DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2008; **24**: 41–51.
Published online 16 May 2007 in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/dmrr.755

RESEARCH ARTICLE

Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled overweight to obese patients with type 2 diabetes

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Received: 8 November 2006
Revised: 28 February 2007
Accepted: 1 April 2007

Abstract

Background Chromium and biotin play essential roles in regulating carbohydrate metabolism. This randomized, double-blind, placebo-controlled study evaluated the efficacy and safety of the combination of chromium picolinate and biotin on glycaemic control.

Methods Four hundred and forty-seven subjects with poorly controlled type 2 diabetes ($HbA_{1c} \geq 7.0\%$) were enrolled and received either chromium picolinate (600 μg Cr^{+3}) with biotin (2 mg), or matching placebo, for 90 days in combination with stable oral anti-diabetic agents (OADs). Major endpoints were reductions in HbA_{1c}, fasting glucose, and lipids. Safety and tolerability were assessed.

Results Change in HbA_{1c} was significantly different between treatment groups ($p = 0.03$). HbA_{1c} in the chromium picolinate/biotin group decreased 0.54%. The decrease in HbA_{1c} was most pronounced in chromium picolinate/biotin subjects whose baseline $HbA_{1c} \geq 10\%$, and highly significant when compared with placebo (−1.76% *vs* −0.68%; $p = 0.005$). Fasting glucose levels were reduced in the entire chromium picolinate/biotin group *versus* placebo (−9.8 mg/dL *vs* 0.7 mg/dL; $p = 0.02$). Reductions in fasting glucose were also most marked in those subjects whose baseline $HbA_{1c} \geq 10.0\%$, and significant when compared to placebo (−35.8 mg/dL *vs.* 16.2 mg/dL; $p = 0.01$). Treatment was well tolerated with no adverse effects dissimilar from placebo.

Conclusions These results suggest that the chromium picolinate/biotin combination, administered as an adjuvant to current prescription anti-diabetic medication, can improve glycaemic control in overweight to obese individuals with type 2 diabetes; especially those patients with poor glycaemic control on oral therapy. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords biotin; chromium; picolinate; diabetes; glucose; hemoglobin A_{1C}

Introduction

The prevalence of type 2 diabetes (type 2 DM) is increasing in the United States and worldwide [1]. Insulin resistance, a major causative factor for the early development of type 2 DM and cardiovascular disease (CVD), is

even more widespread [2–4]. In addition, there is an increasing prevalence of adult and childhood obesity that markedly contributes to the development of type 2 DM [5–7]. Although pharmacological options for the management of insulin resistance and type 2 DM in obese individuals have been increasing [8,9], not all patients have benefited, as the cost and adverse effects of new pharmacologic agents preclude their use in many patients [10,11]. Though a majority of diabetic patients are being treated, many patients are unable to achieve the currently recommended goal of $HbA_{1c} < 7\%$, especially those who are obese. Obese patients are likely to be the most insulin resistant and, therefore, the most difficult to control with currently available standard therapies. Thus, there is a need to identify and evaluate adjunctive therapies that are safe, efficacious, and cost-effective [10]. One adjunctive therapy commonly used by patients to manage their type 2 DM is chromium, alone or in combination with biotin.

Chromium is an essential trace mineral required for carbohydrate and lipid metabolism [12–14]. The link between chromium and carbohydrate metabolism was proposed more than 40 years ago, when it was identified as a component of the biologically active 'glucose tolerance factor' [15]. There is a growing body of evidence from both animal [16–19] and human studies [12,20], suggesting that dietary supplementation with trivalent chromium, especially in the form of chromium picolinate, is a safe [12,21–24] and effective adjunctive therapy in the management of insulin resistance and type 2 DM.

At present, chromium is widely used as a dietary supplement in individuals with type 2 DM [12,24]. Most, but not all, studies report beneficial effects of chromium on glycaemic control, lipid metabolism, and insulin sensitivity [12,25–27]. Differences in study design, subjects evaluated, dose administered, statistical power, and the forms of chromium evaluated may explain the difference in outcomes. A review of the literature reveals that the form and dose of chromium studied may predict treatment efficacy [12,24,28,29]. For instance, virtually all trials studying chromium, using the chromium picolinate form, in subjects with type 2 DM demonstrate a benefit on glycaemic control [12,20,24,30–32]. Recent reports suggest that chromium picolinate is more completely absorbed and has increased bioavailability compared to other forms of chromium, which helps explain the findings of the consistent beneficial effect [12,24,33].

Biotin, a water-soluble B vitamin, plays an essential role in carbohydrate and lipid metabolism [34]. Besides its role as a carboxylase prosthetic group, biotin regulates the expression of genes important for metabolism. Biotin has stimulatory effects on genes whose actions favour glycaemic control, including pancreatic and hepatic glucokinases. It also suppresses the expression of hepatic phosphoenolpyruvate carboxykinase, a key gluconeogenic enzyme [34,35]. Biotin administration to diabetic rodents has been reported to improve glycaemic control [36–38]. A recent report indicated that biotin supplementation alone reduced plasma triacylglycerol and very low density lipoprotein -cholesterol in subjects with type 2 DM [39]. There is evidence that patients with type 2 DM have reduced serum concentrations of biotin and that 30-day supplementation with biotin improves fasting glucose [40].

In cultured human skeletal muscle cells, the combination of chromium picolinate and biotin significantly enhanced glycogen synthesis and glycogen synthase mRNA to a greater extent than chromium picolinate or biotin alone. (Wang, Z.Q, *et al*. Chromium picolinate and biotin enhance glycogen synthesis and glycogen synthase gene expression in human skeletal muscle culture. Presented at 17th International Diabetes Federation Congress November 9, 2000. Mexico City, Mexico.) Pre-clinical data suggest that biotin co-administration may enhance chromium picolinate absorption and raise chromium tissue levels in obese insulin-resistant rodents, as well as decrease plasma glucose and plasma lipids to a greater extent than chromium alone (Sahin, K, *et al*. Effect of chromium picolinate/biotin on carbohydrate and lipid metabolism in a rat model of type 2 diabetes. *Diabetes* 2006; **55** (Suppl 1):A387.). Thus, the administration of chromium picolinate formulated with biotin warrants evaluation as a useful adjunctive treatment for patients with type 2 DM [35].

A recently reported 30-day pilot study [41] concluded that the combination of chromium picolinate and biotin improved short-term glycaemic control (oral glucose tolerance, fasting glucose, and fructosamine), and had a favourable effect on lipid parameters in subjects with type 2 DM. The study was a placebo-controlled 30-day intervention in obese to overweight subjects with poorly controlled type 2 DM, who were already receiving oral anti-diabetic medications (OADs). Treatment with chromium picolinate/biotin (600 μg Cr and 2 mg biotin) once daily was well tolerated, and without any adverse event profile dissimilar to placebo. A significant decrease (~6%) in plasma fructosamine was observed in the active group compared to placebo. In subjects receiving chromium picolinate/biotin, the glucose excursion following an oral glucose tolerance test was significantly decreased by approximately 10%. The triglycerides/high density lipoprotein (HDL)-cholesterol ratio, a proposed metabolic marker of insulin resistance [42,43], was significantly decreased in the chromium picolinate/biotin group. These encouraging results provided the rationale for conducting this 90-day trial to evaluate the effects of chromium picolinate/biotin on glycaemic control.

Materials and methods

This randomized, double-blind, placebo-controlled study was conducted at 17 geographically diverse sites in the United States. The objective of this 90-day study was to determine whether the combination of chromium picolinate and biotin, as an adjunct to a stable regimen

DOI: 10.1002/dmrr

of OADs, improves glycaemic control and blood lipids in subjects with poorly controlled type 2 DM. In addition, safety and tolerability were assessed. The study was designed to reflect an actual clinical practice setting; therefore, the length of type 2 DM diagnosis was set at a 1-year minimum with no limit to duration defined, and the type, class, or duration of OAD therapy was not controlled for at entrance.

Obese to overweight men and women between the ages of 18 and 70 years, with a documented diagnosis of type 2 DM (according to American Diabetes Association criteria) $\geq$12 months, who were poorly controlled ($HbA_{1c} \geq 7.0\%$) on OAD therapy were eligible. A list of the major concurrent medications is provided in Table 1. The inclusion criteria included the following: (1) $HbA_{1c} \geq 7.0\%$, (2) diagnosis of type 2 DM $\geq$12 months, (3) body mass index (BMI) $\geq$25 kg/m^2 and $<$35 kg/m^2, (4) currently taking OADs (stable for $\geq$60 days prior to entry), and (5) fasting triglycerides $<$400 mg/dL.

The exclusion criteria were as follows: (1) diagnosis of type I diabetes, (2) hypoglycemic event requiring emergency transport $\leq$12 months, (3) supplementation with chromium picolinate within 90 days and/or any form of chromium $\geq$120 μg/d within 30 days, (4) daily insulin usage or rescue insulin usage $>$1/week, (5) diabetic ketoacidosis $\leq$12 months, (6) creatinine $\geq$2.0 $\times$ upper limit of normal (ULN); aspartate aminotransferase or alanine transaminase $\geq$2.0 $\times$ ULN; total bilirubin $\geq$1.5 $\times$ ULN, (7) cardiovascular conditions requiring hospitalization $\leq$12 months, (8) history of cerebrovascular accident, pulmonary embolism, or an unresolved deep vein thrombosis, (9) uncontrolled high blood pressure (seated: systolic $\geq$160 mmHg or diastolic $\geq$90 mm Hg), (10) serious immunosuppressive disorder or current immunosuppressive therapy, (11) hepatic disease, impaired thyroid, impaired renal function, or diseases known to affect glucose or lipid metabolism, (12) alcoholism or substance abuse, (13) mental health issues that would prevent the subject from completing the study, and (14) women who were pregnant or nursing.

Table 1. Baseline concurrent medications

Concomitant medication	Placebo (n = 122) %	Chromium/biotin (n = 226) %
Anti-diabetic medications[a,b]		
Biguanide	97 (80.2)	174 (76.7)
Sulfonylurea	79 (65.3)	160 (70.5)
Thiazolidinediones	39 (32.2)	53 (23.3)
Non-sulfonylurea secretagogue	2 (1.7)	9 (4.0)
α-Glucosidase inhibitor	0	3 (1.2)
Rescue insulin (freq $\leq$1 week)	4 (3.3)	11 (4.8)
Other Rx medications (top 20)[b]		
Aspirin	37 (24.3)	60 (20.3)
Lisinopril	16 (10.5)	44 (14.9)
Lipitor	21 (13.8)	36 (12.2)
Atenolol	5 (3.3)	15 (5.1)
Zocor	8 (5.3)	14 (4.7)
Lovastatin	5 (3.3)	13 (4.4)
Norvasc	6 (3.9)	11 (3.7)
Ibuprofen	3 (2.0)	10 (3.4)
Altace	9 (5.9)	8 (2.7)
Captopril	6 (3.9)	8 (2.7)
Prevacid	4 (2.6)	8 (2.7)
Avapro	0 (0.0)	7 (2.4)
Celebrex	4 (2.6)	7 (2.4)
Crestor	1 (0.7)	'7 (2.4)
Diovan	1 (0.7)	7 (2.4)
HCTZ	5 (3.3)	7 (2.4)
Pravacol	5 (3.3)	7 (2.4)
Zetia	3 (2.0)	7 (2.4)
Bextra	3 (2.0)	7 (2.4)
Flomax	5 (3.3)	6 (2.0)

[a]Study subjects enrolled were concomitantly using single, dual, and polytherapy. There were 105 active and 64 placebo subjects on dual or polytherapy. The most common combinations were, respectively, sulfonylurea plus biguanide, TZD plus sulfonylurea, and TZD plus biguanide.
[b]Subjects' concomitant OADs were not dissimilar between groups at study entrance (p = 0.85; Student's t-test).

The study protocol was approved by a central Institutional Review Board [New England Institutional Review Board (IRB), Wellesley, MA]. Subjects were recruited from the Principal Investigators' database, referrals from area physicians, and through advertisements. All consent forms, advertisements, flyers, and posters were IRB approved prior to use. Prior to enrollment, all subjects were informed of the purpose and risks of the study and gave voluntary written consent to participate. The study was conducted in accordance with all federal, state, and local requirements and in compliance with Good Clinical Practice/International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.

Patient contacts during the study included a prescreening phone contact, a Day-0 baseline visit, two mid-study phone contacts, and a Day-90 final visit. At the baseline visit, fasting blood and urine samples were collected to determine: HbA_{1c}, blood glucose, serum insulin, serum lipid profile, blood chemistries, urinalysis (*via* dipstick), and a urine pregnancy test on women of childbearing potential. Subjects received a physical examination including vital signs and measurements of height and weight. Qualified subjects continued on their existing medications and were randomized blindly (2 : 1 ratio) to receive either chromium picolinate (600 μg Cr) + biotin (2 mg) (Diachrome, supplied by Nutrition 21, Inc., Purchase, NY), or placebo, taken once daily prior to the morning meal as an adjunct to their stable OAD regimen. Treatment continued for 90 days. Subjects were instructed not to change their diet or level of physical activity. The office visit procedures outlined in the preceding text were repeated during the final visit.

To facilitate compliance with study protocol, a central call centre contacted the subjects twice (at Days 30 and 60) *via* telephone to reinforce subject-dosing compliance by reminding the subjects to take their treatment daily, along with their other prescription medications. The subjects were also reminded to perform all diary-related tasks (including recording date and time of each dose) and to inform the study coordinator in case they experienced an adverse event. The call also allowed the subjects an opportunity to ask study-related questions. To assess compliance, subjects were instructed to return at their final visit with all unused capsules and bottles. The

DOI: 10.1002/dmrr

subjects' bottles were checked for capsule count at Day 90 (final visit) to calculate percent dosing compliance and their diaries' were checked for completeness of dosing entries as an additional verification of dosing compliance. Owing to logistical complexities, the subjects' urine or blood samples were not screened for supplement metabolites. Concomitant medication usage was also assessed at the final visit and compared to baseline to ensure that there were no medication changes.

Randomization

Subjects were randomized in a 2 : 1 ratio of active treatment to placebo. The randomization schedule was developed using standardized computer software with block sizes of six subjects per unit. All subjects' study medication kits were pre-randomized by the provider of the test product thereby assuring that all study site personnel were blinded throughout the trial. Each site was allocated study treatment kits by complete randomization blocks. The randomization and blinding codes were archived at the manufacturer's facility by unblinded personnel who were unaffiliated with the study.

Assays

Laboratory analyses (HbA_{1c}, glucose, insulin, lipids, chemistry panel, etc.) of fasting blood samples obtained at the baseline and final visits were performed by Physician's Reference Lab (Overland Park, KS; www.prlnet.com); these data were used for all statistical analyses. The observed coefficient of variation for HbA_{1c} analyses was 1.7% of the value reported.

Safety and tolerability

Safety parameters included a physical examination, vital signs, and laboratory evaluation before entering the study. Physical examinations and laboratory tests were repeated at the final visit. Subjects were monitored on a regular basis for adverse experiences. Subjects were contacted at Days 30 and 60 by the call centre and reminded to report all adverse events and serious adverse events to the study coordinator. Patients were canvassed verbally at their final visit as to whether they had experienced an adverse event(s). The results of the clinical laboratory assessments, vital signs, and detailed adverse event profile are reported elsewhere.

Statistical analyses

The primary and secondary endpoints of this 90-day study were the reduction in HbA_{1c} and fasting glucose, compared to placebo, respectively. HbA_{1c} provides an index of long-term glycaemic control and fasting plasma glucose is an index of acute, short-term glycaemic control.

HbA_{1c} is the biomarker that is recommended by the American Diabetes Association as a measure of overall glycaemic control. The intent to treat (ITT) population was defined as any subject who took at least one dose of study product and who had at least one post-randomization HbA_{1c} assessment. The modified intent to treat (MITT) population included all ITT subjects, regardless of the length of study participation or dosing compliance, who were without significant protocol entrance violations. Data analyses as stated were planned for the overall MITT group ($n = 348$), and for a subset of subjects whose baseline $HbA_{1c} \geq 10.0\%$ ($n = 55$). For all applicable efficacy outcome measures, a Student's *t*-test was used where appropriate. For analysis purposes, the null hypothesis was defined as no overall treatment effect when compared to placebo; statistical significance was accepted at $p \leq 0.05$. All values were expressed as the mean $\pm$ SE, unless otherwise indicated. Chi-square tests were conducted for all categorical variables.

An analysis of covariance (ANCOVA) was also used when evaluating change in HbA_{1c} from baseline to control for the potential effects of other variables in the overall MITT analysis set. Results from previous intervention studies [44] and guidelines recently published for evaluating HbA_{1c} data for diabetic interventions [45] suggest using an ANCOVA model to assess baseline HbA_{1c} as a covariate, since baseline glycaemic control appears to modulate treatment outcome [46]. Briefly, the ANCOVA model was conducted by regression of the response variable *versus* the covariate separately for each treatment group. The slopes and intercepts of these two models were compared statistically, with the difference in intercepts being interpreted as the treatment difference, and the difference in slopes being interpreted as the difference in the amount of effect the covariate exerted on the response.

The group sample size of subjects included in the final data analyses ($n = 226$ for the chromium/biotin group and $n = 122$ for placebo) had 30% power to detect a 0.2% mean difference, 80% power to detect a 0.4% mean difference, and 99% power to detect a 0.6% mean difference in HbA_{1c} change from baseline (treatment *vs* placebo; $p \leq 0.05$; two-tailed). Data analyses were performed using SAS(R) Version 8.2.

Results

The CONSORT flow diagram shows the progress of subjects through the study (Figure 1). A total of 447 subjects were enrolled in the study (295 active; 152 placebo). Seventy-eight subjects did not return for further assessments, and were therefore dropouts from the ITT population and excluded from the data analysis. There was no significant difference in the attrition rates between the treatment and placebo groups. Further review of the ITT group identified 21 subjects who had one or more significant inclusion/exclusion violations; these

DOI: 10.1002/dmrr

subjects were excluded from the data analyses. The study entrance violations occurred due to human error during the screening and enrollment process; subject entrance violations discovered during the course of the trial were allowed to continue unless there was a medical or ethical reason to discontinue the patient. Of the 21 subjects excluded, 7 had histories of significant CVD, coronary heart disease, or hypertension, 4 subjects had significantly abnormal lab values, 3 subjects failed BMI requirements, 2 subjects failed HbA_{1c} requirements, 2 subjects' OADs were not stable for $\geq$60 days, 2 subjects were >70 years old, and 1 subject's diagnosis of type 2 DM was $\leq$1 year. There was no significant difference in the attrition rates between the treatment and placebo groups. The remaining 348 subjects were used for the final data analyses (MITT; *n*: active 226; placebo 122).

Demographic data for the MITT population including age, gender, ethnicity, weight, height, body mass index, and blood pressure are shown in Table 2. Approximately 50% of the subjects were White, 30% were Hispanic, and 10% were Black; there were no between-group ethnic distribution differences noted. The groups were similar and without significant differences in age, sex, weight, height, BMI, blood pressure, or glycaemic control (Tables 2 and 3).

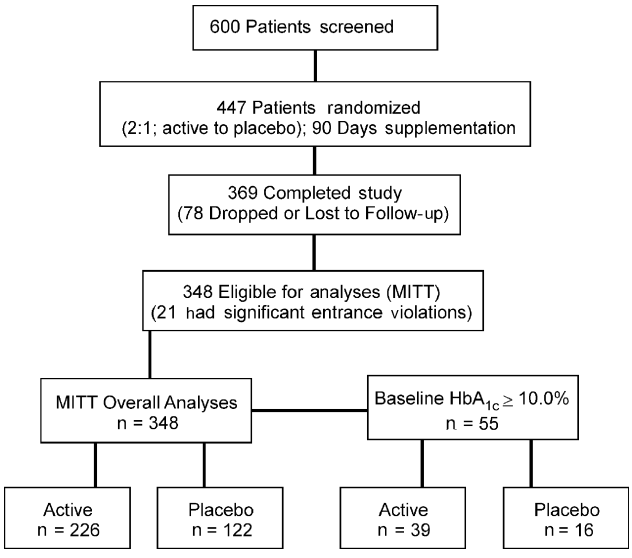

Figure 1. CONSORT study subject flow diagram. (See text for details)

Safety and tolerability

Treatment with chromium picolinate/biotin for 90 days was well tolerated. The adverse effects and clinical safety profile for the active group was not significantly different from placebo. There were no changes in blood pressure or blood chemistries, and no weight gain or sexual dysfunction was noted (Table 4). There was no evidence of fasting or episodic hypoglycemia in either treatment group.

Glycemic control

Chromium picolinate/biotin treatment for 90 days produced modest but significant improvements in **glycaemic** control compared to placebo, as judged by a reduction in both fasting glucose and HbA_{1c}. At baseline, HbA_{1c} in the active group was $8.73 \pm 0.09\%$ (mean $\pm$ SEM). After 90 days of treatment, HbA_{1c} decreased to $8.19 \pm 0.09\%$; an absolute decrease of 0.54%. In the placebo group, HbA_{1c} decreased by 0.34%. The difference between the two groups was significant. ($p = 0.03$ *vs* placebo; Table 3).

At baseline, the mean fasting glucose level in the chromium picolinate/biotin group was 169.7 ± 3.1 mg/dL (mean $\pm$ SEM); declining after 90 days of treatment to 159.9 ± 3.1 mg/dL, a decrease of 9.8 mg/dL, or approximately 6%. In contrast, subjects on placebo experienced an increase of 0.7 mg/dL in glucose levels.

Table 2. Subject baseline demographics

	MITT			$HbA_{1c} \geq 10\%$		
	Placebo	Chromium/Biotin	*p*	Placebo	Chromium/Biotin	*p*
Subjects	$n = 122$	$n = 226$		$n = 16$	$n = 39$	
Age (years)	59.6 ± 0.8	57.6 ± 0.7	0.06	59.4 ± 1.9	55.4 ± 1.1	0.06
Gender	(65% = M)	(56% = M)	0.08	(75% = M)	(51% = M)	0.01
Ethnicity (%)	–	–	0.38	–	–	0.38
White	57.0%	52.4%	–	31.3%	48.7%	–
Hispanic	30.6%	28.6%	–	50%	25.6%	–
Black	6.6%	11.5%	–	12.5%	20.5%	–
Asian	3.3%	6.2%	–	0%	5%	–
Other	2.5%	1.3%	–	6.3%	0%	–
Weight (kg)	89.6 ± 1.3	88.5 ± 1.0	0.25	91.9 ± 3.9	85.6 ± 1.9	0.09
Height (cm)	171.5 ± 0.9	170.0 ± 0.7	0.10	172.7 ± 3.1	167.6 ± 1.5	0.08
Body mass index (kg/m^2)	30.4 ± 0.3	30.3 ± 0.2	0.41	30.6 ± 0.7	30.5 ± 0.5	0.45
Blood pressure (mm Hg)	–	–	–	–	–	–
Systolic	131.9 ± 1.2	129.7 ± 0.9	0.07	136.3 ± 3.9	127.4 ± 2.6	0.04
Diastolic	78.7 ± 0.9	78.8 ± 0.6	0.53	80.9 ± 2.5	78.7 ± 1.6	0.22

Data are means $\pm$ SEM and were analyzed by the Student's *t*-test.
n = number of subjects.

DOI: 10.1002/dmrr

Table 3. Effect of chromium/biotin on glycaemic control

Outcome variable	Placebo ($n = 122$)	Chromium/biotin ($n = 226$)	*p versus* placebo
HbA_{1c} (%)			
Baseline	8.46 ± 0.12	8.73 ± 0.09	–
Final	8.12 ± 0.12	8.19 ± 0.09	–
Change	-0.34 ± 0.15	-0.54 ± 0.15	–
p	0.0001	0.0001	0.03
Fasting Glucose (mg/dL)			
Baseline	171.7 ± 4.5	169.7 ± 3.1	–
Final	172.3 ± 5.2	159.9 ± 3.1	–
Change	0.7 ± 5.9	-9.8 ± 8.5	–
p	0.84	0.002	0.02
Fasting insulin (μU/mL)			
Baseline	14.8 ± 1.4	13.5 ± 0.6	–
Final	13.5 ± 1.0	14.0 ± 0.7	–
Change	-1.5 ± 1.4	0.5 ± 0.5	–
p	0.29	0.25	0.90
	Subjects with baseline $HbA_{1c} \geq 10.0\%$		
Outcome variable	Placebo ($n = 16$)	Chromium/biotin ($n = 39$)	*p versus* placebo
Baseline $HbA_{1c} \geq 10.0\%$			
Baseline	11.14 ± 0.28	11.08 ± 0.16	–
Final	10.46 ± 0.46	9.32 ± 0.27	–
Change	-0.68 ± 0.30	-1.76 ± 0.23	–
p	0.006	0.0001	0.005
Fasting glucose (mg/dL)			
Baseline	230.3 ± 13.8	222.0 ± 9.0	–
Final	246.5 ± 22.8	186.2 ± 9.7	–
Change	16.2 ± 18.4	-35.8 ± 9.1	–
p	0.63	0.017	0.01
Fasting insulin (μU/mL)			
Baseline	12.6 ± 1.5	11.1 ± 1.1	–
Final	11.6 ± 1.3	12.3 ± 1.3	–
Change	-0.49 ± 1.5	1.4 ± 0.8	–
p	0.51	0.17	0.23

Data are means ± SEM and were analysed by Student's *t*-test.
n = the number of subjects.

This difference in response between the two groups was significant ($p = 0.02$ *vs* placebo; Table 3). No difference between groups in fasting insulin was observed (Table 3).

As the study was designed not only to evaluate efficacy but also to identify those subjects who responded the most to chromium picolinate/biotin, an analysis was conducted on those subjects whose HbA_{1c} was ≥10.0% at baseline ($n = 55$; active 39; placebo 16). Reductions in HbA_{1c} and fasting glucose were significantly greater in subjects receiving chromium picolinate/biotin in this set of subjects than were seen in the overall study population. In subjects whose baseline $HbA_{1c} \geq 10\%$, the final HbA_{1c} fell $-1.8 \pm 0.2\%$ in those receiving chromium picolinate/biotin compared to $-0.7 \pm 0.3\%$ in those receiving placebo ($p = 0.005$ *vs* placebo; Table 3). Fasting glucose also fell significantly more in this set of patients in the chromium picolinate/biotin group compared to placebo (-35.8 ± 9.1 mg/dL *vs* 16.2 ± 18.4 mg/dL; $p = 0.01$).

An ANCOVA was performed on the HbA_{1c} change from baseline data using the baseline HbA_{1c} data as the covariate. The fitted regression equations were as follows: *change from baseline in* $HbA_{1c} = 2.99 - 0.40$ *(baseline* HbA_{1c}*)* for the chromium picolinate/biotin group, and *change from baseline in* $HbA_{1c} = 1.34 - 0.20$ *(baseline* HbA_{1c}*)* for the placebo group (Figure 2). The slope coefficients were significantly different from zero ($p = 0.002$), and significantly different from each other ($p = 0.008$) as were the *y*-intercept coefficients ($p = 0.01$). The differences in the slope coefficients indicate

Table 4. Prevalence of adverse events

Parameter	Placebo $n = 122$ %	Chromium/biotin $n = 226$ %	*p versus* placebo
Any AE[a]	42 (34.7)	78 (34.4)	0.95
Any SAE[b]	5 (4.1)	2 (0.9)	0.04
Potentially related AE	8 (6.6)	27 (11.9)	0.12
AE leading to drop-out	4 (3.3)	4 (1.8)	0.36
AE potentially related			
Nervous system	1 (0.8)	10 (4.4)	0.07
Gastrointestinal	4 (3.3)	5 (2.2)	0.54
Skin and tissue	2 (1.8)	4 (1.7)	0.94
Musculoskeletal	0 (0.0)	3 (1.3)	0.20
General medicine	0 (0.0)	3 (1.3)	0.20
Metabolism	0 (0.0)	2 (0.9)	0.30
Immune	1 (0.8)	0 (0.0)	0.17
Hypoglycaemia	0 (0.0)	0 (0.0)	NA

[a]AE, adverse event.
[b]SAE, serious adverse event.
n = number of subjects.

DOI: 10.1002/dmrr

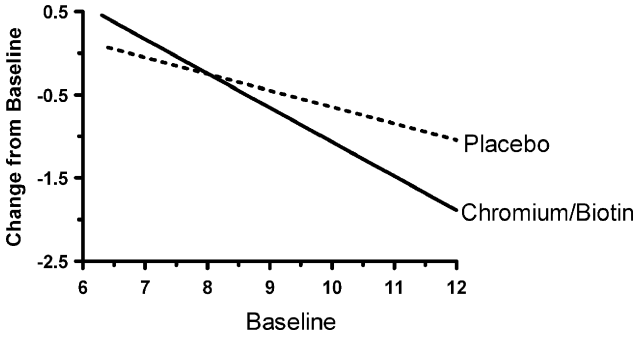

Figure 2. HbA_{1c} ANCOVA regression model for treatment and placebo groups. The fitted regression equations were as follows: *change from baseline in $HbA_{1c} = 2.99 - 0.40$ (baseline HbA_{1c})* for the chromium picolinate/biotin group, and *change from baseline in $HbA_{1c} = 1.34 - 0.20$ (baseline HbA_{1c})* for the placebo group

that, for the chromium picolinate/biotin group, for every unit increase in baseline HbA_{1c} there was a 0.4-unit decrease in the change from baseline HbA_{1c}.

A review of covariates by demographic variables revealed that being Black was predictive of modestly better reductions in HbA_{1c} ($p = 0.03$). An analysis of covariance using other baseline subject characteristics, such as demography, weight, BMI, gender and age, did not reveal any modulation of treatment effect on HbA_{1c} or fasting glucose.

Anti-diabetic medications

Concomitant OAD usage was similar between treatment groups at baseline, as were other prescription medications including statins and antihypertensive agents ($p = 0.85$). A preliminary comparison of the baseline OADs *versus* treatment outcomes revealed that chromium picolinate/biotin worked well with all subjects' OAD medications. However, it is interesting to note that a subset analysis of metformin users demonstrated modestly better treatment outcomes than the overall MITT results. A total of 91 subjects met the criteria of taking only a biguanide (*n*: active 56; placebo 35); HbA_{1c} was significantly reduced in the active group by $0.64\% \pm 0.15$, while placebo was reduced by only $0.24\% \pm 0.12$ ($p = 0.02$ *vs* placebo). No additional differences or trends were noted when performing similar analyses for other OADs. Overall, the combination of chromium picolinate and biotin worked well adjunctively with all concurrent OADs.

Lipids

There was no significant difference in absolute values of total cholesterol, low density lipoprotein cholesterol, HDL cholesterol, and triglyceride levels between groups in the MITT population (Table 5) or the subjects whose baseline HbA_{1c} was greater than 10%. A reduction in lipids or lipid ratios would not be expected in this sample as only a subset of subjects who entered the study was hypercholesterolemic. A more comprehensive analysis of lipid data from subjects with elevated cholesterol levels at baseline (>200 mg/dL) showed significant improvements in lipids and lipid ratio results, as well as markers of CVD disease risk, in the chromium picolinate/biotin group; data are reported elsewhere (Juturu *et al*. 2007 *J Cardiometabolic Syndrome.* In press). No change in body mass index was observed in either group.

However, the triglycerides/HDL ratio was significantly decreased in the chromium picolinate/biotin group. The mean change in this ratio after treatment was -0.13 ± 0.25 in the chromium picolinate/biotin group and 0.52 ± 0.30 in the placebo group ($p = 0.05$ *versus* placebo). A review of covariates by demographic variables revealed that being Hispanic was predictive of modestly better reductions in the triglycerides/HDL ratio ($p = 0.02$). There were no other observations of demographic predictors of treatment success for lipid parameters.

Table 5. Effect of chromium/biotin on lipids and lipid ratios

Outcome variable	Placebo ($n = 122$)	Chromium/biotin ($n = 226$)	*p versus* placebo
Total cholesterol (mg/dL)			
Baseline	197.8 ± 4.4	193.5 ± 2.95	
Final	195.8 ± 5.0	187.6 ± 4.2	
Change	-1.99 ± 2.9	-5.96 ± 2.3	0.14
p	0.45	0.01	
HDL-chol (mg/dL)			
Baseline	46.2 ± 1.0	44.8 ± 0.7	
Final	46.3 ± 1.1	44.97 ± 0.66	
Change	0.12 ± 0.57	0.16 ± 0.46	0.48
p	0.71	0.86	
LDL-chol (mg/dL)			
Baseline	106.5 ± 2.7	110.8 ± 2.8	
Final	103.2 ± 3.0	104.4 ± 2.7	
Change	-4.4 ± 2.0	-5.4 ± 2.8	0.39
p	0.02	0.05	
VLDL-chol (mg/dL)			
Baseline	44.2 ± 2.8	41.6 ± 2.0	
Final	47.9 ± 3.6	42.1 ± 2.7	
Change	3.7 ± 2.2	0.5 ± 2.3	0.15
p	0.90	0.18	
TG (mg/dL)			
Baseline	220.7 ± 13.9	207.9 ± 10.2	
Final	239.4 ± 18.2	210.6 ± 13.5	
Change	18.7 ± 10.8	2.7 ± 11.4	0.15
p	0.09	0.81	
TG/HDL-chol Ratio			
Baseline	5.2 ± 0.4	5.05 ± 0.3	
Final	5.7 ± 0.05	4.9 ± 0.3	
Change	0.52 ± 0.3	-0.13 ± 0.25	0.05
p	0.09	0.63	

Data are means $\pm$ SEM and were analysed by the Student's *t*-test.
n = the number of subjects.

Discussion

This 90-day study was designed to determine whether the combination of chromium picolinate and biotin, as an adjunct to a stable regimen of OADs, reduced HbA_{1c} and fasting glucose in subjects with poorly controlled type 2

DOI: 10.1002/dmrr

DM. In addition, safety and tolerability were assessed. This study was designed to reflect an actual clinical practice setting, therefore baseline class, type, and duration of OAD were not controlled for and only two study visits, 90 days apart, were conducted.

Chromium picolinate/biotin significantly lowered HbA_{1c} and fasting plasma glucose when compared to placebo. The greatest improvements were observed in those individuals who had the poorest degree of control at baseline, as defined by a $HbA_{1c} \geq 10\%$. No safety or tolerability issues were identified in this study. Results from an open-label patient experience program [44] evaluating the effects of chromium picolinate/biotin on subjects with type 2 DM and $HbA_{1c} > 7.0\%$ reported significant reductions in HbA_{1c} from baseline ($n = 30$; -1.0% HbA_{1c}, $p = 0.01$). The results suggested that the reductions in HbA_{1c} observed were greatest in those subjects who had the highest baseline HbA_{1c} levels ($n = 18$; -1.8% HbA_{1c}, $p = 0.001$). On the basis of this information and accepted guidelines for assessing the effect of baseline glycaemic control on glycaemic outcome measures, we performed an ANCOVA using baseline HbA_{1c} as the covariate [44,45]. The results of the analysis suggest a positive relationship between baseline HbA_{1c} and the magnitude of HbA_{1c} reductions. The ANCOVA results reported here demonstrate that the chromium picolinate/biotin combination provides significant HbA_{1c} reductions along the entire range of entrance HbA_{1c} values, but most profoundly in those patients with the poorest control when compared to placebo. Lowering HbA_{1c}, especially in those patients with poor control, results in significant reductions in diabetes-related deaths, all cause mortality, and myocardial infarcts, and improve other diabetes-related healthcare outcomes such as micro- and macro-vascular complications [47,48]. However, the costs of these interventions may be prohibitively expensive [10]. A recent report suggests that the improvements in HbA_{1c} from using the chromium picolinate/biotin combination as an adjunct to current OADs may not only be inexpensive, but result in substantial overall reductions in diabetes-related costs, most especially in those patients with poor glycaemic control [10].

Another recent report [41] from a well-controlled acute 30-day intervention study indicated that the combination of chromium picolinate and biotin resulted in improvements in fasting glucose, post-prandial glucose excursions, and fructosamine. This pilot study did not include a measure of HbA_{1c} since the duration of the intervention was only 30 days; therefore, fructosamine was selected as a measure of sustained glycaemic control. Since the duration of the 90-day placebo-controlled intervention study was of a more protracted length, HbA_{1c} was selected as the gold standard measure of sustained glycaemic control. In both of these studies, the two markers of sustained glycaemic control were significantly reduced, implying similar and supporting evidence of sustainable change.

It is well established that many patients with type 2 DM, especially obese individuals on multiple OADs, do not achieve adequate diabetic control, as evidenced by elevated HbA_{1c} levels. These patients often present an ongoing clinical challenge to physicians. Epidemiological evidence exists that implies the risk of CVD begins well below the current HbA_{1c} target goal of 7.0% [49]. Therefore, additional reductions in HbA_{1c} are desirable, even when close to the target goal. Additional incremental reductions are difficult to obtain, often require poly oral therapy with the addition of insulin, and have an increased risk of emergent hypoglycemic events. Bloomgarden *et al*. reported that, for subjects whose HbA_{1c} is $\leq 8.0\%$, a modest reduction of $\sim 0.5\%$ HbA_{1c} was difficult to achieve in controlled trials using OADs [46,49]. For those subjects whose HbA_{1c} was $<8.0\%$ ($n = 2030$), the reduction was only -0.1 to -0.2% using OAD therapy [46]. Although it is challenging to achieve additional incremental reductions without side effects, by adding chromium/biotin as an adjunctive therapy we report an overall reduction of 0.5% HbA_{1c}, without hypoglycaemic events or other side effects dissimilar from placebo. In fact, for subjects whose HbA_{1c} was between 8.0 and 8.9% ($\boldsymbol{n} = 5269$), the mean reduction amongst all OAD studies analysed was -0.6% [46], confirming the importance of the results reported here.

As noted in the preceding text, the lack of hypoglycaemic side effects is not unexpected. In over 34 clinical trials evaluating chromium picolinate, there has never been any evidence of hypoglycemia whether administered alone, or in combination with other prescription medications including sulfonylureas [12,24].

In the meta-analysis by Bloomgarden *et al*. [46], the group whose HbA_{1c} was $>10.0\%$ ($n = 266$) experienced a reduction in HbA_{1c} of $\sim 1.2\%$; whereas we report a reduction in HbA_{1c} of $\sim 1.76\%$ in a similar sample without an increase in deleterious effects. Findings from this trial suggest that the chromium picolinate/biotin intervention, when used as an adjuvant therapy, was as good as or better than that reported on by Bloomgarden *et al*. [46], which used OADs. Results of this trial indicate that chromium picolinate/biotin supplementation had a beneficial effect on HbA_{1c}, especially in those subjects with the poorest control on current OAD therapy.

In contrast to the results reported here and by others [12,31], a recent study has found that chromium picolinate (500 and 1000 μg daily for 6 months) was ineffective in reducing HbA_{1c} in obese, poorly controlled, insulin-dependent individuals with type 2 diabetes [26]. Several possible explanations for these contrasting results were the limited statistical power in the latter study to detect a significant change due to the small number of subjects ($n = 17$ for placebo group; $n = 14$ for 500 μg group; $n = 15$ for 1000 μg group, *vs* $n = 226$ for the chromium/biotin group and $n = 122$ for placebo group in this study), and the greater degree of obesity and insulin resistance at baseline (BMI = 33–35 kg/m^2 *vs* BMI = 30 kg/m^2 in this study). Furthermore, these subjects [26] were unable to achieve adequate glycaemic control, even with OAD therapy and concomitant high doses of insulin (>90 IU/day insulin).

DOI: 10.1002/dmrr

Although the mechanism of chromium action has not been definitively established, data from a recent *in vivo* study suggest that chromium might exhibit its insulin-sensitizing effect by reducing the content and activity of the tyrosine phosphatase PTP-1B [19]. PTP-1B has long been implicated in the regulation of insulin receptor tyrosine phosphorylation and tyrosine kinase activity [50], and has been validated as a *bona fide* pharmacological target for increasing insulin sensitivity [51–55]. In animals, small molecules that inhibit PTP-1B increase insulin sensitivity and lower plasma glucose [56–58]. Alternatively, chromium might act directly on the insulin receptor, and increase its tyrosine kinase activity [59], as has been observed with other small molecules [60,61].

Reviews of the recent literature reveal additional potential chromium mechanisms of action that may be complementary to those discussed in the preceding text. Researchers from the Elmendorf laboratory have reported a novel potential mechanism of chromium action [62]. Work from this laboratory has indicated that chromium picolinate (and chromium chloride) stimulated GLUT4 translocation to the plasma membrane in cultured 3T3-L1 adipocytes. Concomitant with an increase in GLUT4 at the plasma membrane, insulin-stimulated glucose transport was enhanced by chromium treatment. Chromium-stimulated GLUT4 translocation did not involve known insulin signaling intermediates such as the insulin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, or Akt, but was associated with decreased plasma membrane cholesterol. Subsequent work from this group has found that the GLUT4 redistribution in cultured adipocytes treated with chromium picolinate occurred only in adipocytes cultured in the presence of high glucose (25 mM), but not in those cultured under normoglycemic (5.5 mM glucose) conditions [63]. Examination of the effect of chromium picolinate on proteins involved in cholesterol homeostasis [62,63] revealed that the activity of sterol regulatory element-binding protein, a membrane-bound transcription factor ultimately responsible for controlling cellular cholesterol balance, was up-regulated by chromium picolinate. In addition, ABCA1, a major player in mediating cholesterol efflux was decreased, consistent with sterol regulatory element-binding protein transcriptional repression of the *ABCA1* gene.

Glucokinase, expressed in hepatocyte and pancreatic β cells, has a central regulatory role in glucose metabolism [64]. Efficient glucokinase activity is required for normal glucose-stimulated insulin secretion, post-prandial hepatic glucose uptake, and the appropriate suppression of hepatic glucose output and gluconeogenesis by elevated plasma glucose. Hepatic glucokinase activity is subnormal in diabetes, and glucokinase may also be decreased in the β cells of individuals with type 2 DM [64]. In supra-physiological concentrations, biotin promotes the transcription and translation of the glucokinase gene in hepatocytes; and more recent evidence indicates that biotin increases glucokinase activity in pancreatic islet cells [34,65].

Furthermore, researchers (Wang, Z.Q, *et al*. Chromium picolinate and biotin enhance glycogen synthesis and glycogen synthase gene expression in human skeletal muscle culture. Presented at 17th International Diabetes Federation Congress November 9, 2000. Mexico City, Mexico.) using a human skeletal muscle cell line have investigated whether chromium picolinate, biotin, or the combination stimulate increased glycogen production *versus* control. In this *in vitro* model, it was noted that chromium picolinate alone and biotin alone stimulated glycogen synthase mRNA production, as well as glycogen production, to a greater extent than the control alone. Interestingly, although the effect was more pronounced in the chromium picolinate group than the biotin group, the effect appeared to be synergistic using a combination of chromium picolinate and biotin together *versus* control.

In conclusion, chromium picolinate was combined with biotin in a 90-day double-blind placebo-controlled study in overweight to obese subjects with poorly controlled type 2 DM patients currently on OAD therapy. The results of this study indicated that this combination provided a significant improvement in a validated index of long-term glycaemic control (HbA_{1c}) with results similar to those reported in the literature. Future studies are ongoing to confirm the effectiveness of this supplement in patients with other forms of metabolic dysfunction.

Acknowledgements

This study, and subsequent statistical analyses, was supported by a grant from Nutrition 21, Inc. (Purchase, NY). Statistical analyses of all data were independently performed by Dr Dennis W. King (STATKING Consulting, Inc, Fairfield, OH; www.statkingconsulting.com). Tables were reviewed by Joseph Fuhr, Ph.D., Widener University, Chester, PA 19013-5792. All authors (CAA, BCF, JLE, and IDG) had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis. Trial registered on clinicaltrials.gov; registry # NCT00289354.

Conflict of interest

None declared.

References

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782–787.
2. Reaven GM. Insulin resistance and its consequences: type 2 diabetes mellitus and coronary heart disease. In *Diabetes Mellitus: A Fundamental and Clinical Text* (2nd edn), LeRoith D, Taylor SI, Olefsky JM (eds). Lippincott Williams and Wilkins: Philadelphia, PA, 2000; 604–615.
3. Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595–1607.
4. DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabet Rev* 1997; **5**: 177–269.

5. Kimm SY, Obarzanek E. Childhood obesity: a new pandemic of the new millennium. *Pediatrics* 2002; **110**: 1003–1007.
6. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *J Am Med Assoc* 2004; **291**: 2847–2850.
7. Speiser PW, Rudolf MC, Anhalt H, *et al*. Childhood obesity. *J Clin Endocrinol Metab* 2005; **90**: 1871–1887.
8. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. *Ann Intern Med* 1999; **131**: 281–303.
9. Evans JL, Youngren JF, Goldfine ID. Effective treatments for insulin resistance: trim the fat and douse the fire. *Trends Endocrinol Metab* 2004; **15**: 425–431.
10. Fuhr JP, He H, Goldfarb N, Nash DB. Use of chromium picolinate and biotin in the management of type 2 diabetes: an economic analysis. *Dis Manag* 2005; **8**: 265–275.
11. Altman SH, Parks-Thomas C. Controlling spending for prescription drugs. *N Engl J Med* 2002; **346**: 855–856.
12. Cefalu WT, Hu FB. Role of chromium in human health and in diabetes. *Diabetes Care* 2004; **27**: 2741–2751.
13. Mertz W. Chromium research from a distance: from 1959 to 1980. *J Am Coll Nutr* 1998; **17**: 544–547.
14. Anderson RA. Chromium, glucose intolerance and diabetes. *J Am Coll Nutr* 1998; **17**: 548–555.
15. Schwartz K, Mertz W. Chromium III and the glucose tolerance factor. *Arch Biochem Biophys* 1959; **85**: 292–300.
16. Cefalu WT, Wang ZQ, Zhang XH, Baldor LC, Russell JC. Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. *J Nutr* 2002; **132**: 1107–1114.
17. Kim DS, Kim TW, Kang JS. Chromium picolinate supplementation improves insulin sensitivity in Goto-Kakizaki diabetic rats. *J Trace Elem Med Biol* 2004; **17**: 243–247.
18. Shindea UA, Sharma G, Xu YJ, Dhalla NS, Goyal RK. Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus. *J Trace Elem Med Biol* 2004; **18**: 23–32.
19. Wang ZQ, Zhang XH, Russell JC, Hulver M, Cefalu WT. Chromium picolinate enhances skeletal muscle cellular insulin signaling in vivo in obese, insulin-resistant JCR : LA-cp rats. *J Nutr* 2006; **136**: 415–420.
20. Martin J, Wang ZQ, Zhang XH, *et al*. Chromium picolinate supplementation attenuates body weight gain and increases insulin sensitivity in subjects with type 2 diabetes. *Diabetes Care* 2006; **29**: 1826–1832.
21. Lukaski HC. Chromium as a supplement. *Annu Rev Nutr* 1999; **19**: 279–302.
22. Lamson DS, Plaza SM. The safety and efficacy of high-dose chromium. *Altern Med Rev* 2002; **7**: 218–235.
23. Haslacker AR, Cupp MJ, Tracy TS. Chromium picolinate. In *Dietary Supplements: Toxicology and Clinical Pharmacology* (1st edn), Cupp MJ, Tracy TS (eds). Totowa: Humana: 2003; 41–52.
24. Broadhurst CL, Domenico P. Clinical studies on chromium picolinate supplementation in diabetes mellitus–a review. *Diabetes Technol Ther* 2006; **8**: 677–687.
25. Althuis MD, Jordan NE, Ludington EA, Wittes JT. Glucose and insulin responses to dietary chromium supplements: a meta-analysis. *Am J Clin Nutr* 2002; **76**: 148–155.
26. Kleefstra N, Houweling ST, Jansman FG, *et al*. Chromium treatment has no effect in patients with poorly controlled, insulin-treated type 2 diabetes in an obese Western population: a randomized, double-blind, placebo-controlled trial. *Diabetes Care* 2006; **29**: 521–525.
27. Trumbo PR, Ellwood KC. Chromium picolinate intake and risk of type 2 diabetes: an evidence-based review by the United States Food and Drug Administration. *Nutr Rev* 2006; **64**: 357–363.
28. Komorowski J, Juturu V. Chromium supplementation does not improve glucose tolerance, insulin sensitivity, or lipid profile: a randomized, placebo-controlled, double-blind trial of supplementation in subjects with impaired glucose tolerance: response to Gunton. *Diabetes Care* 2005; **28**: 1841–1842.
29. Gunton JE, Cheung NW, Hitchman R, *et al*. Chromium supplementation does not improve glucose tolerance, insulin sensitivity, or lipid profile: a randomized, placebo-controlled, double-blind trial of supplementation in subjects with impaired glucose tolerance. *Diabetes Care* 2005; **28**: 712–713.
30. 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* 1997; **46**: 1786–1791.
31. Anderson RA. Chromium in the prevention and control of diabetes. *Diabetes Metab* 2000; **26**: 22–27.
32. Cefalu WT, Bell-Farrow AD, Stegner J, *et al*. Effect of chromium picolinate on insulin sensitivity in vivo. *J Trace Elem Exp Med* 1999; **12**: 71–83.
33. Anderson RA, Polansky MM, Bryden NA. Stability and absorption of chromium and absorption of chromium histidinate complexes by humans. *Biol Trace Elem Res* 2004; **101**: 211–218.
34. Fernandez-Mejia C. Pharmacological effects of biotin. *J Nutr Biochem* 2005; **1**: 424–427.
35. McCarty MF. High-dose biotin, an inducer of glucokinase expression, may synergize with chromium picolinate to enable a definitive nutritional therapy for type II diabetes. *Med Hypotheses* 1999; **52**: 401–406.
36. Zhang H, Osada K, Sone H, Furukawa Y. Biotin administration improves the impaired glucose tolerance of streptozotocin-induced diabetic Wistar rats. *J Nutr Sci Vitaminol* 1997; **43**: 271–280.
37. Zhang H, Osada K, Maebashi M, Ito M, Komai M, Furukawa Y. A high biotin diet improves the impaired glucose tolerance of long-term spontaneously hyperglycemic rats with non-insulin-dependent diabetes mellitus. *J Nutr Sci Vitaminol* 1996; **42**: 517–526.
38. Reddi A, Deangelis B, Frank O, Lasker N, Baker H. Biotin supplementation improves glucose and insulin tolerances in genetically diabetic KK mice. *Life Sci* 1988; **42**: 1323–1330.
39. Revilla-Monsalve C, Zendejas-Ruiz I, Islas-Andrade S, *et al*. Biotin supplementation reduces plasma triacylglycerol and VLDL in type 2 diabetic patients and in nondiabetic subjects with hypertriglyceridemia. *Biomed Pharmacother* 2006; **60**: 182–185.
40. Maebashi M, Makino Y, Furukura Y. Therapeutic evaluation of the effect of biotin on hyperglycemia in patients with non-insulin dependent diabetes mellitus. *J Clin Biochem Nutr* 1993; **14**: 211–218.
41. Singer GM, Jeff G. The effect of chromium picolinate and biotin supplementation on glycemic control in poorly controlled patients with type 2 diabetes mellitus: a placebo-controlled, double blinded, randomized trial. *Diabetes Technol Ther* 2006; **8**: 636–643.
42. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven GM. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003; **139**: 802–809.
43. McLaughlin T, Reaven GM, Abbasi F, *et al*. Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? *Am J Cardiol* 2005; **96**: 399–404.
44. Juturu V, Anne R, Hudson M, Komorowski JR. Improved glycemic control after diabetes education and chromium picolinate/biotin supplementation in type 2 diabetes: results from patients experience pilot program. *Trace Elem Electrol* 2006; **23**: 66–72.
45. The European Agency for the Evaluation Medicinal Products (Committee for Proprietary medicinal Products; CPMP). 2002; Note for guidance on clinical investigation of medicinal products in the treatment of diabetes mellitus. *Available* online from http://www.emea.europa.eu/pdfs/human/ewp/108000en.pdf.2002.
46. Bloomgarden ZT, Dodis R, Viscoli CM, Holmboe ES, Inzucchi SE. Lower baseline glycemia reduces apparent oral agent glucose-lowering efficacy: a meta-regression analysis. *Diabetes Care* 2006; **29**: 2137–2139.
47. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977–986.
48. UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837–853.
49. The American Association of Clinical Endocrinologists. 2005; Implementation conference for ACE outpatient diabetes mellitus consensus conference recommendations: position statement. *Available* online from http://www.aace.com/pub/pdf/guidelines/OutpatientImplementationPositionStatement.pdf.

50. Byon JCH, Kusari AB, Kusari J. Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol Cell Biochem* 1998; **182**: 101–108.
51. Elchebly M, Payette P, Michaliszyn E, *et al*. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 1999; **283**: 1544–1548.
52. Klaman LD, Boss O, Peroni OD, *et al*. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 2000; **20**: 5479–5489.
53. Evans JL, Jallal B. Protein tyrosine phosphatases: their role in insulin action and potential as drug targets. *Exp Opin Invest Drugs* 1999; **8**: 139–160.
54. Goldstein BJ. Protein-tyrosine phosphatase 1B (PTP1B): a novel therapeutic target for type 2 diabetes mellitus, obesity, and related states of insulin resistance. *Curr Drug Targets Immune Endocr Metabol Disord* 2001; **1**: 265–275.
55. Ramachandran C, Kennedy BP. Protein tyrosine phosphatase 1B: a novel target for type 2 diabetes and obesity. *Curr Top Med Chem* 2003; **3**: 749–757.
56. Malamas MS, Sredy J, Gunawan I, *et al*. New azolidinediones as inhibitors of protein tyrosine phosphatase 1B with antihyperglycemic properties. *J Med Chem* 2000; **43**: 995–1010.
57. Cheon HG, Kim SM, Yang SD, Ha JD, Choi JK. Discovery of a novel protein tyrosine phosphatase-1B inhibitor, KR61639: potential development as an antihyperglycemic agent. *Eur J Pharmacol* 2004; **485**: 333–339.
58. Liu G, Szczepankiewicz BG, Pei Z, *et al*. Discovery and structure-activity relationship of oxalylarylaminobenzoic acids as inhibitors of protein tyrosine phosphatase 1B. *J Med Chem* 2003; **46**: 2093–2103.
59. Wang H, Kruszewski A, Brautigan DL. Cellular chromium enhances activation of insulin receptor kinase. *Biochemistry* 2005; **44**: 8167–8175.
60. Pender C, Youngren JF, Manchem VP, *et al*. Regulation of insulin receptor function by a small molecule insulin receptor activator. *J Biol Chem* 2002; **277**: 43565–43571.
61. Li M, Youngren JF, Dunaif A, *et al*. Decreased insulin receptor (IR) autophosphorylation in fibroblasts from patients with PCOS: effects of sering kinase inhibitors and IR activators. *J Clin Endocrinol Metab* 2002; **87**: 4088–4093.
62. Chen G, Liu P, Pattar GR, *et al*. Chromium activates glucose transporter 4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. *Mol Endocrinol* 2006; **20**: 857–870.
63. Pattar GR, Tackett L, Liu P, Elmendorf JS. Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. *Mutat Res* 2006; **610**: 93–100.
64. Matschinsky FM. Glucokinase, glucose homeostasis, and diabetes mellitus. *Curr Diab Rep* 2005; **5**: 171–176.
65. Dakshinamurti K. Biotin-a regulator of gene expression. *J Nutr Biochem* 2005; **16**: 419–423.

DOI: 10.1002/dmrr