Chromium Picolinate Enhances Skeletal Muscle Cellular Insulin Signaling In Vivo in Obese, Insulin-Resistant JCR:LA-cp Rats


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ABSTRACT Chromium is one of the few trace minerals for which a specific cellular mechanism of action has not been identified. Recent in vitro studies suggest that chromium supplementation may improve insulin sensitivity by enhancing insulin receptor signaling, but this has not been demonstrated in vivo. We investigated the effect of chromium supplementation on insulin receptor signaling in an insulin-resistant rat model, the JCR:LA-corpulent rat. Male JCR:LA-cp rats (4 mo of age) were randomly assigned to receive chromium picolinate (CrPic) (obese n = 6, lean n = 5) or vehicle (obese n = 5, lean n = 5) for 3 mo. The CrPic was provided in the water, and based on calculated water intake, rats randomized to CrPic received 80 μg/(kg·d). At the end of the study, skeletal muscle (vastus lateralis) biopsies were obtained at baseline and at 5, 15, and 30 min postinsulin stimulation to assess insulin signaling. Obese rats treated with CrPic had significantly improved glucose disposal rates and demonstrated a significant increase in insulin-stimulated phosphorylation of insulin receptor substrate (IRS)-1 and phosphatidylinositol (PI)-3 kinase activity in skeletal muscle compared with obese controls. The increase in cellular signaling was not associated with increased protein levels of the IRS proteins, PI-3 kinase or Akt. However, protein tyrosine phosphatase 1B (PTP1B) levels were significantly lower in obese rats administered CrPic than obese controls. When corrected for protein content, PTP1B activity was also significantly lower in obese rats administered CrPic than obese controls. Our data suggest that chromium supplementation of obese, insulin-resistant rats may improve insulin action by enhancing intracellular signaling. J. Nutr. 136: 415–420, 2006.

KEY WORDS: cellular signaling · chromium · obesity · insulin resistance

Nutritional supplementation with over-the-counter agents, such as chromium, is commonly practiced by the general public and by patients with diabetes in particular. Unfortunately, considerable controversy exists regarding the effectiveness of chromium supplementation to improve carbohydrate metabolism in individuals with diabetes. The controversy surrounding chromium supplementation stems from the lack of definitive randomized trials because many of the earlier studies evaluating chromium were open label and therefore generated substantial bias. Additional concerns were the lack of "gold standard" techniques to assess glucose metabolism, the use of differing doses and formulations, and the study of heterogeneous study populations. As such, conflicting data were reported and they have contributed greatly to the confusion among health-care providers concerning the routine use of chromium as a dietary supplement. More recent evidence, however, supports the concept that chromium supplementation yields more consistent clinical effects on carbohydrate metabolism, particularly when consumed at higher doses, e.g., ≥200 μg/d (1–3).

Despite the considerable clinical interest, chromium is one of the only trace minerals for which a specific cellular mechanism of action has not been identified. Early reports suggested that chromium enhanced insulin binding, insulin receptor number, insulin internalization, and β cell sensitivity (4). In addition, chromium was reported to modulate the activity of phosphotyrosine phosphatase, the enzyme that cleaves phosphate from the insulin receptor (5). We reported that chromium supplementation enhanced skeletal muscle glucose transporter 4 (GLUT-4) translocation and increased insulin sensitivity in a rat model of obesity and insulin resistance, i.e., the JCR-LA-corpulent (JCR-LA-cp) rat, suggesting an improvement in insulin receptor signaling (6). Recent studies reporting the isolation of a protein termed chromodulin that avidly binds chromium also linked chromium to receptor signaling (7–9). It was proposed that chromium-bound chromodulin participates as part of an insulin signal amplification system as the complex binds to insulin-activated insulin

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MATERIALS AND METHODS

Study design. The effect of chromium supplementation on cellular insulin signaling in skeletal muscle was assessed in JCR:LA-cp rats, a strain incorporating the autosomal recessive cp gene that induces obesity (10,11). JCR:LA-cp rats that are homozygous for the autosomal recessive cp gene (cp/cp) lack membrane-bound leptin receptors, leading to marked obesity (12). The cp/cp rats are hyperphagic and become insulin resistant, hyperinsulinemic, and hypertglycemic. In addition, they develop advanced atherosclerotic disease as well as myocardial lesions consistent with an ischemic origin (13,14). The hyperinsulinemia develops rapidly after 4 wk of age, with an age of 5.5 wk for half-maximum. Breeding is done using heterozygous rats (cp/+), and the rats were randomly assigned to receive chromium picolinate (CrPic) (cp/cp) or to the control group (cp/cp). To assess phosphorylation for the substrate of interest, during the treatment phase, skeletal muscle from obese, lean, and control rats was obtained before and at the end of insulin stimulation to assess insulin and glucose levels followed by i.p. injection of regular insulin (5 U/kg) at time 0. At 5, 15, and 30 min postinsulin, repeated biopsies of the vastus lateralis were obtained along with repeated tailcuts for assessment of glucose and insulin. The rats were then killed by rapid decapitation. The rate of glucose disappearance [mmol/(L·min)] was determined as described (6).

Insulin signaling studies in skeletal muscle. Skeletal muscle protein content for phosphatidylinositol (PI)-3 kinase, insulin receptor substrate (IRS)-1, IRS-2, insulin receptor, Akt, and PTB1B was determined by Western blotting techniques as previously described (6,19). Specific antibodies used were anti-IRS-1 antibody (IRS-1) (Upstate Biotechnology), anti-IRS-2 (Santa Cruz Biotechnology), anti-Akt (Upstate Biotechnology), anti-PI-3 kinase antibody (Upstate Biotechnology), anti-PTB1B (Upstate Biotechnology), or anti-insulin receptor β-subunit antibody (Santa Cruz Biotechnology).

PTB1B activity. The activity of PTB1B was assayed by hydrolysis of p-nitrophenol phosphate (PNP). Skeletal muscle lysate was prepared as outlined above. PTB1B was immunoprecipitated with specific antibody (Upstate Biotechnology). The immunoprecipitate was incubated in phosphatase reaction buffer (20 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl, 5 mmol/L dithiothreitol, 1 mmol/L PNP) for 20 min at 37°C. The reaction was stopped with 0.2 mol/L NaOH, and the absorbance at 410 nm was measured. The reactions were run in triplicate (20).

Insulin-stimulated tyrosine phosphorylation and PI-3 kinase activity. To assess phosphorylation for the substrate of interest, biopsies were taken at baseline and postinsulin stimulation (5, 15, and 30 min postinsulin), quickly frozen in liquid nitrogen, and stored at −70°C. Homogenates made from muscle tissue as outlined above were precipitated with an antiphosphotyrosine antibody (PY-20) (Transduction Laboratories, 1:250). Immunoprecipitate collected with protein A-agarose was washed 5 times in ice-cold 50 mmol/L HEPES buffer containing 150 mmol/L NaCl, 0.1% Triton X-100, and 10 μmol/L Na3VO4. The pellets were treated with Laemmli sample buffer containing 100 mmol/L dithiothreitol and heated in a boiling water bath for 5 min. The proteins were separated by 10% SDS-PAGE, transferred to nitrocellulose and immunoblotted. For determination of PI-3 kinase activity, the immune complex was washed, and PI-3 kinase measured as described (21).

Statistical analysis. Data were analyzed by 2-way ANOVA and the Scheffé F-test for post hoc analysis when the interaction was significant. Differences were considered significant at P < 0.05.

RESULTS

The effects of CrPic on clinical variables in this animal cohort were reported previously (6). Specifically, CrPic did not affect daily food and water intakes or body weights over the 12-wk treatment period. Based on the measured food and water intake, the control groups had elemental Cr intake ranging from 6 to 20 μg/kg, whereas the Cr-supplemented groups had daily elemental Cr intake ranging from 33 to 38 μg/kg. The variables assessed at the end of the study in lean and obese rats for control and CrPic groups are outlined in Table 1 (6). Obese rats administered CrPic had significantly reduced insulin concentrations after food deprivation than obese controls, whereas insulin levels did not differ between the 2 groups of lean rats (Table 1) (6). Obese rats administered CrPic had a significantly reduced area under the curve for insulin during the glucose tolerance test compared with obese controls (data...
not shown) (6). In addition, obese rats administered CrPic had lower plasma cholesterol levels, improved total: HDL cholesterol ratios, and higher membrane-associated GLUT-4 levels in skeletal muscle after insulin stimulation than obese control rats (6). Lean rats had significantly greater glucose disposal rates than obese controls (Fig. 1). Cr supplementation did not affect glucose disposal rates in the lean rats. However, the attenuation in glucose disposal rates in the obese rats was significantly improved with CrPic supplementation.

Representative blots from Western Blot analysis of skeletal muscle protein content for IRS-1, IRS-2, PI-3 kinase, and Akt for all experimental groups are shown in Figure 2. Although there appeared to be a higher content in the CrPic-treated obese rats than obese control rats, these differences were not significant (P = 0.15). In addition, the insulin receptor content did not differ between experimental groups (data not shown).

Insulin stimulation significantly increased skeletal muscle IRS-1 phosphorylation in lean control rats compared with obese controls (Fig. 3). Cr supplementation did not increase IRS phosphorylation in the lean rats relative to levels in lean controls. However, in obese rats administered CrPic, IRS-1 phosphorylation after insulin stimulation was significantly greater than in obese controls at 5 min postinsulin (Fig. 3).

IRS-1–associated PI-3 kinase activity was not increased in lean rats administered CrPic compared with lean control rats (see lanes 2–4 compared with lanes 6–8, Fig. 4A and Fig. 5). IRS-1–associated PI-3 activity was lower in obese control rats than in lean controls (see lanes 10–12 compared with lanes 2–4, Fig. 4A and Fig. 5). However, obese rats administered CrPic had significantly greater activity than obese controls (see lanes 14–16 compared with lanes 10–12, Fig. 4A; see lanes 10–12 and 14–16 compared with lanes 2–4 and 6–8, Fig. 4B and Fig. 5).

Obese control rats had significantly higher protein content for PTP1B than lean controls (Fig. 6A). In the lean rats, CrPic supplementation did not alter protein content of PTP1B. However, PTP1B levels were significantly reduced in obese rats administered CrPic compared with obese controls (Fig. 6A). When corrected for protein content, the lean rats did not differ in PTP1B activity, whereas obese rats administered CrPic had significantly lower PTP1B than obese controls (Fig. 6B).

DISCUSSION

This study demonstrated that CrPic supplementation improved insulin action in vivo in an insulin-resistant rat model by enhancing signaling through the insulin receptor. Specifically, increases in IRS-1 phosphorylation and IRS-1–associated PI-3 kinase activity were observed in vivo in skeletal muscle after insulin stimulation in obese JCR:LA-cp rats administered CrPic compared with obese controls. The enhanced signaling occurred...
without an increase in the content of proteins involved in the insulin signaling cascade, e.g., IRS, PI-3 kinase, and Akt. However, the modulation of a protein tyrosine phosphatase, i.e., PTP1B, by chromium levels was suggested by the decreases in enzyme activity in obese rats administered CrPic.

The effect of chromium to improve carbohydrate metabolism in subjects thought to be Cr deficient, and representative of a Cr-deficient state, is well documented (22,23). However, the effect of chromium on insulin action in subjects not likely to be Cr deficient is an area of great controversy. The controversy stems in large part from the conflicting data reported in previous studies. Many of the early studies evaluating chromium were open label and therefore generated substantial bias, did not employ “gold standard” techniques to assess glucose metabolism, used differing doses and formulations, and evaluated heterogeneous study populations (1). The controversy is further fueled by the observation that a specific mechanism for chromium’s effects has not been elucidated. Thus, chromium remains a trace mineral whose mechanism of action is not precisely known.

To gain a better understanding of the mechanism by which chromium may improve insulin action, it is important to understand the mechanisms contributing to insulin resistance in JCR:LA-cp rats. It was reported that a specific cellular signaling abnormality, i.e., enhanced serine kinase activity, in JCR:LA-cp rats was similar to that in a genetically unrelated insulin-resistant rat model, i.e., the Zucker fatty rat, suggesting that the findings apply generally to insulin-resistant states (24,25). Recent studies demonstrated that IRS-1 becomes serine phosphorylated after prolonged exposure to many factors, e.g., tumor necrosis factor-α, glucose, or free fatty acids, and does not become tyrosyl-phosphorylated, resulting in attenuation of insulin signaling (24–28). Therefore, an attractive hypothesis has emerged, suggesting that serine phosphorylation of IRS proteins contributes to insulin resistance in JCR:LA-cp rats. The data obtained from this study would support this hypothesis. Specifically, we demonstrated that obese rats had attenuated IRS-1 phosphorylation and PI-3 kinase activities after insulin stimulation compared with lean controls. The observation that IRS-1 phosphorylation and PI-3 kinase activities were increased in obese rats administered CrPic suggests that one mechanism by which Cr enhances cellular signaling in JCR:LA-cp rats may be secondary to reducing serine kinase activity. Unfortunately, serine phosphorylation of IRS-1 was not measured in this study. Furthermore, an increase in serine phosphorylation of IRS-1 was shown to target the protein for ubiquitin-proteasome-mediated degradation, resulting in

![FIGURE 3](image-url)  
**FIGURE 3** Skeletal muscle insulin stimulated IRS-1 phosphorylation in lean and obese JCR:LA-cp rats that consumed water with or without CrPic for 12 wk. Values are means ± SEM, n = 5–6. Means at a time without a common letter differ, P < 0.05.

![FIGURE 4](image-url)  
**FIGURE 4** IRS-1 associated PI-3 kinase activity in skeletal muscle in lean and obese JCR:LA-cp rats that consumed water with or without CrPic for 12 wk.

![FIGURE 5](image-url)  
**FIGURE 5** IRS-1 associated PI-3 kinase activity in skeletal muscle in lean and obese JCR:LA-cp rats that consumed water with or without CrPic for 12 wk. Values are means ± SEM, n = 5–6. Means at a time without a common letter differ, P < 0.05.

![FIGURE 6](image-url)  
**FIGURE 6** PTP1B abundance (A) and PTP1B activity (B) in vastus lateralis muscle in lean and obese JCR:LA-cp rats that consumed water with or without CrPic for 12 wk. Values are means ± SEM, n = 5–6. Means without a common letter differ, P < 0.05.

![Other figures and tables]
a decreased content of IRS-1 (29,30). In this study, however, the cytosolic content of IRS proteins, PI-3 kinase, or Akt, did not differ between the lean, insulin-sensitive and obese, insulin-resistant rats.

The data also suggest that phenotype must be considered when evaluating the effects of chromium on insulin action in vivo. As observed, the lean rats were much more insulin sensitive than the obese rats, and insulin sensitivity was not increased further in the lean rats administered CrPic. However, the attenuation in insulin action in the obese rats was partially restored with CrPic. Therefore, a very relevant question would be whether hyperinsulinism, insulin resistance, and/or obesity plays a role in Cr metabolism and/or excretion. Such an observation, if validated, may partially explain the reported discrepancies in response to Cr in the human population and why Cr supplementation appears to have a more predictable response in hyperinsulinemic or obese states (1,31,32).

The in vivo data observed in JCR:LA-Cp rats correlates well with the cellular signaling data. Specifically, IRS-1 phosphorylation and PI-3 kinase activity did not differ in the lean rats administered CrPic compared with controls. However, obese rats had reduced IRS phosphorylation and activation of PI-3 kinase after insulin stimulation compared with lean controls. Both of these cellular pathways were partially restored in obese rats administered CrPic, but not to the levels in lean rats. The enhanced insulin signaling resulting from CrPic supplementation in obese rats would be expected to enhance the regulated movement of GLUT-4 and, subsequently, increase glucose disposal. As previously reported (see Table 1), enhanced GLUT-4 translocation in skeletal muscle was observed in obese rats administered CrPic (6). Thus, it appears that the responsible upstream cellular signals for the enhanced GLUT-4 translocation (i.e., IRS phosphorylation, PI-3 activity) were increased in the present study with CrPic. Our observations are in agreement with the findings from a recent in vitro study in mouse muscle cells suggesting that chromium increases both receptor kinase and IRS-1 phosphorylation (33).

Our finding that skeletal muscle from obese JCR:LA-cp rats had increased protein phosphatase contents compared with lean controls is of great interest. Protein tyrosine phosphorylation is controlled through coordinated actions of both protein tyrosine kinases (PTK) and phosphatases (PTP) (34). A disturbance of the normal balance between PTK and PTP function results in aberrant tyrosine phosphorylation and is implicated in the etiology of a number of human diseases, including diabetes. The enzyme PTP1B appears to be the prototypical member of the PTP family, which dephosphorylates and inactivates the insulin receptor (35). Additional evidence supporting a role of PTP1B in insulin signaling comes from gene deletion studies in which PTP1B knockout mice were more insulin sensitive, and this was associated with enhanced tyrosine phosphorylation of the insulin receptor (36). PTP1B levels and activity were elevated in obese JCR LA-cp rats compared with lean rats, but PTP1B levels and activity were decreased in the obese rats administered CrPic. The reasons for the decrease in PTP1B levels in rats administered CrPic are not known but it is possible that CrPic modulates gene expression and/or protein degradation. The data also demonstrated that chromium had no effect on phosphatase activity in the lean rats, suggesting that chromium-mediated effects of phosphatase activity may be secondary to a change in insulin sensitivity rather than the mediator of a change in insulin sensitivity. To the best of our knowledge, this observation regarding PTP1B levels and activity was not reported previously for in vivo studies. However, our findings appear to be in contrast to an earlier report, suggesting that a chromium complex actually increased PTP activity in membranes, an activity that should result in attenuated, rather than enhanced insulin action (5). In addition, Wang et al. (37) reported no effect of Cr on PTP1B activity in tissue culture studies. The differences in our findings and those of Wang et al. may be secondary to the characteristics of the tissue studied. Specifically, PTP1B levels or activity in skeletal muscle from lean rats did not differ because no attenuation in insulin action in vivo was observed. The effect of CrPic to modulate insulin action and PTP1B activity was seen entirely in the obese rats. In the studies of Wang et al. (37), a Chinese hamster ovary-IR cell culture was used, which may not have expressed the same defect in insulin action that occurred in our obese rats. Thus, it appears that in insulin-resistant states, the abnormalities in insulin action are partially reversible with chromium supplementation. However, in insulin-sensitive states in which the abnormalities are not present, chromium has no observable effect.

Finally, the dose of CrPic used in this study must be put in perspective. For humans, adequate intake of Cr for men may be \( \sim 35 \mu g/d \). Assuming an average 75 kg body mass, this would relate to an intake of elemental Cr of \( \sim 0.47 \mu g/kg \). In our recent human trial, we demonstrated improved insulin sensitivity with 1000 \( \mu g/d \) of Cr as CrPic (2) and, given the range of body weights of subjects, intake of Cr ranged from 10 to 13 \( \mu g/kg \). On the basis of the measured food and water intakes, rats treated with Cr in this study were receiving twice as much elemental Cr from the water as from the diet and had a total approximate daily intake \( > 30 \mu g/kg \). Thus, the rats in this study were administered supplemental Cr at levels greater than those observed to be effective in our human trials and could be considered a pharmacologic dose.

In summary, this study confirmed that chromium supplementation may increase glucose transport by enhancing cellular signaling through the insulin receptor in skeletal muscle of an insulin-resistant rat model. We observed an increase in IRS phosphorylation, PI-3 kinase activation, and a reduction in protein content of a specific protein phosphatase, PTP1B, in obese rats administered CrPic. The molecular targets specifically modulated by chromium are those that are responsible for activating these mechanisms have not been elucidated but are the focus of ongoing studies.

LITERATURE CITED


