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Characterization of the metabolic and physiologic response to chromium supplementation in subjects with type 2 diabetes mellitus

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Abstract

The objective of the study was to provide a comprehensive evaluation of chromium (Cr) supplementation on metabolic parameters in a cohort of type 2 diabetes mellitus subjects representing a wide phenotype range and to evaluate changes in "responders" and "nonresponders." After preintervention testing to assess glycemia, insulin sensitivity (assessed by euglycemic clamps), Cr status, and body composition, subjects were randomized in a double-blind fashion to placebo or 1000 μg Cr. A substudy was performed to evaluate 24-hour energy balance/substrate oxidation and myocellular/intrahepatic lipid content. There was not a consistent effect of Cr supplementation to improve insulin action across all phenotypes. Insulin sensitivity was negatively correlated to soleus and tibialis muscle intramyocellular lipids and intrahepatic lipid content. Myocellular lipids were significantly lower in subjects randomized to Cr. At preintervention, *responders*, defined as insulin sensitivity change from baseline of at least 10% or greater, had significantly lower insulin sensitivity and higher fasting glucose and A_{1c} when compared with placebo and *nonresponders*, that is, insulin sensitivity change from baseline of less than 10%. Clinical response was significantly correlated ($P < .001$) to the baseline insulin sensitivity, fasting glucose, and A_{1c}. There was no difference in Cr status between responder and nonresponders. Clinical response to Cr is more likely in insulin-resistant subjects who have more elevated fasting glucose and A_{1c} levels. Chromium may reduce myocellular lipids and enhance insulin sensitivity in subjects with type 2 diabetes mellitus who do respond clinically independent of effects on weight or hepatic glucose production. Thus, modulation of lipid metabolism by Cr in peripheral tissues may represent a novel mechanism of action.

1. Introduction

Although lifestyle modification combined with pharmacologic intervention is the primary strategy to meet glycemic targets in patients with type 2 diabetes mellitus (DM), alternative strategies, for example, nutritional supplementation with over-the-counter agents, continue to be extensively practiced by a large number of patients [1,2]. There are more than 29 000 nutritional supplements available, and patients pay more than 12 billion dollars per year on these supplements [1,2]. One supplement that remains controversial, particularly as it relates to improving glycemia, is chromium (Cr); and to date, routine clinical use has not been suggested [3]. However, several studies suggest that Cr may have more consistent effects in certain clinical states and

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conditions [4-6]. We reported that Cr supplementation, provided as 1000 μg/d as chromium picolinate (CrPic), improved glycemia, attenuated body weight gain, and enhanced insulin sensitivity in subjects with type 2 DM [6]. When compared with studies evaluating a similar dose and formulation of Cr, the data agreed with some, but not all, studies and suggested that subject characteristics may be important when assessing clinical response [6-8]. For example, Kleefstra et al [7] reported that Cr had no benefit in subjects with type 2 DM; but when compared with the studies suggesting a benefit, for example, Martin et al [6] and Wang et al [8], the subjects evaluated appeared to be more obese, were more advanced in their disease process, and were taking other medications, for example, metformin, in addition to high-dose insulin [7].

Given the recent observations, several questions regarding Cr remain. In particular, if Cr does improve glycemia, what are the mechanisms and which patient population is more likely to respond? In pilot studies, a major parameter predicting clinical response to Cr was the pretreatment insulin sensitivity; and additional studies suggest more consistent effects in the presence of insulin resistance and poorer glycemic control [5,8]. Does Cr status of the patient determine who does or does not respond? What is the mechanism for the reported weight effects if indeed Cr modulates weight? In this regard, definitive energy balance studies, that is, dietary intake and energy expenditure (EE), using well-validated techniques have not been reported. As such, we sought to provide a comprehensive assessment of Cr supplementation by conducting randomized, double-blind, placebo-controlled evaluations in individuals with type 2 DM and representing a wide phenotype range and levels of insulin sensitivity. The overall effects of Cr were evaluated in addition to characterizing the specific differences in metabolic and physiologic parameters, that is, hepatic glucose production, Cr status, body weight/distribution, energy balance, and myocellular/intrahepatic lipid content, in those who did or did not respond clinically to Cr.

2. Research design and methods

2.1. Study design

The design was double-blinded, randomized, and placebo-controlled. Type 2 DM subjects (age, 30-70 years) with a body mass index (BMI) range of 25 to 40 and with a fasting plasma glucose of at least 6.94 mmol/L (125 mg/dL) at the time of screening were evaluated. Exclusions were as follows: (1) medications known to affect glucose metabolism; (2) untreated thyroid or chronic liver, renal, or cardiovascular disease; and (3) a history of drug and/or alcohol abuse, or psychiatric disease prohibiting adherence to protocol. All procedures were approved and conducted in compliance with institutional human research guidelines.

The main cohort of subjects was evaluated at the Pennington Biomedical Research Center (Parent Trial, n = 111 randomized, n = 93 completers). After meeting entry criteria, subjects were instructed on a weight maintenance diet by the study dietitian; and the following assessments were obtained: (1) glycemic control, that is, A_{1c} and oral glucose tolerance testing (OGTT); (2) body weight/fat distribution as assessed by abdominal computed tomography and dual-energy x-ray absorptiometry scans; (3) Cr status, that is, urinary and plasma Cr levels; and (4) insulin sensitivity as assessed with hyperinsulinemic-euglycemic clamps. After completing pre-intervention testing, each subject was randomized to receive either 1000 μg of Cr as CrPic (two 500-μg tablets daily) or matching placebo (dicalcium diphosphate). In a substudy conducted only in subjects enrolled in the Parent Trial and at the Pennington site, dietary intake studies; myocellular and intrahepatic lipids, as assessed with magnetic resonance spectroscopy scans; and 24-hour energy expenditure (EE) and respiratory quotient (RQ), as assessed with use of whole-body metabolic chambers, were completed before randomization and at end of study. Subjects were evaluated monthly for routine clinic visits, adverse event monitoring, and study medication compliance. Twenty-four weeks after randomization, subjects underwent another physiologic testing identical to that described for the preintervention testing. As the major objective of the study was to evaluate the role of Cr supplementation on metabolic parameters in a cohort representing a wide phenotype range and range of glycemic control, the entire cohort consisted of subjects randomized as part of the Parent Trial in addition to subjects evaluated at the additional site for which glycemic control, insulin sensitivity, and body weight/adiposity were the primary measures obtained. A brief interim analysis for characteristics defining a clinical response was reported for 73 of the subjects [8].

2.2. Study variables

2.2.1. Insulin sensitivity

Hyperinsulinemic-euglycemic clamps were used to assess insulin sensitivity after an overnight fast [6,9]. After placement of catheters, a primed infusion of [6,6-$^2H_2$] glucose (75 μg/kg lean body mass per minute) was started at 7:00 AM and continued for 5 hours. An insulin infusion (120 mU/[m^2 min]) was started at 10:00 AM and continued for 2 hours. Blood glucose was monitored every 5 minutes, and euglycemia was maintained throughout the clamp by infusing 20% dextrose at a variable rate. The mean rate of exogenous glucose infusion during steady state (last 30 minutes) was corrected for changes in glycemia and divided by fat-free mass (FFM) to assess insulin sensitivity [9]. Basal hepatic glucose production was calculated as previously described [6]. Before and during the last hour of the clamp, resting EE and RQ were assessed for each subject by indirect calorimetry for 45 minutes using the ventilated hood technique; and substrate oxidation was calculated [6].

2.2.2. Glycemic parameters

Glucose tolerance was assessed by performing a 75-g challenge with determination of glucose at 0, 0.5, 1, 1.5, 2,

and 3 hours after challenge. Glucose was measured using a glucose oxidase electrode on the Beckman Coulter DXC600 (Brea, CA). Insulin was measured by enzyme immunoassay on the Siemens 2000 (Los Angeles, CA). Hemoglobin A_{1c} was determined spectrophotometrically on the Beckman Coulter DXC600. Day-to-day precision was assessed as less than 1.9% for glucose, less than 2.4% for hemoglobin A_{1c}, and less than 4.0% for insulin.

2.2.3. Body weight/fat distribution

Fat-free mass, fat mass, and body fat percentage were measured by dual-energy x-ray absorptiometry with coefficient of variation for measurements assessed as 0.6%, 1.1%, and 1.1%, respectively. Total abdominal, visceral, and subcutaneous abdominal fat was measured by computed tomography as previously described [6]. Overall, biologic, instrument, and reader variability was assessed at approximately 10%.

2.2.4. Energy balance

Studies to assess energy balance, i.e. dietary intake and energy expenditure were only done on the subset of subjects enrolled in the Parent Trial at the Pennington site.

2.2.4.1. Dietary intake. On test days, participants in the substudy returned to the center and under supervision consumed a standard breakfast (460 kcal, fat 14.5 g [28.4%], protein 13.6 g [11.8%], and carbohydrate 74.3 g [59.8 %], Cr content, estimated <10 μg). Food intake at both lunch (4 hours after breakfast) and dinner (4.5 hours after lunch) was measured. At dinner, food intake was measured using the Macronutrient Self-Selection Paradigm [10]. Satiety ratings were collected using visual analogue scales [11].

2.2.4.2. 24-Hour EE and RQ. Twenty-four–hour EE and substrate oxidation were measured in a whole-room respiratory calorimeter for participants enrolled in the substudy [12]. Participants entered the chamber at 8:00 AM after an overnight fast. Meals were served at 9:00 AM, 1:30 PM, and 7:00 PM. Microwave motion detectors provided continuous monitoring of the participants' spontaneous physical activity. Twenty-four–hour EE and substrate oxidation (24-hour RQ, fat, carbohydrate, and fat oxidation) were calculated from O_2 consumption, CO_2 production, and 24-hour urinary nitrogen excretion. Coefficient of variation was determined to be 5% for 24-hour EE and 7.8% for 24-hour RQ.

2.2.5. Chromium status

Serum was collected in lithium-heparin-Cr–free Monovettes from Sarstedt (Numbrecht-Rommelsdorf, Germany) and was obtained at all time points during the OGTT and at 10 time points during the clamp. The samples were diluted with 1% nitric acid with 0.02% cetyltrimethylammonium chloride and analyzed on a Varian (Walnut Creek, CA) graphite furnace atomic absorption spectrophotometer [13]. Matrix-matched calibration method was used, which resulted in an analytical sensitivity of 0.01 μg/L. Urine samples, after first morning void, were collected in prescreened urine collection cups over the entire duration of the OGTT and clamp studies and analyzed using graphite furnace atomic absorption [13]. Quality control materials were run each day, and between-run precision was assessed as less than 10%.

2.3. Liver and myocellular lipid content

Intramuscular (IMCL), extramuscular (EMCL), and intrahepatic (IHL) lipid stores were measured using ^{1}H magnetic resonance spectroscopy using either a 1.5-T (Picker Edge Eclipse) or GE 3-T whole-body imaging and spectroscopy system (General Electric, Milwaukee, WI). Once corrected for acquisition and relaxation time differences, data were shown to be highly correlated between the 1.5-T and 3-T systems in a subset of subjects run on both machines and were pooled into a common data set. Acquisition and processing techniques used in this project have been previously published by the current investigators [14,15]. To account for day-to-day variation in system performance, peak areas are expressed either relative to the peak area of an external phantom of known constant concentration or relative to an internal water peak assumed to be of constant concentration using the methodology described by Perseghin et al [16] and Krssak et al [17].

3. Statistical analysis

The SAS (Cary, NC) mixed procedure was used for analysis of variance to detect the effects of Cr. The least square means were used to determine significance of all pairwise differences. Unpaired 2-tailed *t* test was used to determine statistical differences between treatments. Changes between pre- and posttreatment were also tested by *t* test with the hypothesis that change is null. Pearson correlation analysis was applied to analyze the relationships between assessments. Subjects randomized to Cr were classified as "responders" if the difference in insulin sensitivity at study end compared with preintervention was at least 10% or greater or as "nonresponders" if the increase was less than 10%. This stratification was based on data suggesting that a 10% increase in insulin sensitivity resulted in a significant clinical glycemic response [6]. All the data analyses were carried out on SAS (SAS 9.1, 2003).

4. Results

A total of 137 subjects (70 Cr, 67 placebo) were evaluated for the entire cohort. As the overall goal was to evaluate a cohort of individuals with type 2 DM over a wide range of phenotypes, insulin sensitivity values were observed to range from extreme insulin resistance to clinically insulin sensitive. Specifically, insulin sensitivity in the entire cohort ranged from 0.96 to 10.7 mg/kg FFM per minute. There were no differences at baseline for

Table 1
Subject characteristics at baseline

Metabolic parameter	Cr	Placebo
Sex (male/female)	37/33	39/28
Age (y)	58.7 ± 1.0	56.1 ± 1.1
Body weight (kg)	89.0 ± 2.1	91.3 ± 1.8
BMI (kg/m^2)	30.8 ± 0.5	31.5 ± 0.6
A_{1c} (%)	6.9 ± 0.2	6.8 ± 0.2
Insulin sensitivity (mg/kg FFM per min)	5.0 ± 0.3	5.2 ± 0.3
Fasting glucose (mmol/L)	7.4 ± 0.3	7.5 ± 0.3
Fasting insulin (IU/mL)	17.4 ± 1.3	18.1 ± 1.7
Triglyceride (mg/dL)	177.4 ± 17.7	175.4 ± 19.0

Data are mean ± SE.

clinical or biochemical characteristics of subjects randomized to either Cr or placebo (Table 1).

4.1. Insulin sensitivity/hepatic glucose production

When evaluating the entire cohort and across all BMI ranges, insulin sensitivity increased in the Cr group (Δ = 0.35 mg/kg FFM per minute) and slightly decreased in the placebo group (Δ =−0.17 mg/kg FFM per minute); but this difference was not considered statistically significant. However, approximately 46% of individuals randomized to Cr were classified as responders. In subjects classified as responders, there was a marked increase in insulin sensitivity from baseline as opposed to placebo (Δ =1.2 mg/[kg min], $P < .0001$ vs Δ = −0.1 mg/[kg min], P = not significant [NS], respectively); and this difference was considered statistically significant between groups (Table 2). In addition, preintervention insulin sensitivity was observed to be significantly less in responders as opposed to both nonresponders and placebo groups (Fig. 1). Preintervention insulin sensitivity was also negatively correlated to the clinical response, that is, Δ insulin sensitivity at end of study compared with baseline ($r = −0.37$, $P < .001$). Basal hepatic glucose production did not differ between treatment groups at end of study; nor were there were significant differences between responders, nonresponders, or placebo (Table 2).

4.2. Glycemic control

In the entire cohort, differences between placebo and Cr groups for A_{1c} (Δ = 0.12%) and fasting glucose (Δ = 5.4 mg/

Table 2
Metabolic and phenotypic parameters at baseline and end of study

Parameter	Placebo			Cr nonresponders			Cr responders			P^2	P^3
	Baseline	End	P^1	Baseline	End	P^1	Baseline	End	P^1		
Glucose/insulin											
Fasting glucose (mmol/L)	7.5 ± 0.3	7.6 ± 0.2	NS	6.7 ± 0.5	7.1 ± 0.3	NS	8.5 ± 0.5	7.4 ± 0.3	.010	.002	.003
Glycated hemoglobin (%)	6.85 ± 0.25	6.98 ± 0.21	NS	6.29 ± 0.18	6.48 ± 0.17	NS	7.57 ± 0.43	6.85 ± 0.25	.035	.024	.025
Glucose AUC	1172 ± 35	1162 ± 31	NS	1096 ± 36	1126 ± 42	NS	1310 ± 66	1196 ± 46	.015	.033	.010
Insulin sensitivity[a]	5.22 ± 0.29	5.11 ± 0.26	NS	5.91 ± 0.36	5.39 ± 0.42	NS	3.98 ± 0.43	5.17 ± 0.51	.001	.001	.001
Body fat											
Weight (kg)	91.3 ± 1.8	92.4 ± 1.8	NS	88.0 ± 2.9	89.0 ± 3.0	NS	89.6 ± 3.1	90.0 ± 3.0	NS	NS	NS
Body fat (%)	32.4 ± 1.1	32.8 ± 1.1	NS	33.0 ± 1.4	33.3 ± 1.3	NS	33.3 ± 1.4	32.7 ± 1.3	NS	NS	NS
FFM (kg)	61.2 ± 1.5	61.8 ± 1.5	NS	58.0 ± 1.7	57.6 ± 1.8	NS	61.0 ± 2.5	62.5 ± 2.7	.02	NS	NS
Visceral fat	5.70 ± 0.33	5.60 ± 0.33	NS	5.66 ± 0.42	5.80 ± 0.48	NS	5.80 ± 0.55	5.82 ± 0.53	NS	NS	NS
SubQ fat	9.97 ± 0.63	10.24 ± 0.64	NS	9.35 ± 0.73	9.15 ± 0.71	NS	10.36 ± 0.87	11.17 ± 0.72	NS	NS	NS
Myocellular lipids											
Soleus EMCL (ASU)	0.014 ± 0.002	0.019 ± 0.003	.055	0.013 ± 0.003	0.016 ± 0.004	NS	0.023 ± 0.008	0.013 ± 0.001	NS	.011	NS
Soleus IMCL (ASU)	0.008 ± 0.001	0.009 ± 0.001	NS	0.007 ± 0.002	0.007 ± 0.001	NS	0.010 ± 0.003	0.006 ± 0.001	NS	.033	NS
Tibialis EMCL (ASU)	0.016 ± 0.002	0.029 ± 0.005	.008	0.027 ± 0.009	0.040 ± 0.010	NS	0.037 ± 0.018	0.018 ± 0.004	NS	.017	.026
Tibialis IMCL (ASU)	0.007 ± 0.001	0.007 ± 0.001	NS	0.005 ± 0.002	0.007 ± 0.002	NS	0.010 ± 0.004	0.006 ± 0.001	NS	NS	.053
Liver parameters											
IHL (ASU)	0.11 ± 0.02	0.16 ± 0.03	NS	0.10 ± 0.04	0.13 ± 0.05	NS	0.20 ± 0.08	0.12 ± 0.05	.039	.055	.035
HGP (mmol/[dL min])	2.37 ± 0.12	2.17 ± 0.09	NS	2.32 ± 0.11	2.21 ± 0.11	NS	2.48 ± 0.10	2.31 ± 0.08	NS	NS	NS
Metabolic chamber											
Energy balance (kcal/d)	-132 ± 46	-116 ± 44	NS	8 ± 42	-76 ± 63	NS	-163 ± 75	-191 ± 70	NS	NS	NS
24-h EE (kcal/d)	2324 ± 72	2311 ± 63	NS	2043 ± 85	2122 ± 104	NS	2203 ± 152	2184 ± 153	NS	NS	NS
Sleep EE (kcal/d)	1903 ± 54	1915 ± 55	NS	1699 ± 72	1757 ± 78	NS	1821 ± 129	1800 ± 121	NS	NS	NS
NENS EE (kcal/d)	1850 ± 60	1837 ± 52	NS	1617 ± 72	1703 ± 91	NS	1747 ± 123	1747 ± 127	NS	NS	NS
Activity %	15.5 ± 1.6	16.3 ± 1.7	NS	13.9 ± 1.9	14.2 ± 1.7	NS	17.5 ± 2.6	15.6 ± 2.3	NS	NS	NS
Fuel utilization											
Fasting RQ	0.83 ± 0.01	0.82 ± 0.01	NS	0.83 ± 0.01	0.82 ± 0.01	NS	0.83 ± 0.01	0.80 ± 0.01	NS	NS	NS
Clamp RQ	0.86 ± 0.01	0.86 ± 0.01	NS	0.87 ± 0.01	0.87 ± 0.01	NS	0.85 ± 0.01	0.84 ± 0.02	NS	NS	NS
24-h RQ	0.87 ± 0.01	0.88 ± 0.01	NS	0.86 ± 0.01	0.88 ± 0.01	NS	0.88 ± 0.01	0.89 ± 0.01	NS	NS	NS
CHO Ox	288.4 ± 15.4	308.2 ± 18.6	NS	255.6 ± 21.5	290.6 ± 23.8	NS	290.0 ± 21.3	299.7 ± 25.7	NS	NS	NS

P^1 is t test P value of change in parameters vs null. P^2 is t test P value of CrPic responders vs placebo. P^3 is t test P value of CrPic responders vs CrPic nonresponders. SubQ indicates subcutaneous; HGP, hepatic glucose production; NENS, nonexercise, nonsleep; CHO Ox, carbohydrate oxidation.

[a] Insulin sensitivity units = milligrams per kilogram FFM per minute.

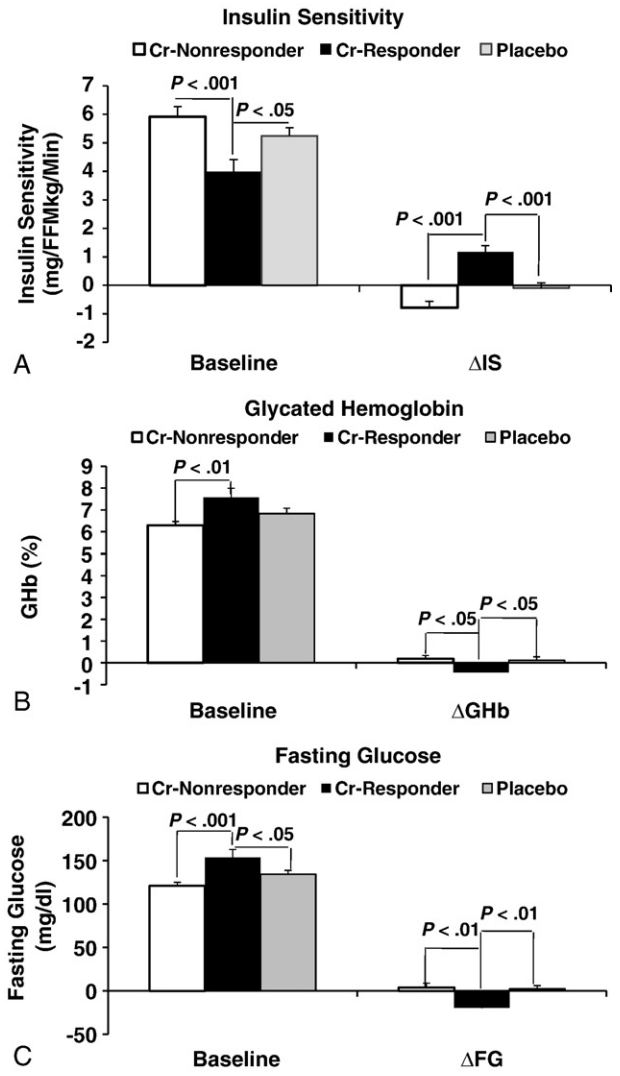

Fig. 1. Demonstrates baseline (preintervention) values and change in each parameter from baseline when assessed at end of study, that is, Δ for insulin sensitivity (A), fasting glucose (B), and glycated hemoglobin (C) in responders, nonresponders, and placebo groups.

dL) were not felt to be statistically significant. However, subjects classified as responders had significantly lower A_{1c} levels, fasting glucose, and glucose area under the curve (AUC) at end of study when compared with preintervention, and when compared with placebo and nonresponders at end of study (Table 2). Fasting glucose and A_{1c} levels, at preintervention, were significantly greater in responders as opposed to both nonresponders and placebo groups (Fig. 1B and C). For both A_{1c} and fasting glucose, the preintervention value significantly correlated to the clinical response as assessed by decrease in A_{1c}, that is, Δ A_{1c}, ($r = -0.57$, $P < .001$), and fasting glucose, that is, Δ fasting glucose, ($r = -0.63$, $P < .001$), for all Cr subjects. To evaluate the role of each subject's response as a function of the baseline value, the percentage change in each glycemic parameter was plotted vs the baseline level for each individual subject. As demonstrated in Fig. 2A, there was a statistically significant and negative correlation for the percentage change in fasting glucose for both Cr responders and placebo groups; but, as expected, there was no correlation of percentage change vs baseline fasting glucose in subjects identified by insulin sensitivity testing as nonresponders ($r = 0.07$, P = NS) (data not shown). In addition, there was a statistically significant and negative correlation for the percentage change of glycated hemoglobin as a function of the individual baseline value for Cr responders; and this value approached significance in the placebo group (Fig. 2B). There was no correlation of percentage change in glycated hemoglobin when plotted vs the baseline level in subjects identified by insulin sensitivity testing as nonresponders ($r = 0.05$, P = NS) (data not shown).

4.3. Chromium status

Chromium levels in serum or urine did not differ at baseline for subjects randomized to either Cr or placebo (Table 1). There was a significant increase in serum Cr in subjects randomized to Cr as opposed to placebo at midpoint (2.40 ± 0.19 vs 0.16 ± 0.05 ng/dL, $P < .0001$) and end of

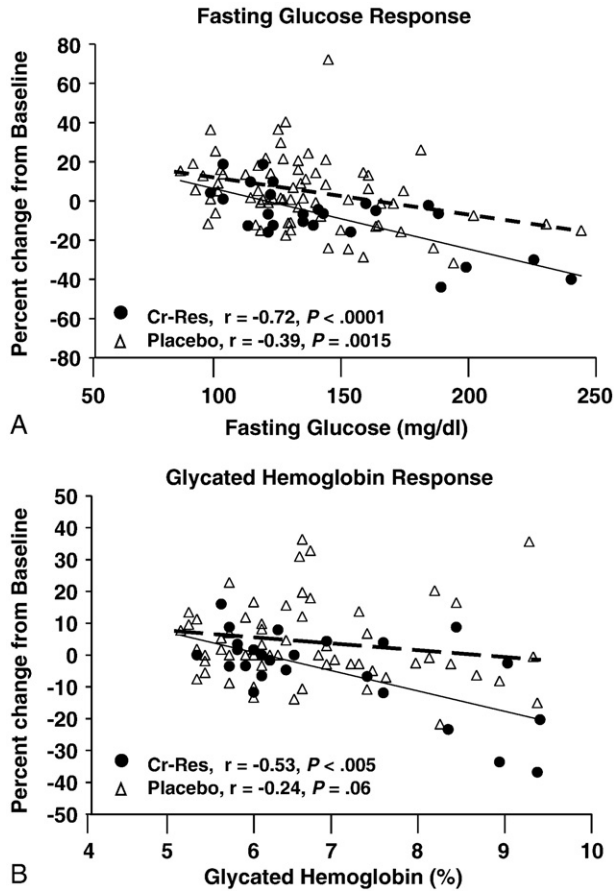

Fig. 2. Demonstrates the percentage change for fasting glucose (A) and glycated hemoglobin (B) when plotted vs the individual baseline value for responders and placebo groups. There was no correlation for percentage change in fasting glucose ($r = 0.07$, P = NS) or glycated hemoglobin ($r = 0.05$, P = NS) for nonresponders (data not shown).

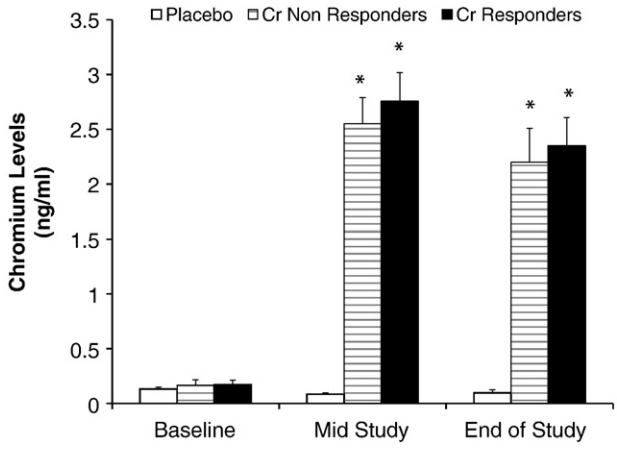

Fig. 3. Demonstrates serum Cr levels in responders, nonresponders, and placebo when assessed at baseline, midpoint, or end of study. *P < .001 vs placebo.

study (2.62 ± 0.09 vs 0.17 + 0.04 ng/dL, P < .0001). In addition, there was a significant increase in urine Cr in subjects randomized to Cr as opposed to placebo at midpoint (11.68 ± 3.39 vs 0.10 ± 0.03 ng/dL, P < .0001) and end of study (6.5 ± 1.13 vs 0.13 ± 0.06 ng/dL, P < .0001). There was no difference in either serum or urine Cr between responders and nonresponders when assessed at baseline, midpoint, or end of study, although both groups differed significantly from placebo (Fig. 3, Table 2).

4.4. Body weight/fat distribution

Body weight did not significantly increase from baseline for subjects randomized to either Cr or placebo (0.8 ± 0.5 vs 0.7 ± 0.6 kg, respectively). There was no difference between treatment groups for body weight, percentage body fat, FFM, or abdominal fat depots, that is, total abdominal fat, visceral abdominal fat, or subcutaneous abdominal fat. In addition, responders did not differ from nonresponders for body fat/composition measures (Table 2).

4.5. Energy balance

Mixed linear models indicated that change in food intake and percentage kilocalories from fat, carbohydrate, and protein did not differ by group. The AUC analysis indicated that change in hunger, desire to eat, fullness, and prospective food consumption did not differ by group (data not shown).

Twenty-four–hour EE, sleep EE, or percentage activity did not differ in subjects randomized to Cr (n = 30) vs placebo (n = 27). Nonexercise/nonsleep EE was increased from preintervention levels in subjects randomized to Cr (63 ± 33, P < .05) as opposed to placebo (−33 ± 30, P = NS), and these differences were statistically different between Cr and placebo groups at study end (P < .03). Subjects randomized to Cr appeared to be in a more negative energy balance compared with preintervention testing as compared with placebo (−63 ± 45, P = .06 vs 12 ± 36, P = NS), but these differences were not statistically significant between groups at end of study. Both responders and nonresponders trended to a more negative energy balance compared with placebo; but there were no significant changes in 24-hour EE, sleep EE, percentage activity, fasting RQ, 24-hour RQ, carbohydrate, or protein oxidation (Table 2).

4.6. Myocellular and intrahepatic lipids

Magnetic resonance spectroscopy scanning was completed on 56 subjects in the substudy (27 Cr, 26 placebo). There was a statistically significant negative correlation between insulin sensitivity and soleus muscle IMCL (r = −0.33, P < .02), tibialis muscle IMCL (r = −0.33, P < .03), and IHL (r = −0.42, P < .01) for all subjects. Compared with preintervention, subjects randomized to placebo had an increase in soleus muscle EMCL (0.006 ± 0.003 arbitrary scanning units [ASU], P = .05) compared with a decrease with Cr (−0.003 ± 0.003 ASU); and the value approached significance between groups (P = .07). There was an increase in tibialis muscle EMCL (0.009 ± 0.003 ASU, P < .01) and IHL (0.17 ± 0.08 ASU, P < .04) in the placebo group, whereas there were no significant changes from preintervention for Cr. When compared with nonresponders, responders had a significant decrease in tibialis muscle IMCL (P = .05), tibialis muscle EMCL (P < .03), and IHL (P < .04). When compared with placebo, responders had a significant decrease in soleus muscle EMCL (P < .02), soleus muscle IMCL (P < .04), and tibialis muscle EMCL (P < .02); and it approached significance for IHL (P = .055) (Table 2).

5. Discussion

This study demonstrated that in a well-characterized cohort of type 2 DM subjects representing a wide phenotype, for example, lean to obese, and wide range of glycemic control, and including both insulin-sensitive and insulin-resistant subjects, there was not a consistent effect of Cr supplementation to improve insulin action or glycemic control. However, in subjects responding to Cr, the effect of Cr to improve glycemia was secondary to enhanced insulin sensitivity in muscle. There was no effect of Cr on hepatic glucose production or on body weight/fat distribution. In addition, in those subjects responding to Cr, fasting glucose and A_{1c} were significantly higher and insulin sensitivity significantly was lower when assessed at preintervention. It was also observed that the clinical response to Cr was significantly related to the baseline A_{1c}, fasting glucose, and insulin resistance and that Cr status did not differentiate between responders and nonresponders. An interesting observation was that myocellular and intrahepatic lipid content was significantly related to insulin sensitivity and that both were reduced in subjects randomized to Cr.

An intriguing finding from this study was the effect of Cr to decrease tissue lipids. Accumulation of lipids in muscle and liver is postulated to impair insulin receptor signaling

and contribute to insulin resistance [18]. Several preclinical studies demonstrate an effect for Cr on lipid metabolism. Specifically, Sreejayan et al [19] reported that Cr treatment decreased liver triglyceride levels and lipid accumulation in animal models. Horvath et al [20] reported that Cr activates Glut-4 trafficking via a cholesterol-dependent mechanism and concluded that Cr^{3+} supplementation may lower blood glucose by altering the plasma membrane composition of cholesterol in fat and muscle cells. However, there have been no reports assessing myocellular lipids in human studies evaluating Cr supplementation. Myocellular and intrahepatic lipid contents were negatively correlated to insulin sensitivity, and both were decreased in subjects randomized to Cr who had improved insulin sensitivity. Tissue lipid content may decrease with lifestyle or drug-induced improvement in insulin sensitivity and, in the case of pharmacologic therapy, can improve tissue lipids without reduction in body weight [21]. The observations support the findings of enhanced insulin sensitivity and reduction in myocellular lipids without an effect on weight in responders. In addition, FFM was increased from baseline in responders and differed significantly from the placebo group. This observation in Cr-treated subjects has been reported previously, but the mechanism is not precisely known [6].

The measured response to an identical pharmacologic or lifestyle intervention will vary greatly among individuals, and the variation may be secondary to differences in genetic or physiologic makeup in addition to differences in other subject characteristics. The data demonstrated that Cr supplementation did not have a consistent and significant effect on metabolic parameters when evaluated over the entire cohort. As stated, the overall goal was to evaluate clinical Cr supplementation across a wide phenotype range and range of glycemia to specifically address the controversy surrounding Cr and recommended clinical use. However, it was concluded that the likelihood for a response to Cr is greater when insulin resistance is present and glucose control is poorer before supplementation, which confirms observations reported in both human and animal studies [5,8,22]. In addition to the data as outlined in Fig. 1 for which statistical differences were noted for baseline insulin sensitivity and fasting glucose in responders and nonresponders, the individual responses for such an effect were assessed. Fig. 2 demonstrates the percentage change for glycemic parameters obtained at end of study when plotted vs baseline value for fasting glucose and glycated hemoglobin. As demonstrated, the data suggest that, below a fasting glucose value of approximately 175 mg/dL and a glycated hemoglobin of 8%, a consistent effect for Cr supplementation to improve glycemia is not observed. Specifically, a considerable overlap is seen for individual responses for fasting glucose and glycated hemoglobin for those classified as responders vs those subjects randomized to placebo. Interestingly, as glycemic control worsens, for example, fasting glucose greater than 175 mg/dL and glycated hemoglobin greater than 8%, it appears that the likelihood for an effect of Cr supplementation was greater. Clearly, there were too few subjects in these higher glycemic ranges to make a definitive statement in this regard. However, the trend appears to be consistent with findings from prior clinical studies suggesting that a formulation of Cr that also contained biotin, and when administered as an adjuvant to current prescription antidiabetic medication, improved glycemic control especially in those patients with poor glycemic control on oral therapy [5]. This observation may partially explain the reported discrepancies in response to Cr in humans and why Cr supplementation appears to have a more predictable response in hyperinsulinemic or obese states [3,8,23]. The mechanism by which hyperinsulinism, insulin resistance, and/or obesity plays a role in Cr response or how Cr contributes to development of these phenotypes is currently unknown. However, this study clearly demonstrates that simply increasing Cr levels in serum is not sufficient for a response, as the Cr status did not differ between responders and nonresponders. Therefore, a very relevant question is the specific mechanism that accounts for the whole-body response to Cr when circulating levels do not appear to differentiate between responders and nonresponders. At this time, the reasons are not known for this observation; and mechanistic studies evaluating gene expression, cellular protein abundance, cytosolic enzyme activity, etc, are clearly required to address this observation.

The decision to use a specified change in insulin sensitivity instead of fasting glucose or A_{1c} levels to stratify subjects as to clinical response was based on the following rationale. Firstly, although A_{1c} represents the criterion standard for assessing glycemic control, it is well known that the decrease in A_{1c} with treatment depends on the baseline level, with a greater percentage decrease observed if the levels are higher before treatment [24]. As such, using a prespecified percentage decrease in A_{1c} to define a responder would have to vary according to the baseline level. Secondly, it has been shown that Cr can improve insulin sensitivity with minimal effect on glycemia as observed in studies evaluating subjects who were nondiabetic but obese and with a family history of type 2 diabetes [25]. Therefore, having to evaluate response on glycemia alone could potentially classify patients incorrectly as nonresponders even though they may have had a significant improvement for insulin sensitivity. Thirdly, in preliminary studies, insulin resistance was demonstrated as the most predictive factor in assessing response to Cr [8]. Thus, the rationale to stratify subjects based on changes in insulin sensitivity seemed not only sound, but highly clinically relevant.

In summary, with use of "state-of-the-art" metabolic techniques and in a well-characterized cohort of individuals with type 2 DM representing a wide range of phenotype, glycemic parameters, and parameters assessing whole-body insulin action, a consistent effect of Cr was not observed. However, this study is the first to show that Cr levels after supplementation do not differ between responders and nonresponders, and provides the first comprehensive

assessment of physiologic and biochemical characteristics of individuals who responded to Cr. Specifically, "response" to Cr is more likely in insulin-resistant individuals who have more elevated fasting glucose and A_{1c} levels. Another novel finding was that tissue lipids are decreased in subjects randomized to Cr. Thus, it may be postulated that Cr alters insulin sensitivity through modulation of lipid metabolism in peripheral tissues from insulin-resistant subjects, and may represent a unique mechanism of action for trace minerals in that setting. The mechanism for this effect is the focus of ongoing studies.

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