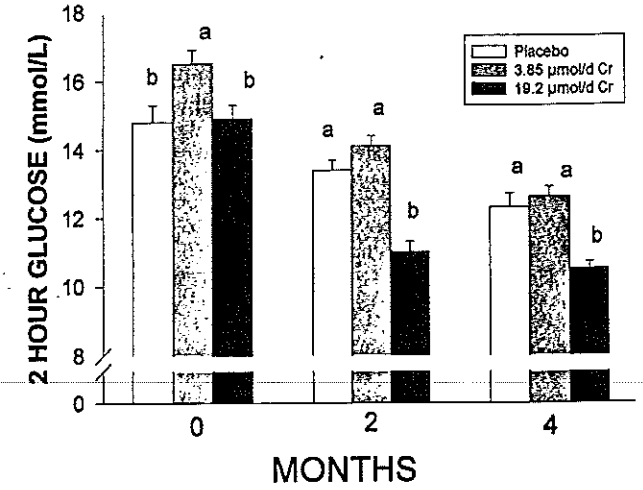

FIG. 2. Supplemental chromium effects on 2-h glucose concentrations. Subjects were given a 75-g glucose challenge at time 0, and blood was drawn 2 h later. Conditions are described in Fig. 1.

and 2-h insulin values of the placebo subjects also decreased over the duration of the study, but the decreases in the chromium groups were much larger. The chromium × time interaction was also significant at $P < 0.0001$.

Decreases in blood glucose and insulin concentrations due to supplemental chromium (Figs. 1–4) were reflected by decreases in HbA_{1c} values, with significant effects of chromium in both chromium groups after 4 months and in the 19.2-µmol group after 2 months (Fig. 5).

Supplemental chromium at 19.2 µmol/day also led to decreased total cholesterol (Fig. 6). The total cholesterol of the men was higher than that of the women, and both sexes responded to supplemental chromium similarly. There were no chromium × sex or time × sex interactions. The chromium × time interaction was significant at $P < 0.02$. There were no significant effects of supplemental chromium on HDL cholesterol, triglycerides, blood urea nitrogen, weight, or BMI (data not shown).

DISCUSSION

These data demonstrate significant effects both statistically and clinically of supplemental chromium at 3.85 and 19.2 µmol/day on glucose and insulin variables in individuals with type 2 diabetes. Improvements in fasting glucose and insulin concentrations as well as those after a glucose challenge document the role of elevated intakes of supplemental chromium in the control of type 2 diabetes. The improvements due to chromium are not due to changes in body weight, since weight did not change significantly over the duration of the study.

The chromium intake of these subjects is not known, but total dietary chromium intake does not accurately reflect chromium status since other factors affect chromium requirements. For example, different forms of stress including diet, exercise, and diabetes all increase chromium requirements (26). Increased intake of simple sugars also increases chromium losses (27). Urinary chromium losses are correlated with the stress hormone cortisol (28), and chromium's effects on morbidity and immune function are only observed in stressed animals (29).

There are no methods to predict chromium status. The only method is to measure glucose, insulin, and lipid variables before and after chromium supplementation. Chromium concentrations in blood, hair, urine, and other tissues or body fluids have not been shown to reflect chromium status.

There have been several studies involving chromium supplementation of people with diabetes. The results of these studies are varied, but in retrospect may be consistent (10–13,30–35). The majority of the studies involving daily chromium supplementation with 4.81 µmol Cr as chromium chloride or less to individuals with diabetes reported no significant consistent improvements (31–33). Improved glucose tolerance and blood cholesterol were reported in roughly half the subjects supplemented daily with 2.89–4.81 µmol Cr as chromium chloride (9–10). Mossop (12) reported significant improvements in fasting blood glucose in 13 people being treated for diabetes. Fasting blood glucose concentrations decreased from 14.4 to 6.6 mmol/l after 2 to 4 months of 11.5 µmol supplemental Cr as chromium chloride daily. Fasting blood glucose, glycosylated hemoglobin, total cholesterol, and LDL cholesterol all improved significantly in 11 individuals with type 2 diabetes who consumed 3.85 µmol/day Cr as chromium picolinate for 6 weeks (35). Ravina et al. (13) also reported improved glucose control in 162 individuals with diabetes after daily chromium supplementation with 200 µg Cr as chromium picolinate.

The reasons for the discrepancy in the response to supplemental chromium appear to be due to the amount and form of chromium consumed. In this study, we used chromium as chromium picolinate, which is utilized more efficiently than chromium chloride (36), used chromium twice per day, and used higher levels than most previous studies. The beneficial effects of 19.2 µmol/day Cr, compared with 3.85 µmol, demonstrate that 3.85 µmol Cr is not sufficient to elicit maximal significant improvements in diabetic subjects.

Chromium picolinate is a convenient form of chromium that is used more efficiently than some other forms of chromium. The active compound is chromium, not picolinate,

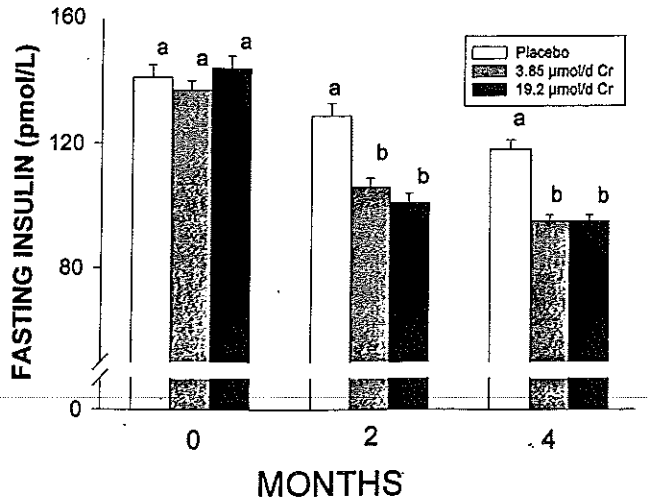

FIG. 3. Supplemental chromium effects on fasting insulin concentrations. Conditions are described in Fig. 1.

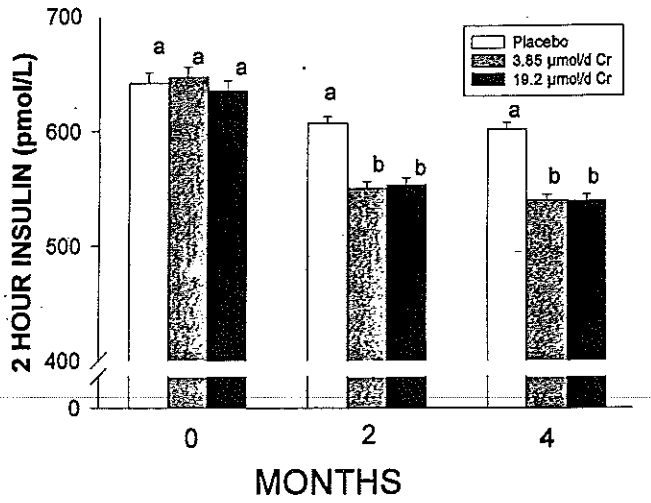

FIG. 4. Supplemental chromium effects on 2-h insulin concentrations. Conditions are described in Fig. 2.

since other studies have shown beneficial effects of chromium as chromium chloride. Chromium chloride is usually the least available of the chromium compounds tested (36). Patients on total parenteral nutrition and individuals with glucose intolerance, hypoglycemia, and diabetes have all been shown to respond to chromium as $CrCl_3$. Several different forms of chromium are likely to elicit similar effects but at different intakes due to the varying absorption, transport, and utilization of the different chromium compounds.

The measurement of glycated proteins, such as HbA_{1c}, is the most reliable method of assessing long-term glycemic control in individuals with diabetes (37–42). HbA_{1c} values were originally postulated to reflect the simple mean plasma glucose level over a certain period, and considering the erythrocyte life span, glycated hemoglobin was thought to be uniformly accumulated in erythrocytes over 120 days. However, theoretical and experimental evidence demonstrates that following a consistent drop in blood glucose, HbA_{1c} values change rapidly in the first 1 to 2 months, followed by a steady-state level after 4 months (41,42). Half of the HbA_{1c} level is determined by the plasma glucose values during the preceding 1-month period and an additional 25% of the HbA_{1c} level in the preceding month (42). Therefore, 75% of the HbA_{1c} level is proportional to the changes in blood glucose over the preceding 2 months. In our study, we saw a rapid drop in HbA_{1c} values in the first 2 months with HbA_{1c} values of 7.4 ± 0.2% for individuals receiving 19.2 µmol Cr daily, compared with 8.6 ± 0.2% for those receiving placebo. The drop in HbA_{1c} value in the group receiving 3.85 µmol Cr daily after 4 months was accompanied by a decrease in both fasting and postprandial insulin, but differences in blood glucose for the corresponding subjects were not significant. However, there were significant drops from the glucose concentrations determined at the onset of the study. Similar results were observed in both male and female subjects.

Changes in serum lipids in this study are consistent with those observed in our previous studies (14,16,17), namely that the effects of supplemental chromium are greater for glucose and insulin than for lipid concentrations. The delayed response of supplemental chromium on blood lipids is con-

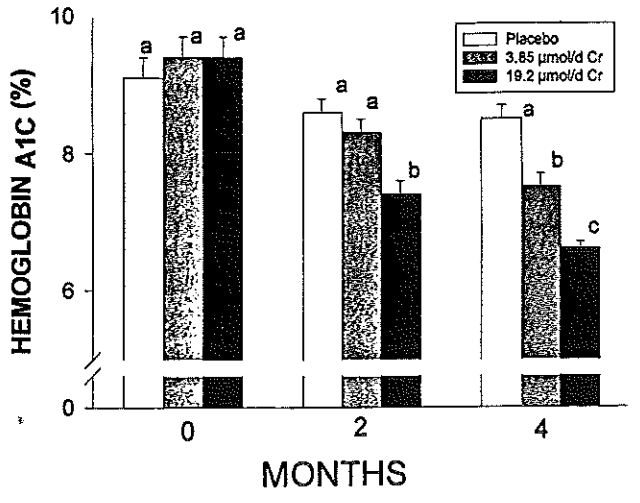

FIG. 5. Supplemental chromium effects on HbA_{1c} values. Conditions are described in Fig. 1.

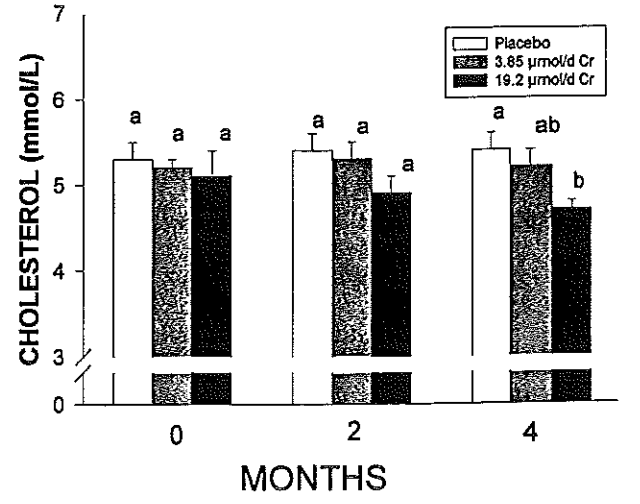

FIG. 6. Supplemental chromium effects on fasting serum cholesterol concentrations. Conditions are described in Fig. 1.

sistent with the study of Abraham et al. (30) that reported no significant effects of supplemental chromium on blood lipids after 3 months but significant decreases in triglycerides and increases in HDL cholesterol after 7–16 months. Similar chromium effects were observed in nondiabetic control subjects and individuals with diabetes.

We did not detect an effect of drug therapy for the control of diabetes and response to supplemental chromium. Diabetic therapy included, in addition to hypoglycemic drugs, traditional Chinese medicines, insulin, and diet alone. Ravina et al. (13) also did not observe an effect of insulin, sulfonylurea, or metformin on improvements in glucose control in diabetic patients receiving 3.85 μmol Cr as chromium picolinate. Supplemental chromium (600 μg/day) was also shown to increase the HDL cholesterol of men taking β-blockers (43). In a separate study, there was a larger effect of chromium on blood lipids of subjects not taking thiazides (44). Martinez et al. (45) also reported no clear effects of 200 μg Cr as chromium chloride daily in women taking medications that affect glucose tolerance but significant effects in 2-h blood glucose concentrations in nonmedicated subjects.

The mechanism of action of chromium on the control of blood glucose concentrations is the potentiation of insulin action. In the presence of chromium in a useable form, much lower levels of insulin are required. In the epididymal fat cell assay, near maximal insulin response can be achieved by adding chromium in a form that potentiates insulin (46). Inorganic chromium is without effect in the epididymal fat cell assay. Supplemental chromium leads to increased insulin binding to cells due to increased insulin receptor number (14). A direct binding of chromium to insulin is postulated (47), and a direct binding of an insulin potentiating form of chromium to insulin has been observed (48). Chromium was also shown to affect β-cell sensitivity measured in euglycemic clamp studies (49). The overall effect of chromium is to increase insulin sensitivity, which is associated with decreased glucose intolerance, decreased risk factors associated with cardiovascular diseases, improved immunity, and increased life span (50).

Trivalent chromium, the form of chromium found in foods and nutrient supplements, is considered one of the least toxic nutrients. The reference dose established by the U.S. Environmental Protection Agency for chromium is 350 times the upper limit of the Estimated Safe and Adequate Daily Dietary Intake of 3.85 μmol (200 μg/day). The reference dose is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects over a lifetime" (51). This conservative estimate of safe intake has a much larger safety factor for trivalent chromium than almost any other nutrient. The ratio of the reference dose to the Estimated Safe and Adequate Daily Dietary Intake or the Recommended Daily Allowance is 350 for chromium, compared to <2 for zinc, roughly 2 for manganese, and 5–7 for selenium (51). Anderson et al. (52) demonstrated a lack of toxicity of chromium chloride and chromium picolinate in rats at levels several thousand times the upper limit of the estimated safe and adequate daily dietary intake for humans (based on body weight). There was no evidence of toxicity in this study, and there have not been any reported toxic effects in any of the human studies involving supplemental chromium.

In summary, supplemental chromium was shown to have pronounced effects on glucose and insulin variables in individuals with type 2 diabetes. A total of 200 μg Cr daily (3.85 μmol) did not appear to be sufficient for the reversal of diabetic symptoms over the 4-month duration of the study, since larger consistent effects were observed in subjects receiving 1,000 μg (19.2 μmol) supplemental Cr daily. Additional studies are needed to establish the form and amount of supplemental chromium required to elicit maximal responses in individuals with diabetes and in the prevention of diabetes.

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