Diss. ETH No. 14970

EFFECTS OF ACUTE CHROMIUM SUPPLEMENTATION ON POSTPRANDIAL METABOLISM IN YOUNG, HEALTHY MEN

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of

Doctor of Natural Sciences

presented by

Marc Frauchiger

Dipl. Lm.-Ing. ETH Zürich

MSc in Nutrition, University of London

born March 27, 1969

citizen of Wyssachen (BE)

accepted on the recommendation of

Prof. Dr. C. Wenk, examiner

Dr. U. Moser, co-examiner

Dr. P. Colombani, co-examiner

2003

Danksagung

Zuerst möchte ich Prof. Dr. Caspar Wenk herzlich danken für die Möglichkeit diese Doktorarbeit zu machen, den mir gewährten Freiraum drei Jahre lang selbständig zu forschen sowie für seine sympathische und unkomplizierte Arbeitsweise.
Besonderer Dank geht an Dr. Paolo Colombani für die tadellose fachliche Betreuung, die interessanten Diskussionen über Sporternährung und die Vorzugsbehandlung bei Computerproblemen.

Ein grosses Dankeschön gebührt auch folgenden Personen oder Institutionen:

-Dr. Ulrich Moser für das Übernehmen des Korreferats
-den Probanden, ohne die die ganzen Versuche nicht möglich gewesen wären
-den DiplomandInnen Esther Frey, Christine Müller, Andrea Obermeier, Philipp Gross und Mirjam Rutz für die Vorversuche und die Mithilfe bei den Studien
-Christian Wenk, Simon Wrann, Thomas Schwab, Myrtha Arnold und Mirjam Mülli für die Blutentnahmen während der Versuche
-Myrtha Arnold und Tony Moses für die Mithilfe bei den Blutanalysen
-Dr. Karina Fischer für die Unterstützung und die Vorarbeit betreffend Studiendesign und Statistik
-Bruno Jörg und Patrick Flütsch für die genialen Ideen und die tatkräftige Hilfe bei Reparaturen und Basteleien an diversen Sportgeräten
-den MitarbeiterInnen, AssistentInnen und OberassistentInnen des INW für die Mitarbeit als Probanden oder HelferInnen bei den Versuchen und für die angenehme Atmosphäre in der Gruppe
-Dr. Kurt Gautschi für das Chrompicolinat und die HbA1c-Analysen
-den Firmen HUG AG und Biofamilia AG sowie der Stiftung zur Förderung der Ernährungsforschung in der Schweiz und der Hochstrasser-Stiftung für die finanzielle Unterstützung.

Zuletzt möchte ich von ganzem Herzen meiner Familie, meinen Freunden und meiner Partnerin Monica für die Unterstützung während der letzten Jahre danken.

Table of contents

Summary

The essential trace element chromium (Cr) enhances insulin action and hence influences glucose metabolism. Chronic Cr supplementation over weeks or months has been shown to improve glucose metabolism in individuals with hypoglycemia, hyperglycemia, insulin resistance, and type 2 diabetes. In contrast, only little data is available on the effects of acute supplementation. According to its mechanism of action we hypothesized that Cr would display an effect on postprandial metabolism after short-term supplementation. One aim of the present thesis therefore was to investigate the acute effects of a single-dose Cr supplementation on postprandial metabolism. Three randomized studies with identical repeated measures crossover design were carried out. The subjects were young, non-smoking, apparently healthy men of normal body mass. In all studies postprandial metabolism after a high-glycemic meal was examined by measuring blood concentration of various metabolites. After baseline collection, Cr or placebo was given orally 30 min before the test meal.

In the first study a supplementation dose of 200 µg Cr as Cr picolinate was evaluated on fourteen young men. We observed no differences in postprandial glucose concentration between Cr and placebo trial, possibly indicating that 200 µg may be an insufficient amount to elicit a response in healthy people. For that reason, higher doses of Cr were supplemented in the second study using similar subjects (n=13). After addition of 400 and 800 µg Cr, postprandial glycemia for capillary glucose, measured as incremental area under the curve (AUC), was 23 ($p = 0.053$) and 20 % ($p = 0.054$), respectively, lower than after the placebo trial. These differences reached significance if the subjects were divided into those who benefited from Cr (responders (n=10)) and those whose glycemia was unchanged after addition of Cr (non-responders (n=3)). For the responders, AUC after 400 and 800 µg Cr was reduced by 36 and 30 %, respectively. Responders and non-responders differed also significantly in their nutrient intake and eating pattern, and total serum iron concentration tended to be lower in the responder group.

As we wanted to corroborate these findings and further investigate the reasons leading to differing responses to supplemental Cr we carried out a third study with a larger study population (n=26) and using 400 µg Cr as a test dose. Again, we found a significant effect of Cr in some but not all subjects. The capillary glucose AUC was significantly reduced by 20 % after Cr supplementation in those subjects with a glucose AUC above the median during the placebo trial, while the AUC

did not differ between trials in the other half of the subjects. We could only partially corroborate the findings of the second study, as the response to supplemental Cr could not be associated to any factors (e.g. iron, transferrin, ferritin, nutrient intake) other than glucose tolerance.

In conclusion, larger doses of Cr given shortly before a meal can positively influence postprandial glucose metabolism in young, apparently healthy men. However, an effect of Cr could not be detected in all subjects. The reasons for the differing response are still not clear. However, we observed that individuals with slightly poorer glucose control were more likely to benefit from supplemental Cr than those with better control. This would suggest that supplemental Cr may be most effective in individuals with poorer glucose tolerance like for example overweight or elderly people. That could be of importance as recent epidemiological studies indicate that postprandial blood glucose may be an independent risk factor for cardiovascular disease and diabetes. Furthermore, as recent evidence suggests that a high dietary glycemic load may lead to impaired glucose tolerance and insulin resistance, lowering the glycemic load in the population may carry an important preventive potential to decrease the incidence of these disorders. So, thanks to its glycemia lowering effect, acute and chronic Cr supplementation could be a promising approach in helping to reduce the prevalence of some of the most common diseases. However, more research has to be done as many questions about Cr remain unanswered.

Zusammenfassung

Chrom ist ein essentielles Spurenelement, welches die Insulinwirkung verstärkt und damit den Kohlenhydratstoffwechsel beeinflusst. Es wurde gezeigt, dass chronische Chromsupplementation über Wochen oder Monate den Glucosestoffwechsel bei Individuen mit Hypo- oder Hyperglycämie, Insulinresistenz oder Typ 2 Diabetes zu verbessern vermag. Über den Effekt akuter Chromsupplementation ist hingegen erst wenig bekannt. In Anbetracht des Wirkungsmechanismus von Chrom haben wir die Hypothese aufgestellt, dass Chrom schon nach kurzfristiger Supplementation einen Einfluss auf den postprandialen Stoffwechsel ausüben sollte. Ein Ziel der vorliegenden Dissertation war deshalb, den akuten Einfluss einer einzelnen Chromdosis auf den postprandialen Metabolismus zu untersuchen. Dazu wurden drei Studien mit einer ähnlichen randomisierten Kreuzversuchsanordnung mit wiederholten Messungen durchgeführt. Die Testpersonen waren normalgewichtige, nichtrauchende und scheinbar gesunde junge Männer. In allen Studien wurde der Stoffwechsel nüchtern und nach einer hoch-glycämischen Mahlzeit mittels Erfassen verschiedener Blutparameter untersucht. Chrom oder Placebo wurde unmittelbar nach den Nüchternmessungen beziehungsweise 30 min vor der Testmahlzeit oral verabreicht.

Im ersten Versuch, bei welchem vierzehn junge Männer teilnahmen, wurde die Wirkung einer Dosis von 200 µg Chrom als Chrompicolinat untersucht. Wir fanden keine Unterschiede im postprandialen Blutglucoseverlauf zwischen Chrom- und Placebovariante, was möglicherweise darauf hinweist, dass 200 µg eine zu geringe Dosis ist, um einen Effekt bei gesunden Männern zu bewirken. Deshalb wurden in einer zweiten Studie, bei ähnlicher Probandenzahl, höhere Dosierungen getestet. Nach Zugabe von 400 und 800 µg Chrom war die postprandiale, kapilläre Glycämie, erfasst als Fläche unter der Kurve, 23 respektive 20 % tiefer als nach Zugabe von Placebo. Diese Unterschiede erreichten statistische Signifikanz, wenn die Probanden in positiv auf das Chrom Reagierende (Responder, n=10) und durch Chrom nicht Beeinflussbare (Non-Responder, n=3) unterteilt wurden. Bei den Respondern war die Fläche unter der Kurve nach 400 und 800 µg Chrom um 36 respektive 30 % verkleinert. Zwischen Respondern und Non-Respondern wurden auch Unterschiede in der Nährstoffaufnahme, im Ernährungsverhalten und bei der Serumeisen-Konzentration beobachtet.

Um diese Resultate zu erhärten und die Gründe für die variierenden Reaktionen auf Chrom weiter zu untersuchen, führten wir eine dritte Studie mit grösserer Probandenzahl (n=26) und einer Versuchsdosis von 400 µg Chrom durch. Wiederum beobachteten wir einen Einfluss von Chrom in manchen aber nicht allen Testpersonen. Die kapilläre Fläche unter der Kurve war bei den Probanden mit einem leicht schlechteren Glucosemetabolismus nach der Chromvariante um 20 % verkleinert, während sie bei den Probanden mit besserem Glucosemetabolismus nicht durch Chrom beeinflusst wurde. Wir konnten die Ergebnisse der zweiten Studie nur teilweise bestätigen. Die Antwort auf das Chrom konnte ausser mit der Glucosetoleranz nicht mit anderen Parametern (z.B. Serumeisen, Transferrin, Ferritin, Nährstoffaufnahme) assoziiert werden.

Schlussfolgernd können grössere Chromdosierungen, die kurz vor einer Mahlzeit eingenommen werden, den postprandialen Glucosemetabolismus in jungen, scheinbar gesunden Männern positiv beeinflussen. Der Einfluss von Chrom konnte aber nicht in allen Testpersonen beobachtet werden. Die Gründe für diese unterschiedlichen Reaktionen sind immer noch unklar. Wir beobachteten jedoch, dass Individuen mit leicht verschlechterter Blutglucoseregulierung vermehrt von der Chrombeigabe profitierten als diejenigen mit besserer Regulierung. Dies deutet an, dass Personen mit schlechterer Glucosetoleranz, wie z.B. Übergewichtige oder ältere Menschen den grössten Nutzen von Chromzusätzen haben könnten. Möglicherweise ist dies von Bedeutung, da neuste epidemiologische Studien darauf hinweisen, dass der postprandiale Blutglucoseverlauf ein unabhängiger Risikofaktor für kardiovaskuläre Krankheiten und Typ 2 Diabetes sein könnte. Zudem legen weitere Erkenntnisse nahe, dass eine hohe glycämische Belastung zu verminderter Glucosetoleranz und Insulinresistenz führen kann. Somit könnte das Senken der glycämischen Belastung in der Bevölkerung eine wichtige präventive Massnahme sein, um die Inzidenz dieser Krankheiten zu verringern. Dank ihres positiven Effekts auf den Glucosestoffwechsel, könnten akute oder chronische Chromzusätze ein viel versprechender Ansatz sein, um dies zu erreichen. Da viele Fragen betreffend Chrom noch offen sind, müsste aber die Forschung auf diesem Gebiete intensiviert werden.

Chapter 1

General introduction

Chromium (Cr) is the second largest-selling mineral supplement in the United States. But while there is a major interest of the public and millions of Americans take Cr supplements the scientific community seems to consider Cr research as a trivial topic. So, it is not surprising that scientific data is limited and that there remain many unanswered questions about Cr.

Cr is a transition element that can occur in different oxidation states of which only 3^+ and 6^+ are found in environmental systems (1). The environmental behavior of Cr is mainly a function of its valence state. Hexavalent compounds are strong oxidizers, considered toxic and originate primarily from industrial sources (2). Toxic effects are mostly observed when hexavalent compounds are inhaled: Consumed orally in small amounts hexavalent Cr is rapidly converted into the trivalent form in the acidic environment of the stomach (2). The trivalent form is relatively inert and is considered to be the most stable form in biological systems (2). It has a low order of toxicity and the safety margin between the amounts usually ingested and the toxic dose is large. The oral reference dose (RfD), which represents the highest daily dose that can be taken in for a lifetime without causing deleterious effects, has been set at 1.5 mg per kg body mass and day by the U.S. Environmental Protection Agency (3). So, for a 70 kg man the RfD equals to 105 mg/d while the adequate intake recently has been estimated to be 35 $\mu g \cdot d^{-1}$ (4), i.e. a dose 3000 times lower.

Trivalent Cr is an essential component in animal and human nutrition needed in trace amounts. A biological function for the trace element Cr was first reported in the late 1950's by Schwartz & Mertz (5). They observed that Cr was needed for maintenance of normal glucose tolerance in rats. This role was reported also for humans a few years later and since then many studies have confirmed that Cr enhances insulin action in glucose uptake and protein metabolism (6). The evidence for an effect of Cr is quite strong for its proposed role on glucose metabolism while its action on body composition is uncertain. The exact mechanism of action of Cr on insulin metabolism is still unclear. In early studies, a Cr-containing compound called glucose tolerance factor was described that improved glucose metabolism in rats and humans (7). More recently, it was suggested that a Cr-containing oligopeptide, named low-molecular-weight Cr-binding

substance or chromodulin, stimulates the protein tyrosine kinase activity of the insulin receptor and thereby influences insulin action (8).

Until now only little is known about the way Cr is metabolized. The biological availability appears to be quite low and depends on the form and amount of Cr in the diet. Organic compounds like Cr picolinate are absorbed at a relatively high efficiency rate of 2 to 5 % (9), while only approximately 0.4 to 1 % of inorganic Cr chloride is absorbed (10). Furthermore, at a daily dietary Cr intake of 40 µg the absorption rate drops from 2 % to 0.4 to 0.5 % when intake is only 10 µg (11). Cr absorption is also influenced by other dietary components. Amino acids, oxalate, and ascorbic acid increase Cr uptake (12-14) while phytate and starch reduce Cr absorption (10;13). The mechanisms of Cr absorption and transport are still uncertain. Little is known about the absorption process in the digestive tract. Dowling et al (15) proposed that Cr was absorbed by the process of passive diffusion while Vincent (9) hypothesized that Cr absorption may be more complicated than simple passive absorption. The absorption of Cr seems to be quite rapid as blood Cr concentration peaks within 90 minutes after intake (16). More is known about the transport of Cr in the blood. The serum protein transferrin appears to be of importance in the transport of Cr in the plasma (17;18). In vitro studies have shown that Cr readily binds to transferrin (18;19). As transferrin saturation with iron is only about 30 %, transferrin could potentially carry other metal ions like Cr. Even if the transport with transferrin has not been proven conclusively in vivo some evidence exists that transferrin is involved in Cr transport (9). Recently, Vincent (20) put forward a model of Cr transport. Increases in insulin concentration cause a translocation of transferrin from the blood to insulin-sensitive cells. There, transferrin binds to its receptor and enters the cell, where Cr is released and then bound to apo-chromodulin. Once in the holo-form chromodulin binds to the insulin-activated insulin receptor, enhancing the activity of the insulin receptor tyrosine kinase up to eightfold. This amplification of insulin signaling leads to greater glucose uptake by the cell. Once blood insulin concentration drops chromodulin is excreted into the blood and ultimately in the urine. Cr is primarily excreted through the kidney and only minor amounts are lost in bile, sweat, and hair (21). Urinary Cr losses are enhanced by different stress factors, like high sugar diets, acute exercise, lactation, and physical trauma (10).

The exact Cr requirements are not known and so it is not surprising that recommended dietary intakes vary from country to country. In 1980 the USA set up the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) to 50-200 µg/d, but just recently reset these recommendations. The Institute of Medicine decided that there was not enough scientific evidence to set an Estimated Average Requirement (EAR) for Cr. Therefore, based on estimated mean intakes, an Adequate

Intake (AI) was set at 35 μg/d for men and 25 μg/d for women. On the other hand the German, Austrian, and Swiss nutrition societies recently set the reference intake for adults at 30-100 μg/d.

Several studies have observed effects of Cr on human metabolism after supplementation periods of several weeks or months (for a review see (22)). But, according to the mechanism of action described earlier, Cr should also display effects on insulin action and as a result metabolism after short-term supplementation. Therefore, the aim of this thesis was to investigate if Cr not only shows effects after chronic but also after acute supplementation, and to determine the most effective dose on postprandial metabolism.

In many studies investigating the effects of supplemental Cr on normal individuals, hyper- or hypoglycemics, and patients with type 2 diabetes it was noted that some but not all subjects benefited from the supplementation. The reasons for this varying reaction are not clear. While Ravina (23) found no clinical signs that would predict the response to Cr, Anderson (24) postulated that only Cr-deficient individuals would benefit from Cr. Unfortunately, there are still no specific tests available to measure Cr status before a supplementation trial, and consequently the response to supplemental Cr cannot be predicted. For this reason, another goal of this thesis was to determine the metabolic factors that might influence or predict the reaction to supplemental Cr.

References

1. Vincent JB. Mechanisms of chromium action: Low-molecular-weight chromium-binding substance. *J Am Coll Nutr*;18:6-12, 1999.
2. Mertz W. Chromium occurrence and function in biological systems. *Physiol Rev*;49:163-239, 1969.
3. U.S.Environmental Protection Agency. Reference Dose for Chronic Oral Exposure. EPA Integrated Risk Information System (IRIS). 1998. Ref Type: Electronic Citation
4. Food and Nutrition Board. Chromium. In: Institute of Medicine, ed. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, D.C.: The National Academy Press:155-76, 2001.
5. Schwarz K, Mertz W. Chromium (III) and the glucose tolerance factor. *Arch Biochem Biophys*;85:292-5, 1959.
6. Mertz W. Interaction of chromium with insulin: A progress report. *Nutr Rev*;56:174-7, 1998.
7. Mertz W. Chromium research from a distance: From 1959 to 1980. *J Am Coll Nutr*;17:544-7, 1998.
8. Davis CM, Vincent JB. Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry*;36 (15):4382-5, 1997.
9. Vincent JB. The bioinorganic chemistry of chromium(III). *Polyhedron*;20:1-26, 2001.
10. Anderson RA. Nutritional factors influencing the glucose/insulin system: chromium. *J Am Coll Nutr*;16:404-10, 1997.
11. Anderson RA, Kozlovsky AS. Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am J Clin Nutr*;41:1177-83, 1985.
12. Dowling HJ, Offenbacher EG, Pi-Sunyer FX. Effects of amino acids on the absorption of trivalent chromium and its retention by regions of the small intestine. *Nutr Res*;10:1261-71, 1990.
13. Chen NS, Tsai A, Duer IA. Effect of chelating agents on chromium absorption in rats. *J Nutr*;103:1182-6, 1973.
14. Offenbacher EG. Promotion of chromium absorption by ascorbic acid. *Tr Elem Electrolytes*;11:178-81, 1994.
15. Dowling HJ, Offenbacher EG, Pi SF. Absorption of inorganic, trivalent chromium from the vascularly perfused rat small intestine. *J Nutr*;119:1138-45, 1989.

16. Kerger BD, Paustenbach DJ, Corbett GE, Finley BL. Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water. *Toxicol Appl Pharmacol*;141:145-58, 1996.
17. Dowling HJ, Offenbacher EG, Pi-Sunyer FX. Effects of plasma transferrin and albumin on the absorption of trivalent chromium. *J Nutr*;10:1251-60, 1990.
18. Ani M. The effect of chromium on parameters related to iron metabolism. *Biol Trace Elem Res*;32:57-64, 1992.
19. Mostaghie AA, Ani M, Bazrafshan MR. Comparative binding study of aluminum and chromium to human transferrin. *Biol Trace Elem Res*;32:39-46, 1992.
20. Vincent JB. The biochemistry of chromium. *J Nutr*;130:715-8, 2000.
21. Lukaski H. Chromium as a supplement. *Annu Rev Nutr*;19:279-302, 1999.
22. Anderson RA. Chromium, glucose intolerance and diabetes. *J Am Coll Nutr*;17:548-55, 1998.
23. Ravina A, Slezak L, Rubal A, Mirsky N. Clinical use of the trace element chromium(III) in the treatment of diabetes mellitus. *J Trace Elem Exp Med*;8:183-90, 1995.
24. Anderson RA. Chromium, glucose tolerance, and diabetes. *Biol Trace Elem Res*;32:19-24, 1992.

Seite Leer /
Blank leaf

Chapter 2

Single-dose chromium supplementation and postprandial metabolism after a high-glycemic meal in healthy men

Based on

Marc T. Frauchiger, Paolo C. Colombani, Caspar Wenk

Submitted to Biological Trace Element Research, September 2002

Abstract

Chromium (Cr) potentiates insulin action. Chronic Cr supplementation improves glucose tolerance in people with impaired insulin sensitivity but it is not known if Cr also acutely influences postprandial glycemia. Therefore, our aim was to evaluate the effects of an acute supplementation on metabolism after food intake. Fourteen young non-diabetic men were tested in a single-blind, randomised crossover study. The subjects were given a high-glycemic meal consisting of white bread with Cr (200 μg as Cr picolinate) or placebo, or a low-glycemic cracker meal, each providing 50 g available carbohydrates. No effects of Cr supplementation on postprandial glucose concentration were apparent for the whole study group but individual responses differed. In all subjects, insulin concentration increased about 70 % less ($P < 0.05$) between 20 and 40 min after meal intake during the Cr supplementation compared with the placebo trial, possibly indicating an effect of Cr on insulin sensitivity. In conclusion, an acute supplementation with 200 μg Cr in young healthy men apparently improved insulin sensitivity with no detectable changes in blood glucose.

Introduction

Cr is an essential trace element required for normal carbohydrate and lipid metabolism in humans. It potentiates the effect of insulin on glucose oxidation by influencing cellular action of insulin [1,2]. The way in which Cr improves insulin sensitivity and reduces postprandial glycemia is still not clearly understood. Mertz *et al.* [3] proposed that Cr may facilitate insulin attachment to the insulin receptor. A few years ago it was suggested that a Cr-containing oligopeptide (low-molecular-weight chromium-binding substance, LMWCr) stimulates the insulin receptor's protein tyrosine kinase activity after that insulin binds to the receptor [4,5].

Cr deficiency in humans leads to symptoms similar to those associated with non-insulin-dependent diabetes mellitus (NIDDM) like decreased glucose tolerance, increased concentration of circulating insulin, elevated cholesterol and triacylglycerol, and reduced HDL cholesterol concentration [6,7]. Dietary Cr intake seems to be suboptimal in the industrialised world. More than 90 % of normal, well-balanced diets provide less than the lower limit of the estimated safe and adequate daily dietary intake for adults (ESADDI, 50 – 200 μg [8,9]). However, the American Institute of Medicine lately set the adequate intake, based on estimated mean Cr intake, at 35 $\mu g \cdot d^{-1}$ for men and 25 $\mu g \cdot d^{-1}$ for women, respectively [10]. In contrast, the German, Austrian, and Swiss nutrition societies recently set the adequate intake for adults at 30-100 $\mu g \cdot d^{-1}$ [11]. This illustrates the uncertainty concerning Cr requirements. Even if the requirements were smaller than previously estimated, there still might be individuals with a marginally deficient Cr status as many people do not eat balanced diets. For that reason it could be expected that at least some apparently healthy individuals could benefit from Cr supplementation.

NIDDM is a major threat to public health. According to the 1998 world health report of the WHO, incidence of diabetes in adults will increase from 143 to 300 millions in the next 25 years largely because of dietary and other lifestyle factors [12]. The type of carbohydrates in the diet may play an important role in this context. There is growing evidence that high-glycemic diets increase the risk of developing insulin resistance and ultimately NIDDM in later life [13,14]. High-glycemic foods lead to a sharp increase in blood glucose concentration and result in higher insulin secretion. Therefore, food or meals with a low glycemic index (GI) or substances lowering postprandial glycemia could have a beneficial role in delaying or even preventing the onset of insulin resistance and NIDDM.

Several studies have shown that long-term Cr supplementation can improve glucose tolerance and insulin sensitivity in people with impaired glucose tolerance or NIDDM (for a review see [7]). In

many of these studies, Cr was supplemented at 200 μg per day, which is at the upper level of the ESADDI, or at higher doses, and subjects were given Cr for periods of several weeks or even months. In contrast, the effects of a short-term supplementation on glucose metabolism have not been investigated yet. The acute glycemic response to a meal could already be lowered by a single Cr dose, as ingested Cr is rapidly absorbed and its blood concentration reaches a maximum 30 to 90 minutes after intake [15]. Also, according to the mechanism of action of Cr proposed by Davis & Vincent [5], binding of insulin to the insulin receptor triggers a movement of Cr from blood into insulin-dependent cells. There, Cr binds to LMWCr which enhances tyrosine activity and glucose uptake up to eightfold. We hypothesized that if Cr concentration in the blood was elevated through acute oral intake, glucose uptake would be potentiated and the glycemic response reduced.

The purpose of this study was, therefore, to investigate the effects of a single Cr dose of 200 μg on postprandial metabolism after a high-glycemic meal in young healthy males, and to evaluate the magnitude of the effects by comparing them to the postprandial response to a low-glycemic meal.

Subjects and methods

Subjects

Fifteen young men were recruited for the study by advertisement at the Swiss Federal Institute of Technology in Zurich. One subject dropped out of the study on his first test day after fainting during the insertion of the catheter into an arm vein. The fourteen subjects were aged 20 to 34 years (mean 26 years), weighed from 62 to 92 kg (mean 72 kg) and had body mass indexes ranging from 20 to 26 $kg \cdot m^{-2}$ (mean 23 $kg \cdot m^{-2}$). They were non-smokers, neither on any medication nor taking 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 2 $h \cdot wk^{-1}$). All participants were apparently healthy and had no family history of diabetes. The aim, procedure, and possible risks of the study were explained to the subjects who then gave their written consent. The research protocol was approved by the scientific ethics committee of the Swiss Federal Institute of Technology in Zurich.

Experimental design

The study was performed as a placebo-controlled, single-blind crossover experiment. All subjects underwent three trials in random order in which different test meals were provided. Test meals consisted either of white bread with supplemental Cr (Cr200) or with placebo (WB), or of wholemeal crackers (Darvida; HUG, Malters, Switzerland), each meal providing 50 g available carbohydrates. Each subject was tested at least five days apart and all three trials were performed within four weeks. Participants were told not to change dietary habits or physical activity level until completion of the study. On the evening before each trial, subjects consumed an identical, standardised pasta meal providing approximately 3.5 MJ energy (112 g CHO, 30 g fat, 26 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 arrived at the laboratory using local transport after a 12 h overnight fast and first completed a short questionnaire assessing food intake and activity patterns of the day before to check compliance with the given instructions. 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 the antecubital vein, a fasting blood sample was

taken. Baseline respiratory measurements were also recorded. After assessment of all baselines, test meals were given and eaten within ten minutes. Postprandial blood samples were taken at 20, 40, 60, 90, and 120 min after beginning of the meal. Postprandial respiration was recorded hourly. After the baseline assessment and 30 min before the test meal, 200 μg Cr as Cr picolinate (GNC, Pittsburgh, PA, USA) were given with the Cr200 trial and a placebo (Hänseler AG, Herisau, Switzerland) with the WB trial. Placebo pills were indistinguishable from the Cr pills and contained 120 mg lactose and 50 mg potato starch.

Blood sampling and analysis

Blood samples were collected in tubes (Monovette, Sarstedt AG, Sevelen, Switzerland) containing either EDTA plus sodium fluoride for the analysis of glucose, lactate, and β-hydroxybutyrate (BHB) or EDTA for the analysis of insulin, glucagon, cortisol, glycerol, fatty acids, and glycosylated haemoglobin (HbA_{1c}). 1 ml of each EDTA blood sample was transferred into a glass tube containing 500 KIU trypsin inhibitor (Aprotinin as Trasylol®, Bayer AG, Leverkusen, Germany) for the analysis of glucagon. All blood samples were immediately placed on ice and then centrifuged at 3000 g for 15 min at 8 °C. Plasma and serum samples were stored at –20 °C until analysis.

HbA_{1c} levels were measured on a Cobas Integra 700 (Roche, Basel, Switzerland) using a Cobas Integra Haemoglobin A1c kit. Plasma metabolites were analysed enzymatically with a Cobas Mira analyser (Roche, Basel, Switzerland) using commercial kits: Glucose (Roche, Basel, Switzerland), glycerol and BHB (Sigma diagnostics, St. Louis, MO, USA), and fatty acids (Wako Chemical GmbH, Neuss, Germany). Plasma hormones were assessed by standard radioimmunoassay kits: Insulin (Pharmacia AB, Uppsala, Sweden), glucagon and cortisol (DPC, Los Angeles, CA, USA).

The incremental area under the curve (AUC) for the blood glucose and insulin response was calculated using the trapezoidal method [16]. The GI of a test meal was defined as the glucose AUC for that meal expressed as a percentage of the AUC for WB.

Respiratory measurements

The respiratory exchange ratio (RER) was determined using a half open system (Oxycon Sigma, Mijnhardt BV, Netherlands). Measurements were made at baseline, and 60 and 120 min after the meals. RER was recorded for 15 min while subjects walked slowly on a treadmill (2.5 $km \cdot h^{-1}$) to

ensure a stable respiration. The mean of the last five minutes of each measurement was defined as the RER of the respective measurement.

Statistical analyses

All results are expressed as means ± standard error of the mean and/or range. Repeated-measures analysis of variance was used to compare the pattern of the postprandial changes in blood variables between treatments. If significant differences were found, they were then located by using Tukey's post hoc test. The plasma glucose and insulin AUC were compared between trials using paired t-tests with Bonferroni adjustment. The level of significance was set at $P < 0.05$. Data were analysed 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 4.6 to 5.6 % (5.0 ± 0.1 %). No significant differences in fasting concentrations of all measured indexes were observed between trials. All fasting values were within the normal range for healthy people.

Glucose

The mean plasma glucose concentration increased after consumption of each meal, attaining a peak level at 40 min, and returned almost to baseline after 120 min (Fig. 1). The glucose concentration was similar for the WB and Cr200 trial at all time points and tended to be smaller for the cracker trial after 60 and 90 min. The plasma glucose AUC was not different between the WB and Cr200 trial (Table 1). However, the AUC during the cracker trial was 19 ($P = 0.18$) and 26 % ($P = 0.08$) smaller compared with the WB and Cr200 trial, respectively. These differences reached the significance level if one subject's AUC values (outlier, i.e. > 2 standard deviations from the mean) were discarded. Glucose AUC was lowered after Cr supplementation compared to WB in six subjects (responders) and was unchanged or increased in eight subjects. For the responders, the GI of the meal (as a measure of the individual glucose response) was lowered by 30 % ($P < 0.05$) after supplementation.

Table 1 Glucose and insulin AUC for the test meals (mean values with standard error of the means)

	WB	Cr200	Cracker
Glucose [mmol · min/l]*	60.4 ± 7.5 [a]	65.2 ± 8.2 [a]	43.7 ± 6.9 [b]
Insulin [pmol · min/l]**	1330 ± 200 [ab]	1350 ± 150 [a]	1070 ± 130 [b]

* n=13, ** n=14. Numbers in the same row not sharing a common superscript are significantly different, $P<0.05$. WB=white bread with placebo, Cr200=white bread with chromium, Cracker=wholemeal cracker.

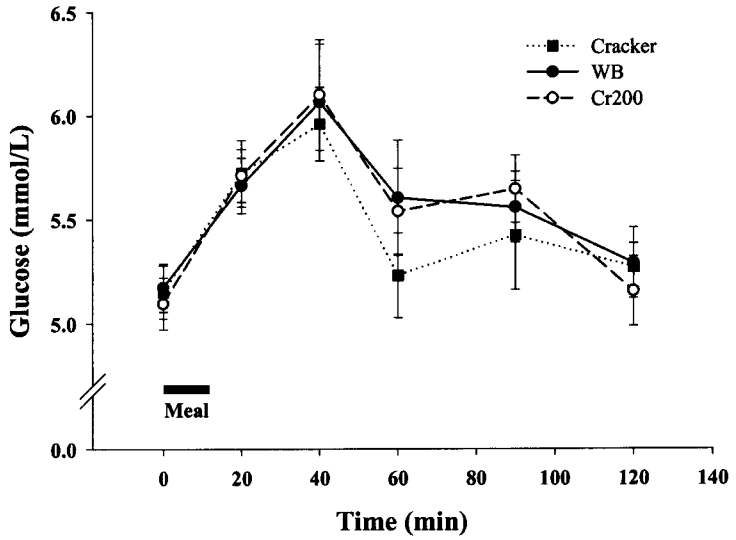

Figure 1. Plasma glucose response to test meals containing 50 g available CHO. Test meals were eaten within 10 min and consisted of white bread with Cr (Cr200, O), white bread with placebo (WB, ●) and wholemeal cracker (Cracker, ■). Values are means for fourteen subjects with standard errors of the means shown by vertical bars.

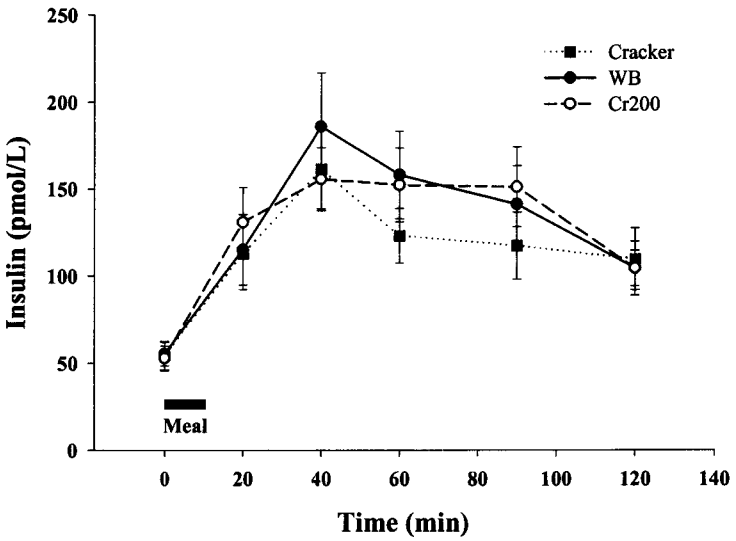

Figure 2. Plasma insulin response to test meals containing 50 g available CHO. Test meals were eaten within 10 min and consisted of white bread with Cr (Cr200, O), white bread with placebo (WB, ●) and wholemeal cracker (Cracker, ■). Values are means for fourteen subjects with standard errors of the means shown by vertical bars.

Insulin

For each trial, plasma insulin concentration peaked 40 min after ingestion and remained above fasting concentrations during the entire observation period (Fig. 2). The increase in insulin concentration between the 20 and 40 min point was 69 % lower for Cr200 than for WB ($P < 0.05$) and peak insulin concentration (at 40 min) tended ($P = 0.11$) to be lower after Cr supplementation than after WB alone. But, no difference in AUC was observed between the WB and Cr200 trial. During the cracker trial the AUC was 24 ($P = 0.18$) and 26 % ($P < 0.05$) smaller than during the WB and Cr200 trials, respectively (Table 1).

Other variables

The decrease in fatty acid and glycerol concentration over the 2 h test period was 40 % and 45 %, respectively, greater for Cr200 compared to WB alone (Fig. 3). However, these differences were not statistically significant ($P = 0.06$ and $P = 0.11$, respectively). No differences between trials were observed for glucagon, cortisol, lactate, and BHB concentrations (Table 2).

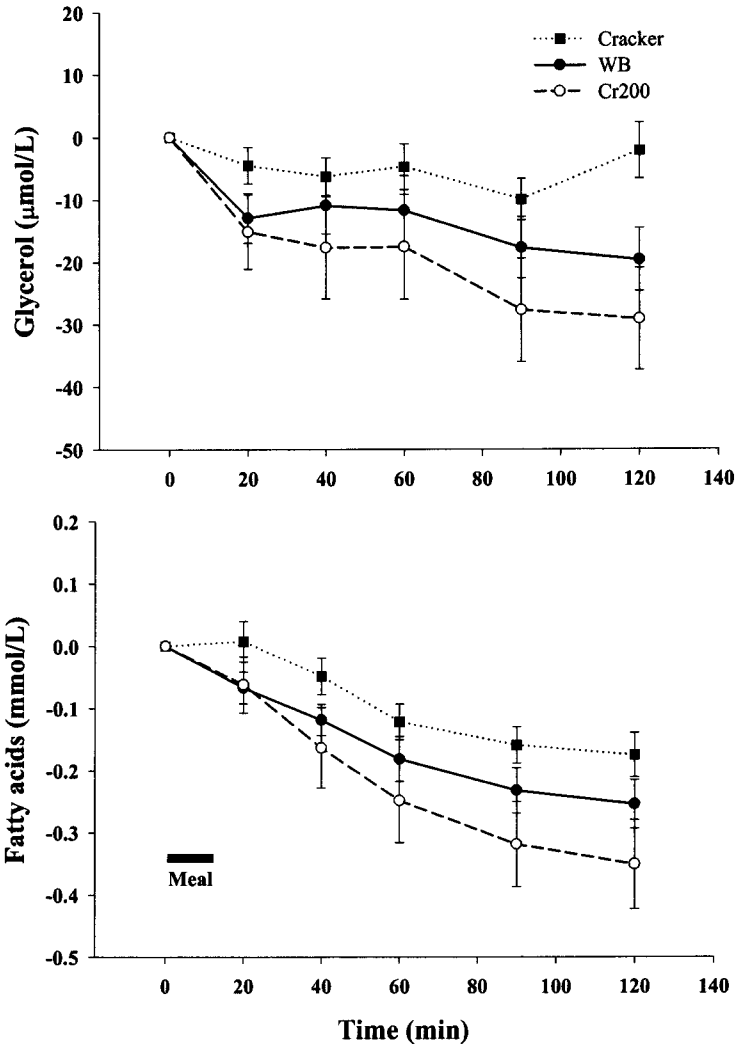

Figure 3. Decremental fatty acids and glycerol concentrations after intake of test meals containing 50 g available CHO. Test meals were eaten within 10 min and consisted of white bread with Cr (Cr200, O), white bread with placebo (WB, ●) and wholemeal cracker (Cracker, ■). Values are means for fourteen subjects with standard errors of the means shown by vertical bars.

Table 2 Temporal changes for glucagon, cortisol, lactate, and β-hydroxybutyrate concentrations after ingestion of test meals containing 50 g available carbohydrates. (Mean values with SEM for fourteen subjects)

	Time of repeated measurements (min)												Repeated-measures ANOVA, P-value	
	0		20		40		60		90		120			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Meal	Meal x Time
Glucagon (pmol/l)														
WB	100	9	99	9	100	9	102	10	100	8	102	8		
Cr200	96	7	97	6	99	7	100	7	97	7	98	7	0.92	0.16
Cracker	96	6	94	6	95	7	100	7	101	7	101	6		
Cortisol (nmol/l)														
WB	462	35	359	27	346	21	326	21	310	23	267	18		
Cr200	425	24	330	19	332	18	319	16	322	22	277	21	0.44	0.53
Cracker	447	23	340	23	327	26	304	26	281	24	260	21		
Lactate (mmol/l)														
WB	1.39	0.14	1.13	0.08	1.18	0.08	1.14	0.07	1.24	0.07	1.06	0.05		
Cr200	1.15	0.07	0.97	0.06	1.08	0.06	1.05	0.06	1.17	0.05	1.03	0.03	0.30	0.23
Cracker	1.29	0.12	1.09	0.05	1.20	0.08	1.12	0.06	1.27	0.07	1.06	0.05		
β-hydroxybutyrate (μmol/l)														
WB	117	11	109	9	103	12	87	3	82	3	83	3		
Cr200	133	22	138	22	114	12	93	4	82	3	93	7	0.56	0.90
Cracker	97	3	102	10	93	6	88	4	85	3	84	3		

WB, white bread meal; Cr200, white bread meal plus 200 μg Cr; Cracker, cracker meal.

Respiratory measurements

The respiratory exchange ratio rose postprandially with all trials, indicating a larger contribution of carbohydrates as a fuel for oxidation (Fig. 4). The increase during the 2 h test period was greater with the WB and Cr200 trial than with the cracker trial (both $P < 0.05$). The peak value tended to be higher with the WB trial than with the Cr200 trial ($P = 0.08$).

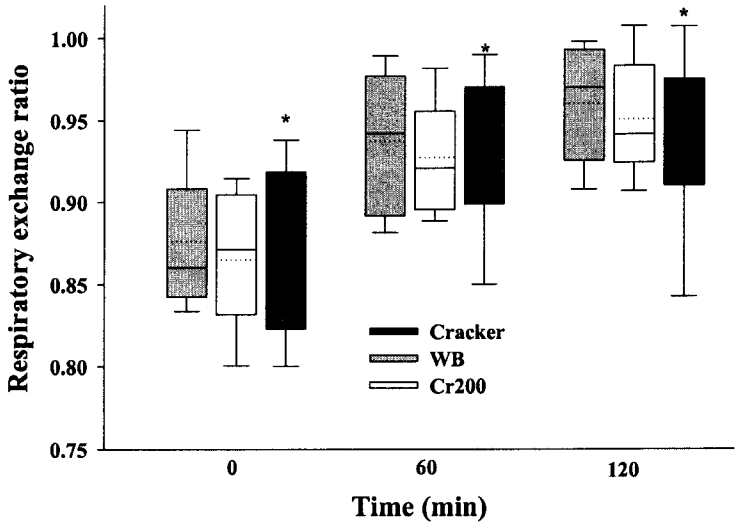

Figure 4. Respiratory exchange ratio after intake of test meals. Results are presented as box and whisker plots. Boxes indicate the 25th, 50th, and 75th percentiles and show the mean as a dotted line. Whiskers extend to the 5th and 95th percentiles. Test meals were eaten within 10 min and consisted of white bread with Cr (Cr200), white bread with placebo (WB) and wholemeal cracker (Cracker). * Increase over the 2 h period was lower for cracker than for WB and Cr200, $P < 0.05$ (n=14).

Discussion

The present study was designed to estimate the effects of acute Cr supplementation on carbohydrate metabolism after ingestion of a high glycemic meal providing 50 g available carbohydrates. We could not detect any statistically significant effect of a single dose of 200 μg Cr given as Cr picolinate on postprandial plasma glucose concentration in young, healthy subjects. Glucose and insulin response after ingestion of a high glycemic white bread snack-like meal did not seem to be influenced by supplemental Cr, except for a less steep increase in circulating insulin between 20 and 40 min after meal ingestion.

Cr picolinate was chosen because it is the most popular Cr supplement and many studies were performed using it. Lately some concerns have been raised about a potential mutagenic effect of Cr picolinate [17,18] and that its chronic intake may have negative effects [19,20]. However, the evidence for this is scarce and long-term studies on the effects of Cr picolinate supplementation are needed to verify these hypotheses.

The reason why we did not observe an effect on glucose metabolism might be that supplementing 200 μg Cr, which represents the upper limit of the ESADDI, was a too small dosage to induce any physiological effects in a group of apparently healthy young men. Accordingly, in a study by Anderson *et al.* [21], lowered fasting and 2 h glucose values were observed after supplementation with 1000 μg but not with 200 μg Cr picolinate for four months. It has been estimated that a large percentage of the population in the western world does not meet the recommendation for Cr intake and that some individuals may be marginally Cr deficient [8,9]. This possibly means that higher amounts would be needed (provided chronically or acutely) to revert a deficiency and to show a beneficial outcome on metabolism. However, other studies reported beneficial effects of supplemental Cr already with a dosage of 200 μg in people with impaired glucose tolerance [22] or with type 2 diabetes [23].

We observed a difference between WB and Cr200 trials in the increase in insulin concentration between 20 and 40 min after the meals. Concomitant to this smaller increase in insulin concentration was a tendency towards a larger decrease in fatty acid and glycerol concentrations in the Cr200 trial compared with the WB trial. This points to increased insulin sensitivity after Cr supplementation. Our study group was probably too small and may not have had the statistical power to show an acute effect. Yet, the 2 h postprandial insulin response, measured as AUC, was not different for the two trials. So, the physiological relevance of this finding may therefore possibly be small.

To our knowledge, the effect of an acute single-dose supplementation of Cr on metabolism was investigated only in a few studies until now: Hopkins [24] observed that an impaired glucose tolerance in malnourished children improved within 18 h of a single oral dose of Cr chloride. In the study by Davis [25], total anaerobic power was increased 20 to 50 min after consumption of a Cr-containing carbohydrate-electrolyte beverage compared to the carbohydrate-electrolyte drink alone. This would suggest a possible effect of Cr after an acute supplementation. In contrast to that, an earlier study by Glinsmann & Mertz [26] found that three out of six type 2 diabetics had improved glucose tolerance after long-term (weeks) but not after short-term (1 - 7 d) supplementation with Cr chloride. Recent studies carried out at our laboratory indicate that Cr may influence postprandial metabolism after a short-term supplementation at higher dosages (400 and 800 μg Cr as Cr picolinate, unpublished data). It is therefore still unclear if Cr needs to be administered chronically rather than acutely to modulate insulin activity, despite the fact that the maximal Cr concentration in the blood is reached 30 to 90 min after intake [15].

In some studies it was noted that some but not all subjects responded to supplemental Cr [26-30]. The results of our study seem to confirm these findings as only six out of 14 subjects responded to the addition of Cr with a decrease in postprandial glycemia. It has been suggested that extra Cr will only benefit individuals with low Cr nutritional status, because Cr acts as a nutrient and not as a therapeutic agent [6,31,32]. Unfortunately, there is still no valid method to assess Cr status and no indicator is known that would predict response to Cr supplementation [27]. So, we can only hypothesise if the subjects responding to supplemental Cr were marginally Cr deficient or not.

It has been shown that Cr supplementation alleviates hypoglycaemic symptoms [30] and improves glucose tolerance in hyperglycemics [22] and in subjects with type 2 diabetes [21,27,33]. Cr supplementation also normalised blood glucose in adults with a tendency towards impaired glucose utilisation but apparently had no effect on individuals with normal blood glucose concentration [22,26,34]. We found an inverse correlation between the AUC after the WB and Cr200 trials ($r = 0.53$, $P = 0.05$, not shown). Subjects with "high" glycemic responses after the WB trial showed a smaller glucose AUC after the Cr200 trial and subjects with "low" AUC after the WB trial displayed an unchanged or increased glycemic response after supplemental Cr. None of our subjects showed irregular postprandial glycemia or abnormal HbA_{1c} values. So it might be that even in normoglycemic people some individuals will respond to Cr, and it seems that those with the highest glycemia after a high glycemic meal will profit the most from supplemental Cr.

We included a wholemeal cracker in the study to be able to compare the magnitude of the potential GI-lowering effect of Cr with a natural low glycemic food. The GI of the cracker was not

determined previously. But, we expected from its chemical composition, physical structure (whole grains) and fibre content that it would have a GI notably lower than WB, which was the case (GI of 73 in comparison to 100 for WB). Consumption of the wholemeal cracker lead to a lower AUC for plasma glucose compared with both WB trials and a concurrent insulin response. Also, the RER was lower after the cracker meal indicating an increased lipid oxidation and a smaller reliance on carbohydrates as an energy substrate than after WB. Beneficial effects of low glycemic diets on glucose homeostasis and lipid profiles are well known. Ingestion of low glycemic foods leads to smaller increases in plasma glucose and insulin concentrations, reduces HbA_{1c} and fasting glucose concentration on the long-term, improves insulin sensitivity, and may decrease the risk of developing NIDDM in later life [13,14,35-39]. Yet, many low glycemic foods except those that are high in fat, do not rank high in the taste preferences of consumers and there is a lack of low glycemic breakfast foods as bread and most cereals (especially ready-to-eat varieties) have high GI [40,41]. Therefore, other means to reduce the mean GI of a diet might be useful.

In conclusion, an acute supplementation with 200 μg Cr in young healthy men had no evident influence on postprandial metabolism except for a small effect on insulin concentration. Further studies are needed to examine if higher amounts of Cr induce beneficial effects after short-term addition or if longer supplementation periods are required.

Acknowledgments

This work was supported by the companies HUG AG (Malters) and Biofamilia AG (Sachseln), and by a grant of the Swiss Foundation for Nutrition Research.

References

1. W. Mertz, Chromium research from a distance: From 1959 to 1980, *J Am Coll Nutr* 17, 544-547, 1998.
2. W. Mertz, Chromium in human nutrition: a review, *J Nutr* 123, 626-633, 1993.
3. W. Mertz, E. Toepfer, E.E. Roginski, and M.M. Polansky, Present knowledge of the role of chromium., *Fed. Proc.* 33, 2275-2280, 1974.
4. C.M. Davis, K.H. Sumrall, and J.B. Vincent, A biologically active form of Chromium may activate a membrane phosphotyrosine phosphatase (PTP), *Biochemistry* 39, 12963-12969, 1996.
5. C.M. Davis and J.B. Vincent, Chromium oligopeptide activates insulin receptor tyrosine kinase activity, *Biochemistry* 36, 4382-4385, 1997.
6. H. Lukaski, Chromium as a supplement, *Annu. Rev Nutr* 19, 279-302, 1999.
7. R.A. Anderson, Chromium, glucose intolerance and diabetes, *J Am Coll Nutr* 17, 548-555, 1998.
8. R.A. Anderson and A.S. Kozlovsky, Chromium intake, absorption and excretion of subjects consuming self-selected diets, *Am J Clin Nutr* 41, 1177-1183, 1985.
9. R.A. Anderson, N.A. Bryden, and M.M. Polansky, Dietary chromium intake. Freely chosen diets, institutional diet, and individual foods, *Biol Trace Elem Res* 32, 117-121, 1992.
10. Food and Nutrition Board, "Chromium, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc,* Institute of Medicine, The National Academy Press, Washington, D.C., 155-176, 2001.
11. *Referenzwerte für die Nährstoffzufuhr*, Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, and Schweizerische Vereinigung für Ernährung, Umschau Braus GmbH, 2000.
12. WHO, *The World Health Report 1998*, WHO Press Release, Geneva, 1998.
13. J. Salmeron, A. Ascherio, EB. Rimm, G. Colditz, D. Spiegelman, D.J. Jenkins, M.J. Stampfer, A.L. Wing, and J.E. Manson, Dietary fiber, glycemic load, and risk of NIDDM in men, *Diabetes Care* 20, 545-550, 1997.
14. J. Salmeron, J.E. Manson, M.J. Stampfer, G. Colditz, A.L. Wing, and W.C. Willett, Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women, *JAMA* 277, 472-477, 1997.

15. M.L. Gargas, S.M. Hays, M.A. Katona, and B.L. Finley. The kinetics of dietary chromium (III). International Symposium on the Health Effects of Dietary Chromium, Boston May 1&2 , 8. 1998.
16. T.M. Wolever, D.J. Jenkins, A.L. Jenkins, and R.G. Josse, The glycemic index: methodology and clinical implications, *Am J Clin Nutr* 54, 846-854, 1991.
17. J.K. Speetjens, R. Collins, and J.B. Vincent, The nutritional supplement chromium trispicolinate cleaves DNA, *Chem. Res. Toxicol.* 12 (6), 483-487, 1999.
18. D.M. Stearns, S.M. Silveira, K.K. Wolf, and A.M. Luke, Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells.," *Mutat Res* 513, 135-142, 2002.
19. D.M. Stearns, J.J. Belbruno, and K.E. Wetterhahn, A prediction of chromium(III) accumulation in humans from chromium dietary supplements, *Faseb J* 9, 1650-1657, 1995.
20. J.B. Vincent, The bioinorganic chemistry of chromium(III), *Polyhedron* 20, 1-26, 2001.
21. R.A. Anderson, N. Cheng, N.A. Bryden, M.M. Polansky, J. Chi, and J. Feng, Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes, *Diabetes* 46, 1786-1791, 1997.
22. R.A. Anderson, M.M. Polansky, N.A. Bryden, and J.J. Canary, Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets, *Am J Clin Nutr* 54, 909-916, 1991.
23. R. Riales and M.J. Albrink, Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men, *Am J Clin Nutr* 34, 2670-2678, 1981.
24. L.L.Jr. Hopkins, O. Ransome-Kuti, and A.S. Majaj, Improvement of impaired carbohydrate metabolism by chromium 3 in malnourished infants, *Am J Clin Nutr* 21, 203-211, 1968.
25. J.M. Davis, R.S. Welsh, and N.A. Alderson, Effects of carbohydrate and chromium ingestion during intermittent high-intensity exercise to fatigue, *Int J Sport Nutr Exerc Metab* 10, 476-485, 2000.
26. W. Glinsmann and W. Mertz, Effect of trivalent chromium on glucose tolerance, *Metabolism* 15, 510-520, 1966.
27. A. Ravina, L. Slezak, A. Rubal, and N. Mirsky, Clinical use of the trace element chromium(III) in the treatment of diabetes mellitus, *J Trace Elem Exp Med* 8, 183-190, 1995.
28. R.A. Levine, D.H. Streeten, and R.J. Doisy, Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects, *Metabolism* 17, 114-125, 1968.

29. R.A. Anderson, M.M. Polansky, W. Mertz, and W. Glinsmann, Chromium supplementation of human subjects: effects on glucose, insulin, and lipid variables, *Metabolism* 32, 894-899, 1983.
30. R.A. Anderson, M.M. Polansky, N.A. Bryden, S.J. Bhathena, and J.J. Canary, Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia, *Metabolism* 36, 351-355, 1987.
31. R.A. Anderson, Essentiality of chromium in humans, *Sci Total Environ* 86, 75-81, 1989.
32. W. Mertz, Interaction of chromium with insulin: A progress report, *Nutr Rev* 56, 174-177, 1998.
33. E.G. Offenbacher and Pi-Sunyer FX, Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects., *Diabetes* 29, 919-925, 1980.
34. R.A. Anderson, M.M. Polansky, N.A. Bryden, K.Y. Patterson, C. Veillon, and W.H. Glinsmann, Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters, *J Nutr* 113, 276-281, 1983.
35. W.H. Saris, N.G. Asp, I. Bjorck, E. Blaak, F. Bornet, F. Brouns, K.N. Frayn, P. Furst, G. Riccardi, M. Roberfroid, and M. Vogel, Functional food science and substrate metabolism, *Br J Nutr* 80 Suppl 1, S47-S75, 1998.
36. G. Frost, J. Wilding, and J. Beecham, Dietary advice based on the glycaemic index improves dietary profile and metabolic control in type 2 diabetic patients, *Diabet Med* 11, 397-401, 1994.
37. G. Riccardi and A.A. Rivellese, Dietary treatment of the metabolic syndrome--the optimal diet, *Br J Nutr* 83 Suppl 1, S143-S148, 2000.
38. J.C. Brand, S. Colagiuri, S. Crossman, A. Allen, D.C. Roberts, and A.S. Truswell, Low-glycemic index foods improve long-term glycemic control in NIDDM, *Diabetes Care* 14, 95-101, 1991.
39. J.C. Brand-Miller, Importance of glycemic index in diabetes, *Am J Clin Nutr* 59, 747S-752S, 1994.
40. K. Foster-Powell and J.C. Brand-Miller, International tables of glycemic index, *Am J Clin Nutr* 62, 871S-893S, 1995.
41. I. Bjorck, H. Liljeberg, and E. Ostman, Low glycaemic-index foods, *Br J Nutr* 83 Suppl 1, S149-S155, 2000.

Chapter 3

Acute chromium supplementation lowers postprandial glycemia after a high-glycemic meal in healthy young men

Based on

Marc T Frauchiger, MSc, Caspar Wenk, PhD, Paolo C Colombani, PhD

Submitted to Journal of the American College of Nutrition, September 2002

Abstract

Chromium (Cr) potentiates the action of insulin in the cell and improves glucose tolerance after long-term supplementation. We hypothesized that Cr may also have acute effects and might be beneficial in lowering the glycemic index of a meal. 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. 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 WB 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 ± 22, Cr400 111 ± 14 ($p < 0.01$), Cr800 122 ± 15 mmol · 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 ($p = 0.07$). Acute Crsupplementation improved postprandial glucose metabolism in some 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 type II diabetes. The type of carbohydrates and the glycemic response to a meal may be important risk factors. There is 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].

Therefore, lowering the glycemic response to a meal or a diet may represent an important preventive approach in delaying the onset of insulin resistance and NIDDM. The trace mineral 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 supplementation on the metabolism [15,16]. However, Cr is rapidly absorbed and the maximal blood concentration is reached 30 to 90 min after ingestion [17]. So, an acute effect of Cr may 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]. Nevertheless, mean Cr intake in the USA and in other developed countries corresponds only to 50-60 % of the minimum Estimated Safe and Adequate Daily Dietary Intake (50 µg) [20]. So, parts of the population may be mildly Cr deficient. 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 [21]. These recommendations were based on the Cr content of well balanced diets. As many people do not consume balanced diets, there might still be individuals with marginal Cr intake 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 ± 0.9 years (mean ± SEM) and with normal body mass indexes (22.5 ± 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 in the morning to avoid any physical exertion and arrived at the laboratory after a 10-12 h overnight fast. There they 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 the 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 was 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 (**Figure 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 ($p = 0.13$), respectively). 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 those subjects who showed a lower postprandial glycemia after both Cr trials compared with the WB trial, whereas non-responders displayed no change or an increase after supplementation. Postprandial capillary glycemia and the glycemic indexes 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 (**Figure 2**).

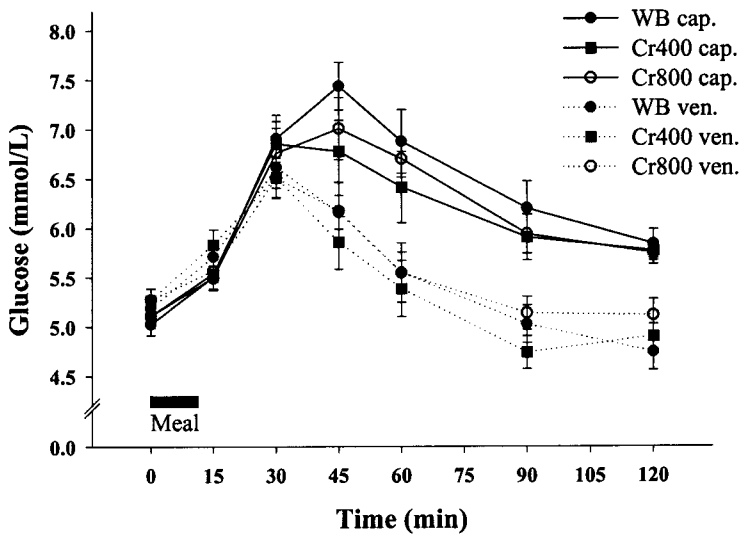

Figure 1. Capillary and venous glucose concentrations after test meals providing 75 g available carbohydrates. Test meals were eaten within 10 min and consisted of white bread with placebo (WB, ●), white bread with 400 μg Cr (Cr400, ■) and white bread with 800 μg Cr (Cr800, O). Values are means for thirteen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

Table 1. Capillary and venous glucose AUC (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.

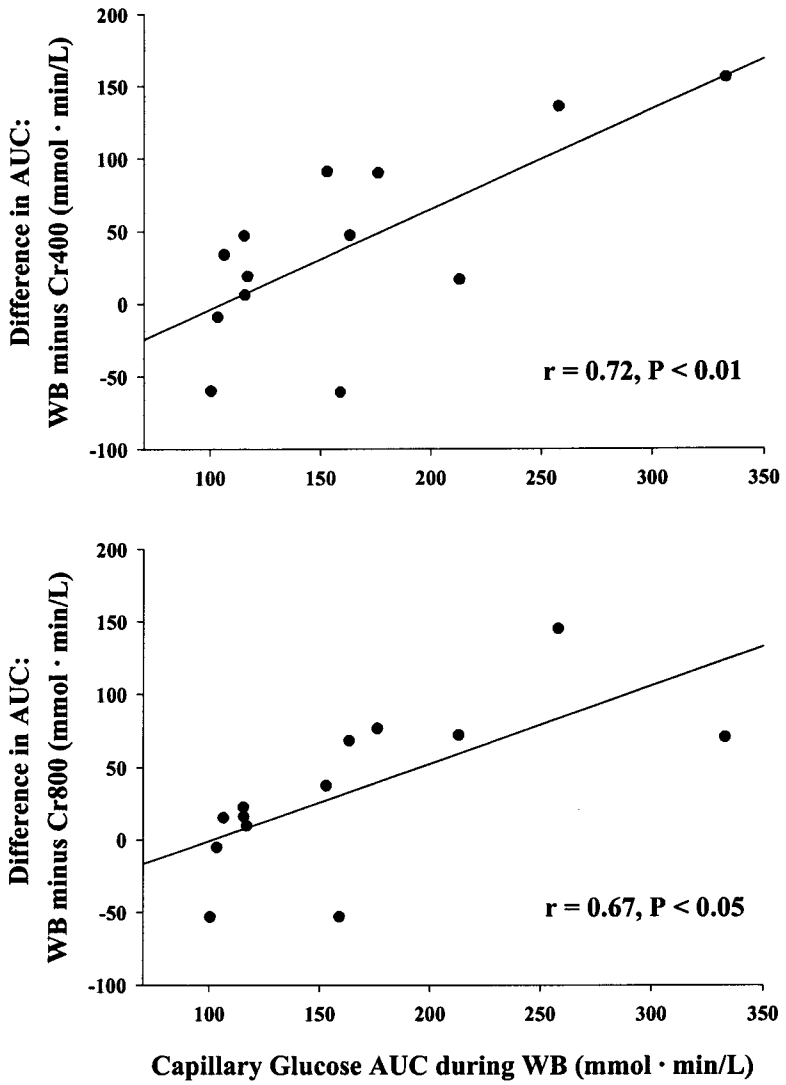

Figure 2. Significant positive correlations were shown between the extent of the capillary glycemic response after the WB trial and the reduction in glycemia during the Cr400 and the Cr800 trial. The dots above zero (y-axis) represent the subjects having shown a decrease in glycemia after Cr supplementation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

Insulin

Insulin concentrations after the Cr trials were not significantly different from concentrations after the WB trial at all time points (**Figure 3**). Accordingly, we observed no differences for the AUC (AUC WB: 13520 ± 920, Cr400: 12840 ± 1240 ($p = 0.53$), and Cr800: 12600 ±1330 ($p = 0.34$) pmol · min/L). Of the ten subjects with a smaller glucose AUC after the Cr trials (responders), six had smaller insulin AUC during the Cr trials and four had smaller insulin AUC during the WB trial. Insulin sensitivity (QUICKI) was similar for responders and non-responders (0.68 ± 0.02 and 0.66 ± 0.01, $p = 0.69$).

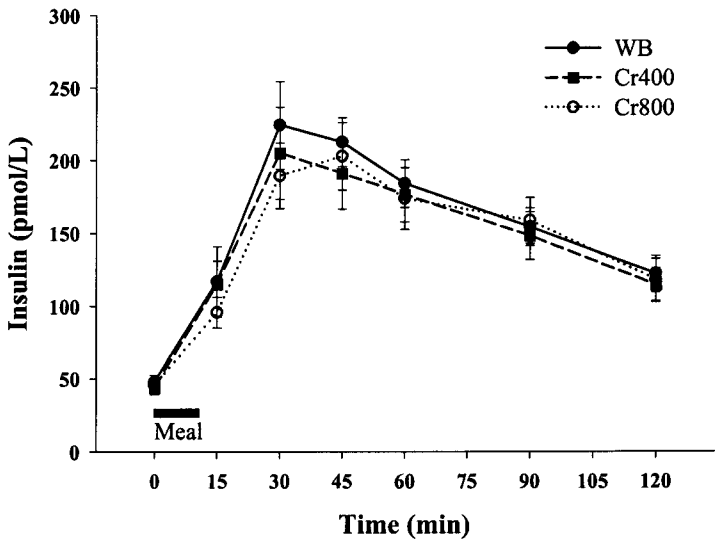

Figure 3. Plasma insulin concentrations after test meals providing 75 g available carbohydrates. Test meals were eaten within 10 min and consisted of white bread with placebo (WB, ●), white bread with 400 μg Cr (Cr400, ■) and white bread with 800 μg Cr (Cr800, O). Values are means for thirteen subjects 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**).

Table 2: Comparison of estimated energy and nutrient intake in responders and non-responders.

	Responders (n = 10)		Non-responders (n = 3)			
	Mean	SEM	Mean	SEM	*p*-value	DRI
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, 2000)

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 3: Fasting iron , ferritin and transferrin concentrations and transferrin saturation in responders and non-responders.

	Responders (n = 10)		Non-responders (n = 3)		
	Mean	SEM	Mean	SEM	P-value
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.

In the present study a substantial reduction in postprandial glycemia was observed after addition of 400 as well as 800 μg Cr compared with the WB trial (-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 M., Colombani P.C. and Wenk C., 2001). This suggests that 200 μg of Cr might be an insufficient amount to influence postprandial metabolism and that in healthy young men a single-dose of Cr of at least twice the maximum ESADDI is needed to affect glucose metabolism. 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.

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. An other 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 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, transforming the high-glycemic food white bread 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. The blood glucose lowering activity of acute Cr supplementation demonstrated in this study supports the role of Cr as a potential antidiabetic agent.

Acknowledgments

This work was supported by a grant of the Swiss Foundation for Nutrition Research.

References

1. WHO. The World Health Report 1998. Geneva: *WHO Press Release*, 1998.
2. Salmeron J., Manson JE, Stampfer MJ, Colditz G, Wing AL, Willett WC: Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* 277:472-477, 1997.
3. Salmeron J., Ascherio A., Rimm EB. et al.: Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 20:545-550, 1997.
4. Saris WH, Asp NG, Bjorck I et al.: Functional food science and substrate metabolism. *Br J Nutr* 80 Suppl 1:S47-S75, 1998.
5. Frost G, Dornhorst A: The relevance of the glycaemic index to our understanding of dietary carbohydrates. *Diabet Med* 17:336-345, 2000.
6. Brand JC, Colagiuri S, Crossman S, Allen A, Roberts DC, Truswell AS: Low-glycemic index foods improve long-term glycemic control in NIDDM. *Diabetes Care* 14:95-101, 1991.
7. Frost G, Keogh B, Smith D, Akinsanya K, Leeds A: The effect of low-glycemic carbohydrate on insulin and glucose response in vivo and in vitro in patients with coronary heart disease. *Metabolism* 45:669-672, 1996.
8. Jarvi AE, Karlstrom BE, Granfeldt YE, Bjorck IE, Asp NG, Vessby BO: Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* 22:10-18, 1999.
9. Jenkins DJ, Wolever TM, Collier GR et al.: Metabolic effects of a low-glycemic-index diet. *Am J Clin Nutr* 46:968-975, 1987.
10. Anderson RA, Polansky MM, Bryden NA, Bhathena SJ, Canary JJ: Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism* 36:351-355, 1987.
11. Anderson RA, Polansky MM, Bryden NA, Canary JJ: Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutr* 54:909-916, 1991.
12. Anderson RA, Cheng N, Bryden NA, Polansky MM, Chi J, Feng J: Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes* 46:1786-1791, 1997.
13. Offenbacher EG, Pi-Sunyer FX: Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. *Diabetes* 29:919-925, 1980.

14. Ravina A, Slezak L, Rubal A, Mirsky N: Clinical use of the trace element chromium(III) in the treatment of diabetes mellitus. *J Trace Elem Exp Med* 8:183-190, 1995.
15. Davis JM, Welsh RS, Alderson NA: Effects of carbohydrate and chromium ingestion during intermittent high-intensity exercise to fatigue. *Int J Sport Nutr Exerc Met* 10:476-485, 2000.
16. Hopkins LL.Jr., Ransome-Kuti O, Majaj AS: Improvement of impaired carbohydrate metabolism by chromium 3 in malnourished infants. *Am J Clin Nutr* 21:203-211, 1968.
17. Gargas M, Hays S, Katona M, Finley B. The Kinetics of Dietary Chromium (III).
18. Anderson RA: Essentiality of chromium in humans. *Sci Total Environ* 86:75-81, 1989.
19. Anderson RA: Chromium, glucose tolerance, and diabetes. *Biol Trace Elem Res* 32:19-24, 1992.
20. Anderson RA: Nutritional factors influencing the glucose/insulin system: chromium. *J Am Coll Nutr* 16:404-410, 1997.
21. Food and Nutrition Board. Chromium. In: Institute of Medicine, ed. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, D.C.: The National Academy Press, 2001:155-76.
22. Katz A, Nambi SS, Mather K, Follmann DA, Sullivan G, Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85:2402-2410, 2000.
23. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG: The glycemic index: methodology and clinical implications. *Am J Clin Nutr* 54:846-854, 1991.
24. Anderson RA: Chromium, glucose intolerance and diabetes. *J Am Coll Nutr* 17:548-555, 1998.
25. Davis CM, Vincent JB: Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry* 36 (15):4382-4385, 1997.
26. Wolever TM, Jenkins DJ: Metabolic response to test meals containing different carbohydrate foods. *Nutr Res* 8:573-581, 1988.
27. Wolever TM, Bolognesi C: Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *J Nutr* 126:2798-2806, 1996.
28. Jackson RA, Blix PM, Matthews PA, Morgan LM, Rubenstein AH, Nabarro JD: Comparison of peripheral glucose uptake after oral glucose loading and a mixed meal. *Metabolism* 32:706-710, 1983.

29. Offenbacher EG, Rinko CJ, Pi SF: The effects of inorganic chromium and brewer's yeast on glucose tolerance, plasma lipids, and plasma chromium in elderly subjects. *Am J Clin Nutr* 42:454-461, 1985.
30. Ani M: The effect of chromium on parameters related to iron metabolism. *Biol Trace Elem Res* 32:57-64, 1992.
31. Vincent JB: The biochemistry of chromium. *J Nutr* 130:715-718, 2000.
32. Anderson RA, Polansky MM, Mertz W, Glinsmann W: Chromium supplementation of human subjects: effects on glucose, insulin, and lipid variables. *Metabolism* 32:894-899, 1983.
33. Bjorck I, Liljeberg H, Ostman E: Low glycaemic-index foods. *Br J Nutr* 83 Suppl 1:S149-S155, 2000.
34. Foster-Powell K, Brand-Miller JC: International tables of glycemic index. *Am J Clin Nutr* 62:871S-893S, 1995.

Seite Leer /
Blank leaf

Chapter 4

Single-dose chromium supplementation improves postprandial glycemia in men with slightly impaired glucose metabolism

Based on

Marc T Frauchiger, Caspar Wenk, Paolo C Colombani

Submitted to Journal of Trace Elements in Medicine and Biology, September 2002

Abstract

The essential trace element chromium (Cr) has an effect on glucose metabolism through its action on insulin. Our aim was to investigate which metabolic and/or dietary factors influence the effects of acute Cr supplementation on postprandial glycemic response. Twenty-six apparently healthy young men were tested in a single-blind, randomized crossover study. Each subject ate on two separate occasions a white bread test meal providing 75 g of highly available carbohydrates, once with a placebo and once supplemented with 400 μg Cr (as Cr picolinate). Blood samples were obtained twice before ingestion of the test meal and six times afterward. The capillary glucose area under the curve (AUC) was significantly reduced (-20 %, $P<0.01$) after Cr supplementation in those subjects with a glucose AUC above the median during the placebo trial, while the AUC did not differ between trials in the other half of the subjects. We found no differences in the insulin AUC between the two trials. The response to supplemental Cr could not be associated to any factor (e.g. iron, transferrin, ferritin, nutrient intake) other than glucose tolerance. In conclusion, acute Cr supplementation improved the glycemic response to a high-glycemic meal only in a subgroup of individuals with slightly poorer glucose control (glucose AUC>median). This suggests that supplemental Cr may be most effective in individuals with poorer glucose tolerance like overweight or elderly people.

Introduction

Recent epidemiological studies have shown that diets resulting in high postprandial glucose responses may over the long-term lead to insulin resistance and play a role in the development of type II diabetes (1;2). So, factors contributing to better glycemic control and lowering postprandial glycemia may be of importance to reduce the incidence of type II diabetes. One of these factors could be the trace element Cr.

Cr potentiates the action of insulin and has been shown to improve glucose tolerance after long-term supplementation (3-7). Recent findings at our laboratory indicate that Cr may also have a beneficial effect on postprandial glycemia after acute supplementation (8).

In many of these studies it has been observed that some but not all individuals responded to Cr supplementation. It has been proposed that, as Cr is a nutrient, only individuals with a deficient or impaired Cr status would respond to a supplementation (9). Unfortunately it is still not possible to assess Cr body stores and this hypothesis cannot be proven conclusively. Anderson (10) observed that people with mild hyper- or hypoglycemia responded to supplemental Cr, whereas the individuals with normal glucose response did not. In a recent study performed by our group we found an association between the response to Cr supplementation and transferrin concentration in our subjects (8). As transferrin is supposed to play a role in Cr transport (11;12), differing transferrin concentrations may possibly indicate variations in Cr status.

This underlines the uncertainty about the factors or mechanisms that influence the individual response to supplemental Cr. Therefore, the aim of the present study was to investigate if and what metabolic or nutritive factors may be associated to or influence the response to Cr after acute supplementation.

Subjects and methods

Subjects

Twenty-six apparently healthy, non-smoking males aged 27.3 ± 0.7 years (mean ± s.e.) with normal body mass indexes (23.4 ± 0.4 $kg \cdot m^{-2}$) participated in the study. Hemoglobin A_{1c} (HbA_{1c}) in these subjects ranged from 4.4 to 5.8 % (normal, 4.0-6.5 %). Individuals were excluded from the study if characterized by at least one of the following: overt illnesses, family history of diabetes, smoking, endurance-trained (exercise volume more than 2 $h \cdot wk^{-1}$), taking medication or mineral supplements, and under- or overweight (BMI <20 or >26 $kg \cdot m^{-2}$). All participants were told the purpose, practical details and risks associated with participation in the study before signing an informed-consent form and were aware of the possibility of withdrawing from the study at any time. The Scientific Ethics Committee of the Swiss Federal Institute of Technology in Zurich gave approval of the study.

Study design

The study was placebo-controlled and had a single-blind crossover design. Subjects underwent two trials in random order. Test meals consisted of white bread with supplemental Cr (Cr400) or placebo (WB), each meal providing 75 g available carbohydrates. Participants were tested at least one week apart to avoid carry-over effects. The two trials were performed within two weeks. Each subject was instructed 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 pasta meal providing approximately 3.9 MJ energy (190 g carbohydrates, 8 g fat, 38 g protein), and were told not to eat anything else until the next morning. Furthermore, subjects were asked not to ingest alcohol or caffeine containing drinks and foods, and were requested to avoid physical exercise the day prior to each trial. Subjects arrived at the laboratory using local transport after a 10-12 h overnight fast and 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. Blood samples were drawn from an antecubital vein. Two basal blood samples were taken 30 min apart. After the first baseline assessment and 30 min before the beginning of the test meal, 400 μg Cr as Cr picolinate in pill form (GNC, Pittsburgh, PA, USA) were given with the Cr trial 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. Immediately after

the second basal blood sample, 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, 2.3 g of fat, and 12.8 g of protein. Postprandial venous blood samples were taken at 15, 30, 45, 60, 90, and 120 min after beginning of the meal. Finger-prick capillary blood samples for glucose analysis were taken at the same times than the venous samples.

Blood sampling and analysis

Venous blood samples were collected in tubes (Monovette, Sarstedt AG, Sevelen, Switzerland) containing EDTA plus sodium fluoride for the analysis of glucose, EDTA for the analysis of insulin, or a coagulation activator for the analysis of iron, ferritin, transferrin, cholesterol, and HDL, and no reagent for the analysis of HbA_{1c}. Tubes with blood for plasma samples were immediately placed on ice and centrifuged (3000 g for 15 min at 8 °C) within 20 min. Tubes for serum samples were left at room temperature for 30 min to allow coagulation before centrifugation. Plasma and serum samples were transferred into cups and stored at –20 °C until analysis.

Metabolites were analyzed enzymatically with a Cobas Mira analyzer (Roche, Basel, Switzerland) using commercial kits: glucose, iron, ferritin and transferrin, cholesterol and HDL-cholesterol (Roche, Basel, Switzerland). Insulin was assessed by a standard radioimmunoassay kit (Pharmacia AB, Uppsala, Sweden). HbA_{1c} was measured on a Cobas Integra 700 (Roche, Basel, Switzerland) using a Cobas Integra Hemoglobin A_{1c} kit. Capillary blood glucose concentration was 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 was explained and then given to them. The booklet included pages to record the food eaten and different sets of color photographs. Each set of photographs showed three portion sizes (small, medium, and large) 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 tomatoes, slices of bread), or in portion sizes (i.e. small, medium or large). Nutrient intake was calculated using the Swiss version of the EBISpro software (University of Hohenheim, Germany).

Insulin sensitivity

The quantitative insulin sensitivity check index (QUICKI = 1/[log (fasting insulin) + log (fasting glucose)]) was used to assess insulin sensitivity (13).

Statistical analysis

ANOVA for repeated measures was used to compare postprandial changes in blood variables between the trials. A Tukey's post hoc test was applied in the event of a significant overall difference between treatments. Calculation of correlation coefficients between variables was performed by using the Pearson product-moment test. Incremental areas under the curve (AUC) were calculated using the trapezoidal method (14) and then compared between trials using Student's paired t-tests. All results were expressed as means ± s.e. and/or range. Statistical significance was set at $P < 0.05$. Data were analyzed using the statistics software SYSTAT 9.01 (SPSS Inc., Chicago, IL, USA).

Results

Glucose

No differences between treatments were found in the glucose measurements for the whole study population (**Fig 1**). Capillary as well as venous concentrations and AUC were similar for WB and Cr400 trial. Ten subjects had higher capillary AUC values after Cr supplementation whereas 16 subjects had a lower glucose AUC compared with placebo (WB). After classifying the subjects according to their glycemic response during the placebo trial, a significant difference in the capillary, but not venous, glucose AUC was found (**Table 1**). Of the one half of subjects (n = 13) with a capillary glucose AUC during the WB trial above the median (135 mmol*min/L) eleven reacted positively to Cr leading to a reduction (-20 %) in postprandial glycemia after Cr supplementation (P = 0.007). The capillary and venous glucose concentrations of this subgroup are shown in **Figure 2**. The 13 participants with an AUC lower than the median showed no significant differences between trials. For all subjects, we observed a significant, positive correlation between the postprandial glycemia during the WB trial and the glycemic response shown after Cr supplementation ($y = -85 + 0.65x$, $r = 0.67$, $P < 0.01$).

Table 1. Capillary and venous glucose area under curve (AUC: mmol · min/L) after test meals consisting of white bread with placebo (WB) and white bread with 400 μg Cr (Cr400) for all subjects, subjects with good glucose control ("good control", WB AUC<median) and subjects with poorer glucose control ("poor control", WB AUC>median).

		WB	Cr400	P-value
All (n=26)	Capillary	137±12	132±9.4	0.72
	Venous	61±8.8	52.±7.1	0.23
Good control (n=13)	Capillary	86±6.9	115±12	0.09
	Venous	32±6.4	39±7.5	0.29
Poor control (n=13)	Capillary	187±11	149±13	**0.007**
	Venous	89±12	64±11	0.07

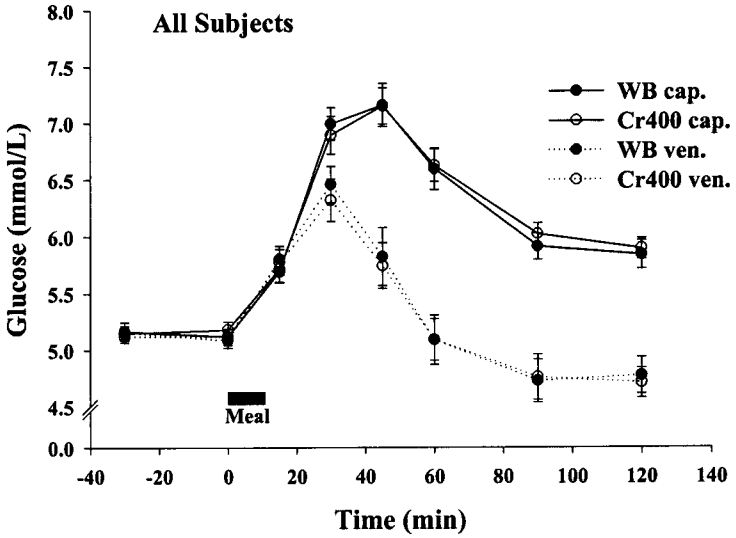

Figure 1. Capillary and venous glucose concentrations after test meals providing 75 g available carbohydrates. Test meals were eaten within 10 min and consisted of white bread with placebo (WB, ●), and white bread with 400 µg Cr (Cr400, □). Values are means for 26 subjects with standard errors of the means shown by vertical bars.

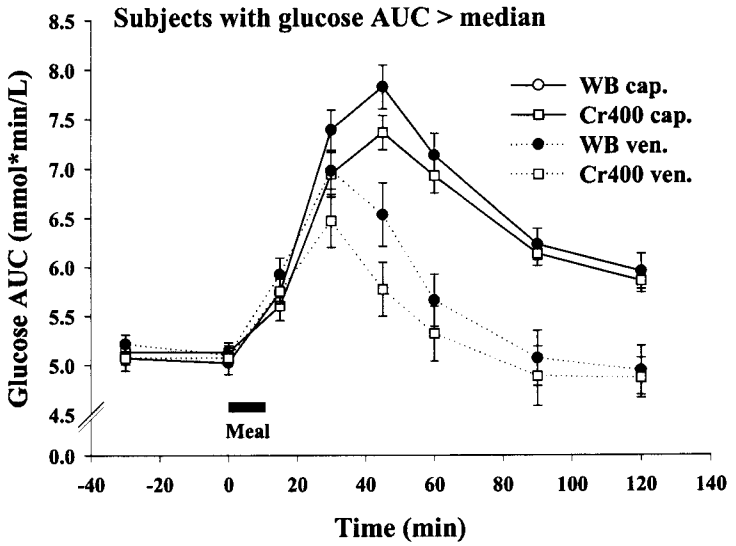

Figure 2. Capillary and venous glucose concentrations after test meals in subjects with poor glucose control. The test meals consisted of white bread with placebo (WB, ●) or with 400 µg Cr (Cr400, □) provided 75 g available carbohydrates, and were eaten within 10 min. Values are means for 13 subjects with standard errors of the means shown by vertical bars.

Insulin

Insulin concentration and AUC were similar for both trials in the whole study population as well as after classification according to the glycemic response (**Fig 3**). Insulin sensitivity was assessed with the quantitative insulin sensitivity check index and was similar for the subjects with good and the ones with poorer glycemic control (0.65 ± 0.02 and 0.63 ± 0.01, respectively, P = 0.40).

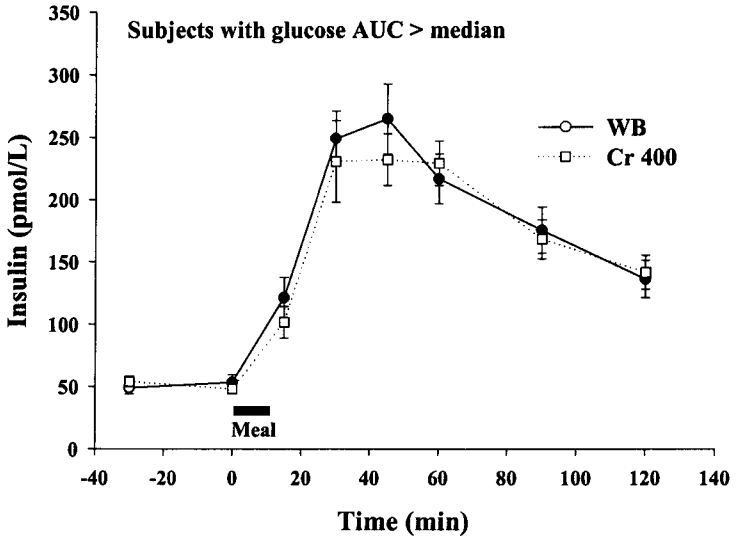

Figure 3. Plasma insulin concentrations after test meals in subjects with poor glucose control. The test meals consisted of white bread with placebo (WB, ●) or with 400 μg Cr (Cr400, □) provided 75 g available carbohydrates, and were eaten within 10 min. Values are means for 13 subjects with standard errors of the means shown by vertical bars.

Physical activity

Eleven of our subjects performed moderate amounts of physical exercise (mean 2.7±0.6 h, endurance training 0-2 h, resistive training 1-5 h) while the other fifteen reported doing no forms of training. A classification into two subgroups according to training status revealed differences in some measured variables (**Table 2**). Postprandial insulinemia (AUC) during the WB trial was 24 % (P = 0.04) lower for the active compared with the sedentary subgroup. There was also a tendency towards smaller glucose AUC (-15 %) for the active individuals during the WB trial. Effects or tendencies of training status were also seen in total and HDL-cholesterol, iron and transferrin concentrations, and transferrin saturation.

Table 2. Comparison of different variables (baseline or during placebo trial) between physically active and sedentary subjects.

	Active (n=11)	Sedentary (n=15)	P-value
Insulin AUC (pmol · min/L)	12130±1240	15946±1170	**0.04**
Glucose AUC (mmol · min/L)	123±22	146±12	0.35
Cholesterol (mmol/L)	4.3±0.25	4.9±0.21	0.08
HDL (mmol/L)	1.14±0.08	1.05±0.07	0.48
LDL (mmol/L)	4.3±0.20	4.9±22	0.09
Iron (μmol/L)	21±2.1	27±2.1	0.06
Ferritin (ng/L)	84±9.2	115±16.5	0.16
Transferrin (g/L)	2.7±0.13	2.2±0.05	**0.001**
Transferrin saturation (%)	32±3.8	51±4.6	**0.008**
BMI (kg/m^2)	24.2±0.51	22.7±0.48	0.06
Body mass (kg)	81±2.9	76±2.0	0.15

Values are means ± s.e., AUC: area under curve.

Diet records

The subjects met the recommended dietary intakes for all analyzed nutrients except folate. No differences in nutrient intake between subgroups were observed after classification according to glycemic response (except for zinc) or activity level.

Iron variables

Variables of iron metabolism (iron, ferritin, transferrin, transferrin saturation) were not different for the individuals with good glucose control compared with those with poorer control. Concentrations were in the normal range for all subjects.

Discussion

In the present study we investigated the effects of an acute Cr supplementation on postprandial glycemic response and metabolism in young men. We found no differences between treatments in the whole study population but observed an effect of Cr on postprandial glucose concentration in those individuals with poorer blood glucose control (glucose AUC > median).

Our subjects were all apparently healthy and their mean capillary glycemic response (measured as AUC) to a 75 g load of available carbohydrates in form of white bread was quite low (137 mmol · min/L) compared with those assessed in other studies. In a previous study we measured a mean capillary glucose AUC of 163 mmol · min/L (8) and with a similar CHO load Wolever also measured higher values in normal subjects (15-17). So, it seems that the subjects in the present study had very good glucose control which could explain why we observed no beneficial effects of Cr supplementation on glycemic response in the whole study population. If we divided our subjects into two subgroups according to the glycemic response shown during the placebo trial, i.e. according to their "white bread tolerance", those above the median (glucose AUC > 135 mmol · min/L, group mean: 187 mmol · min/L) had a significantly reduced glycemia (-20 %) after Cr supplementation. Of this subgroup eleven out of thirteen subjects showed a positive reaction to Cr. The probability of this is 0.009, i.e. there was only a 0.9 % chance to observe this if the responses to Cr and placebo had been equal. So, it is highly likely that Cr supplementation had an effect. On the other hand, we observed a non-significant increase in the glucose AUC after Cr supplementation, probably reflecting the normal variation in glucose tolerance, for the subjects with good glucose control (AUC < median). This is in agreement with the findings of Anderson et al (10), who found an effect of Cr supplementation on glucose response in people with slightly impaired glucose tolerance but not in individuals with normal glucose tolerance.

We found a positive correlation between postprandial glycemia after the WB trial and the glucose response observed after addition of Cr. That is, individuals with "poorer" blood glucose control after the WB meal were more likely to react positively to Cr and showed greater reductions in glycemia after supplementation than those with "better" blood glucose control. This suggests that people with impaired glucose tolerance or insulin resistance may benefit even more from acutely supplemented Cr than individuals with normal glucose tolerance. This could be of particular importance as recent epidemiological studies indicate that postprandial blood glucose may be an independent risk factor for cardiovascular disease and diabetes (18-20). The intake of large quantities of high-glycemic index foods leads to high postprandial hyperglycemia and may

contribute to the risk of developing the above mentioned diseases (1;2). It is known that lowering the dietary glycemic load improves hyperglycemia and dyslipidemia in diabetics (21-23). Furthermore, as recent evidence suggests that a high dietary glycemic load may lead to impaired glucose tolerance and insulin resistance, lowering the glycemic load in the healthy population may carry an important preventive potential to decrease the incidence of these disorders. Thanks to its insulin sensitivity enhancing effect, acute and chronic Cr supplementation could be a promising approach to improve glucose tolerance in people at risk or even in the total population.

In a previous study performed at our laboratory with a similar design and comparable subjects we observed that some but not all individuals responded to Cr (8). The responders and non-responders not only differed in their response to supplemental Cr but also in their nutrient intake and eating pattern as well as in their serum iron concentration. The differences in iron metabolism were particularly interesting as it has been proposed that Cr is transported from mobile pools to insulin sensitive cells with transferrin (12). However, in the present study the response to Cr supplementation was only correlated with the glucose tolerance of the subjects. We could not corroborate the differences in serum iron metabolism and dietary intake. So, the reasons why there are Cr responders and non-responders remain unclear (6). It has been postulated that people eating healthy diets would have adequate Cr intake and status and therefore not respond to additional Cr (9;24). All our subjects consumed diets that met the recommended dietary intakes of most nutrients (see Table 3). Consequently, it may be assumed that they were also getting sufficient amounts of Cr with their diet. The observation that some of the subjects nevertheless responded to Cr seems to be in contrast with the above mentioned studies. Especially, as we used a single-dose supplementation and it may be assumed that a higher percentage of individuals would have responded to longer-term addition of Cr. Anderson also reported that most people with marginally impaired glucose tolerance would respond to Cr supplementation and suggested that the varying response to supplemental Cr is probably due to the diet and dietary Cr intake, selection of subjects, the degree of glucose intolerance and form and amount of supplemental Cr (25).

One of our exclusion criteria for the test subjects was endurance exercise (not more than 2 h weekly). It is known that endurance-trained athletes have better insulin sensitivity than sedentary people (26;27). Apparently, exercise training per se has no independent impact on insulin action and it seems that the improved insulin sensitivity is only a transient effect due to an acute exercise session and that the improved glucose tolerance lasts for less than five days (28;29). As it is quite difficult to find young, non-smoking, healthy men who are not involved in any forms of exercise, we allowed individuals doing resistive training to participate. The influence of resistance training on insulin sensitivity is much less evident than for aerobic exercise (30;31). So, we did not expect

to find any effects of resistive exercise on glucose metabolism. It was interesting to note that the subjects participating in weight training showed better insulin sensitivity than the subjects not reporting any forms of exercise. It has been proposed that improved insulin sensitivity in resistance-trained women was due to increased lean body mass (32). We did not measure lean body mass in our subjects. However, our active individuals had a higher BMI and on average weighed 5 kg more than the sedentary subjects. As most of our physically active subjects were doing resistive training it is likely that this additional mass was mostly lean body tissue. So, this may be the reason for their better insulin response. We also observed that our active individuals tended to have lower cholesterol and LDL values than our sedentary subjects, which would suggest that already moderate amounts of physical exercise may improve important health-related variables.

We conclude that in a normal, apparently healthy population of young men those showing a larger glycemic response after a white bread meal are more likely to respond beneficially to supplemental Cr. It seems that in healthy individuals with poor blood glucose control Cr supplementation may improve glycemic control, and by doing so possibly lessen the impact of an important risk factor in the development of insulin resistance and type II diabetes.

Acknowledgments

This work was supported by grants of Hochstrasser Foundation and the Swiss Foundation for Nutrition Research.

References

1. Salmeron J., Manson J.E., Stampfer M.J., Colditz G., Wing A.L., Willett W.C., Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA*; 277:472-7, 1997.
2. Salmeron J., Ascherio A., Rimm EB. et al, Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care*; 20:545-50, 1997.
3. Hopkins LL.Jr., Ransome-Kuti O., Majaj A.S., Improvement of impaired carbohydrate metabolism by chromium 3 in malnourished infants. *Am J Clin Nutr*; 21:203-11, 1968.
4. Levine R.A., Streeten D.H., Doisy R.J., Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. *Metabolism*; 17:114-25, 1968.
5. Anderson R.A., Polansky M.M., Bryden N.A., Canary J.J., Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutr*; 54:909-16, 1991.
6. Ravina A., Slezak L., Rubal A., Mirsky N., Clinical use of the trace element chromium(III) in the treatment of diabetes mellitus. *J Trace Elem Exp Med*; 8:183-90, 1995.
7. Anderson R.A., Cheng N., Bryden N.A., Polansky M.M., Chi J., Feng J., Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes*; 46:1786-91, 1997.
8. Frauchiger, M.T., Effects of acute Chromium supplementation on postprandial metabolism in young, healthy men. Swiss Federal Institute of Technology, Zurich, 2002.
9. Anderson R.A., Chromium, glucose intolerance and diabetes. *J Am Coll Nutr*; 17:548-55, 1998.
10. Anderson R.A., Polansky M.M., Mertz W., Glinsmann W., Chromium supplementation of human subjects: effects on glucose, insulin, and lipid variables. *Metabolism*; 32:894-9, 1983.
11. Ani M., The effect of chromium on parameters related to iron metabolism. *Biol Trace Elem Res*; 32:57-64, 1992.
12. Vincent J.B., The biochemistry of chromium. *J Nutr*; 130:715-8, 2000.
13. Katz A., Nambi S.S., Mather K., Follmann D.A., Sullivan G., Quon M.J., Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*; 85:2402-10, 2000.
14. Wolever T.M., Jenkins D.J., Jenkins A.L., Josse R.G., The glycemic index: methodology and clinical implications. *Am J Clin Nutr*; 54:846-54, 1991.

15. Wolever T.M., Jenkins D.J., Metabolic response to test meals containing different carbohydrate foods. *Nutr Res*; 8:573-81, 1988.
16. Wolever T.M., Vuksan V., Palmason C., Less variation of postprandial blood glucose after starchy test meals than oral glucose. *Nutr Res*; 16 (6):899-905, 1996.
17. Wolever T.M., Bolognesi C., Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *J Nutr*; 126:2798-806, 1996.
18. Bonora E., Muggeo M., Postprandial blood glucose as a risk factor for cardiovascular disease in Type II diabetes: the epidemiological evidence. *Diabetologia*; 44:2107-14, 2001.
19. Liu S., Manson J.E., Dietary carbohydrates, physical inactivity, obesity, and the 'metabolic syndrome' as predictors of coronary heart disease. *Curr Opin Lipidol*; 12:395-404, 2001.
20. Liu S., Willett W.C., Stampfer M.J. et al, A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr*; 71:1455-61, 2000.
21. Brand-Miller J.C., Importance of glycemic index in diabetes. *Am J Clin Nutr*; 59:747S-52S, 1994.
22. Frost G., Wilding J., Beecham J., Dietary advice based on the glycaemic index improves dietary profile and metabolic control in type 2 diabetic patients. *Diabet Med*; 11:397-401, 1994.
23. Frost G., Leeds A.A., Dore C.J., Madeiros S., Brading S., Dornhorst A., Glycaemic index as a determinant of serum HDL-cholesterol concentration [see comments]. *Lancet*; 353:1045-8, 1999.
24. Offenbacher E.G., Rinko C.J., Pi S.F., The effects of inorganic chromium and brewer's yeast on glucose tolerance, plasma lipids, and plasma chromium in elderly subjects. *Am J Clin Nutr*; 42:454-61, 1985.
25. Anderson R.A., Nutritional factors influencing the glucose/insulin system: chromium. *J Am Coll Nutr*; 16:404-10, 1997.
26. Henriksson J., Influence of exercise on insulin sensitivity. *J Cardiovasc Risk*; 2:303-9, 1995.
27. Stallknecht B., Larsen J.J., Mikines K.J., Simonsen L., Bulow J., Galbo H., Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. *Am J Physiol*; 279:E376-85, 2000.
28. King D., Baldus P.J., Sharp R.L., Kesl L.D., Feltmeyer T.L., Riddle M.S., Time course for exercise-induced alterations in insulin action and glucose tolerance in middle-aged people. *J Appl Physiol*; 78:17-22, 1995.

29. Segal K.R., Edano A., Abalos A. et al, Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol*; 71:2402-11, 1991.
30. Goodyear L.J., Kahn B.B., Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med*; 49:235-61, 1998.
31. Takala T.O., Nuutila P., Knuuti J., Luotolathi M., Yki-Jarvinen H., Insulin action on heart and skeletal muscle glucose uptake in weight lifters and endurance athletes. *Am J Physiol*; 276:E706-11, 1999.
32. Poehlman E.T., Dvorak R.V., DeNino W.F., Brochu M., Ades P.A., Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial. *J Clin Endocrinol Metab*; 85:2463-8, 2000.

Chapter 5

Summarising discussion

The major aim of this thesis was to determine the effects of a short-term Cr supplementation on postprandial metabolism in apparently healthy humans. In addition, we wanted to assess the most beneficial dose and to investigate the metabolic factors in relation to the response to supplemental Cr.

Effects of acute Cr supplementation on postprandial metabolism

Why did we choose to examine postprandial and not fasting metabolism? Assuming a recommended eating pattern of three main meals plus two snacks, a typical Western individual remains in the postprandial state for a major part of the day, which illustrates the importance of metabolism after food intake.

So, the effects of a short-term Cr supplementation on metabolism after a high-glycemic meal were investigated in young, apparently healthy men of normal body mass in studies using a randomized crossover design. In our first study, the intake of 200 μg Cr, given as Cr picolinate, shortly before a high-glycemic meal did not influence postprandial glucose metabolism in our subjects (see Chapter 2). The glycemic response was similar for the placebo and the supplementation trial. This could be due to the fact that Cr intrinsically does not have an acute effect on postprandial metabolism and chronic intake over days, weeks or months is needed to elicit a response. Another explanation would be that Cr has an acute effect but that 200 μg was a too small amount to induce any changes. The latter theory was substantiated in our second study, when postprandial glucose AUC were considerably reduced after 400 and 800 μg supplemental Cr compared with placebo (see Chapter 3). This indicates that 200 μg may indeed be an insufficient dose and that at least 400 μg are needed to show an acute effect on metabolism in healthy, young men.

A smaller rise in insulin concentration, concomitant with a larger reduction in FFA concentration during the Cr trial, suggests that insulin sensitivity may have been increased by the addition of Cr (Chapter 2). In the other studies insulin concentration was apparently not influenced. But the smaller glycemic response after supplementation indicates that insulin action must have been

enhanced and therefore insulin sensitivity improved compared with the placebo trial. These results are in accordance with the hypothesis that Cr potentiates the action of insulin at the cellular level without having any effects on insulin secretion.

Metabolic factors influencing response to supplemental Cr

We observed in all our experiments that individual responses to Cr intake varied and that some but not all subjects reacted to Cr supplementation. This was noted also in studies using chronic Cr supplementation but until now no compelling explanation why people reacted differently has been put forward. The percentage of subjects responding with a reduction in glycemia after Cr intake was higher in the trials with 400 and 800 μg compared with the one with 200 μg. This suggests that the response to Cr may be dose-dependent. It has been proposed that the amount needed to elicit a response is depending on the Cr status of the subjects. That is, individuals with marginal Cr deficiency would be more likely to respond than the ones with adequate Cr intake and stores. Unfortunately, no biomarker of Cr status exists and there is still no simple method available to identify subjects who are Cr depleted and thus would be expected to respond to supplemental Cr.

We found a correlation between the outcome of Cr supplementation and concentration of iron variables in our subjects (Chapter 3). As transferrin is involved in the transport of Cr in the blood, we speculated that transferrin concentration may be an indicator of the reaction to supplemental Cr. But these findings could not be corroborated in the follow-up study (Chapter 4). So, it is still unclear if there are metabolic factors other than Cr status that would predict the response to a Cr load. Another interesting observation, seen in all three studies, was that the extent of the beneficial response to Cr was depending on the glucose tolerance (defined as glycemia after white bread intake) of the subjects. Individuals with poorer glucose tolerance were more likely to respond positively to supplemental Cr and showed a greater reduction in glucose concentration than the subjects with better glucose control. It is known that Cr has a beneficial effect on people with impaired glucose tolerance and type 2 diabetes. Still, it is interesting to note that even in a young, apparently healthy population with normal glucose tolerance those with slightly poorer glucose control are more likely to benefit from Cr supplementation. This suggests that acute effects of Cr would probably be more pronounced in population groups with poorer glucose tolerance, like overweight or elderly people.

Concluding remarks

The studies performed during this thesis have shown that the trace element Cr may have beneficial effects on postprandial glucose metabolism in healthy people. This is possibly significant as recent epidemiological studies have demonstrated that elevated postprandial glucose concentration is a risk factor for cardiovascular disease and type 2 diabetes (see Chapter 3 and 4). Thus, factors reducing the glycemic load in a diet may be important in delaying the onset or even preventing these diseases. Cr may be one of these factors.

Curriculum vitae

Marc Frauchiger was born on the 27th of March 1969 in Uster as a citizen of Wyssachen, Switzerland

Education

1982-88	Gymnasium Typ B ("High School"), Kantonsschule Oerlikon Zurich
1989-94	Study of Lebensmittel-Ingenieur (Food Science), Swiss Federal Institute of Technology Zurich
1995-96	Study of Human Nutrition, King's College, University of London
1999-02	Doctoral Thesis at the Swiss Federal Institute of Technology Zurich, Institute of Animal Sciences, Research Group of Nutrition Biology

Degrees

1988	Matura Typ B ("High School")
1994	Diploma Lebensmittel-Ingenieur ETH (Engineer in Food Science), Swiss Federal Institute of Technology Zurich
1996	MSc in Nutrition, King's College, University of London

Professional Experience

1990-94	9 months of practical training in the food industry (dairy plant, bakery)
1994-95	6 months traineeship at Nestle R&D centre (Vitoreco, Kempttal, Switzerland)
1997-99	Scientific employee in quality management at COOP (Basel, Switzerland)

Research

1994	Student research assistant at the Institute of Animal Sciences, Research Group of Nutrition Biology, Swiss Federal Institute of Technology Zurich, Switzerland. *Carbohydrates and lipids metabolism in physically active and sedentary young men.*

1996	Student research assistant at the Institute of Human Nutrition, King's College, University of London, Great Britain. *Influence of process technology on flavour components in Soy milk.*
1999-02	Research assistant at the Institute of Animal Sciences, Research Group of Nutrition Biology, Swiss Federal Institute of Technology Zurich, Switzerland. *Chromium and postprandial metabolism in young, healthy men.*

Professional Society Membership

Since 1997	Swiss Society for Food Science and Technology
Since 1999	Swiss Nutrition Society
Since 1999	Swiss Society of Nutrition Research

Teaching

1999 to now	Supervision of semester and diploma theses
1999 to now	Occasional lectures in Nutrition with Prof. Dr. C. Wenk
2000	Lectures in Sports Nutrition at the Agricultural school in Zollikofen

Other

1998/99	Twice finisher at Ironman Hawaii Triathlon
2001	Member of a scientific mountaineering expedition to –and successful climber of- Shisha Pangma (Tibet, 8046mts)