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Chromium picolinate supplementation improves cardiac metabolism, but not myosin isoenzyme distribution in the diabetic heart

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Because chromium (Cr) containing compounds are thought to improve glucose homeostasis, we hypothesized that chromium picolinate (CrP) could partially reverse diabetes-induced damage to cardiac tissue. Young, adult female rats were fed either a basal diet (CONT), a basal diet containing no CrP and made diabetic (DIAB-CONT), or a basal diet containing 600 ng/g of CrP (3 times the suggested daily chromium intake) and made diabetic (DIAB-CrP). Diabetes was induced by a single streptozotocin injection, 55 mg/kg i.p. After 8 weeks animals were sacrificed, hearts removed, and spectrophotometrically analyzed for citrate synthase (CS), hexokinase (HK), and beta hydroxyacyl CoA dehydrogenase activity (HOAD). Cardiac myosin isoenzymes were separated from crude myofibril extracts by PAGE electrophoresis. Diabetes did not alter CS activity relative to the CONT group, but did significantly ($P < 0.05$) reduce HK and HOAD activity and expression of the high ATPase myosin isoenzyme V1. In contrast, DIAB-CrP animals displayed normal HK activity and greater HOAD activity relative to CONT animals. Surprisingly, the addition of CrP to the diet further reduced expression of the V1 myosin isoenzyme. These results demonstrate that dietary CrP supplementation has diverse effects on the subcellular properties of the diabetic heart. The functional impact of these CrP-induced changes remains to be defined. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:617–622, 1996.)

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Introduction

Chromium is considered a nutritionally essential trace element that appears to influence glucose homeostasis and lipid metabolism by potentiating the action of insulin.[1,2] Chromium, once absorbed, acts to potentiate the cellular action of insulin. Because diabetes causes a defect in glucose homeostasis, attempts have been made to identify a chromium deficiency in diabetics and to employ dietary chromium supplementation to ameliorate diabetic glucose intolerance. Diabetic patients have been found to have lower serum chromium levels[3,4] and a higher chromium excretion rate.[3,4] Treatment with chromium has been found by some to improve glucose tolerance in diabetic patients,[1,2,4] while other investigators have not found chromium supplementation to be effective in improving glucose tolerance in these patients.[5,6] Lack of controls, poor analytical techniques for measuring chromium, the heterogeneity of the diabetic population, and the use of pharmacologic rather than physiologic doses of chromium have been cited as reasons for these equivocal findings.[1] As a result, the chromium status of diabetics and the therapeutic value of chromium supplementation for this population remains somewhat controversial.

The impact of diabetes on the body is extremely widespread, producing defects and functional deficits in a variety of tissues, including the kidney, eye, blood, nerves, and blood vessels. These pathologies result from changes in a

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variety of cellular and subcellular processes. In the heart, diabetes is known to depress a number of subcellular processes including sarcoplasmic ATPase activity, control of calcium influx into cardiac myocytes, myosin ATPase activity, myosin isoenzyme distribution, and mitochondrial metabolic activity.[7] These changes, which significantly reduce cardiac functional capacity, result in a condition characterized as a diabetic cardiomyopathy. Treatment with exogenous insulin has proven successful in ameliorating these defects, suggesting that they are the direct result of an insulin deficiency.[8,9] If chromium supplementation can potentiate the action of insulin, then it is reasonable to speculate that chromium might improve cardiac specific changes caused by diabetes. Therefore, this study tested the hypothesis that dietary chromium supplementation would ameliorate subcellular defects present in the diabetic heart. Chromium was provided in the form of chromium picolinate, and the defects investigated included the well characterized diabetic-induced shifts in cardiac myosin isoenzyme distribution and cardiac metabolic potential.[7,9,10] Results demonstrate that dietary Cr supplementation impacted these properties of cardiac myocytes, but in a contrasting manner. The functional impact of these changes remains to be determined.

Methods and materials

Animal care

Immediately upon arrival, 45 young female Sprague-Dawley rats were placed on a semipurified AIN-76A basal diet.[11] After 3 days on this diet, the animals were placed in one of three dietary groups: control (CONT), diabetic control (DIAB-CONT), or diabeticchromium treated (DIAB-CrP). Chromium picolinate was added to the basal diet given to the DIAB-CrP animals (600 ng/g of basal diet), an amount of Cr that exceeds optimal daily intake by approximately three fold.[12] No chromium picolinate was added to the basal diet fed either the CONT or the DIAB-CONT animals. Despite using chromium-free mineral mix, analysis of the basal diet revealed that it was contaminated with approximately 180 ng of Cr/g of basal diet. This level of chromium contamination is consistent with previous reports of chromium concentrations present in presumably chromium-free, semipurified diets.[13,14] Animals had ad libitum access to food and water and were maintained under a 12:12-hr light cycle in a temperature-controlled facility. Animals were weighed three times per week and daily food intake was determined between the second and seventh weeks of the study. All procedures were approved by the appropriate Louisiana State University animal welfare committee.

Diabetes was induced in the DIAB-CONT and DIAB-CrP animals by a single intraperitoneal injection of streptozotocin (55 mg/kg). Diabetic animals were placed on the appropriate diet on the day before the streptozotocin injection. Four days after injection, a diabetic condition was confirmed by determining fasting serum glucose levels in all animals using a commercially available assay kit (No. 315, Sigma Chemical Co., St. Louis, MO USA). This route of streptozotocin injection, which has been shown to create a moderate diabetic state,[15] resulted in a mortality rate of 20%. Two DIAB-CONT animals and three DIAB-CrP animals died before the study was completed. Eight weeks after inducing diabetes, animals were anaesthetized, weighed, and sacrificed by exsanguination. Hearts were removed, trimmed free of connective tissue and the great vessels, weighed, and stored at −80°C for later biochemical analysis.

Maximal enzyme activities

Portions of the left ventricle were homogenized in a cold 175 mM KCl, 2 mM EDTA, 10 mM Tris HCl (pH 7.4) buffer. Protein content of the resulting homogenates was determined by the biuret method.[16] Aliquots were then removed from the homogenate and spectrophotometrically analyzed for maximal hexokinase (marker of glycolytic activity), citrate synthase (marker of Krebs cycle activity), and 3-hydroxyacyl CoA Dehydrogenase (HOAD) activity (marker of beta oxidation activity) as described by Haddad et al.[17] Activities of these enzymes are expressed as μmol/g min^{-1}.

Myosin isoenzyme distribution

Crude myofibrills were isolated from 200–250 mg of the left ventricle according to the methods of Tsika et al.[18] Individual myosin isoenzymes were separated using the PAGE electrophoretic methods of Hoh et al.[19] After separation, gel bands were visualized by means of commassie blue staining and quantified by means of a Zeineh Soft Laser Scanning Densitometer connected to an IBM personal computer equipped with a program for integration of peak areas. Quantification of the relative proportions of the individual myosin isoenzymes was achieved by first summing the densities of the three individual bands representing the three isoenzymes. This sum represented the total amount of myosin on a gel. The density of each individual band was then divided by the summed density to yield the relative quantity of the individual myosin isoenzymes, which is expressed as a per cent of the total myosin.

Statistical analysis

All data are presented as means ± standard deviation. Data were analyzed by means of a one-way analysis of variance (ANOVA) (GB Stat, Silver Springs, MD USA). When differences were detected, a Tukey's protected *T*-test was used to determine where those differences lie. Levels of significance were set at 0.05.

Results

Serum glucose levels

Four days after ip injection of streptozotocin serum glucose levels were 393 and 406 mg/dL for the DIAB-CONT and DIAB-CrP groups respectively, levels that were approximately three times higher than the serum glucose levels of the controls ($P < 0.05$). After 21 days, serum glucose levels were further elevated in the DIAB-CONT and DIAB-CrP groups, 509 and 567 mg/dL, respectively. These values were significantly different from that of the CONT group (129 mg/dL), but not significantly different between themselves. Both groups of diabetic animals suffered polyuria, polydipsia, and polyphagia, which combined with their elevated serum glucose levels, demonstrates that this method of streptozotocin injection-induced a moderate diabetic state.

Body weight, organ weight, and food intake

Diabetes resulted in a 5% reduction ($P < 0.05$) in body weight in both the DIAB-CONT and the DIAB-CrP groups in the first week after the streptozotocin treatment (*Figure 1*). Body weights of these animals remained relatively unchanged for the duration of the study but were significantly less than the body weight of the CONT animals who increased their body weight by approximately 18% over the

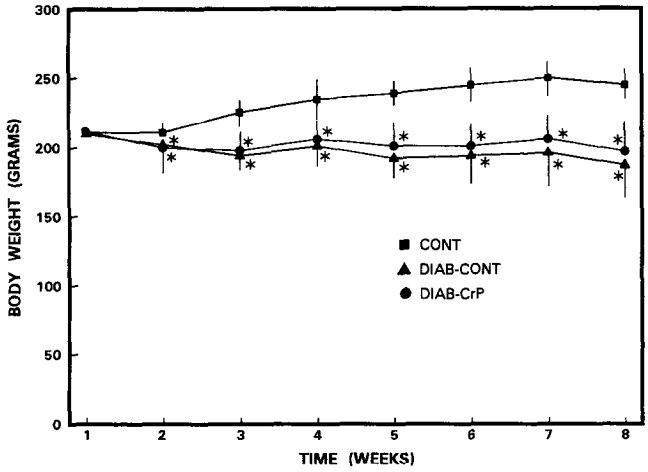

Figure 1 Body weight changes over the 8-week period of this study. Groups are as follows: CONT, untreated animals fed a basal diet; DIAB-CONT, group made diabetic by ip injection of streptozotocin (55 mg/Kg) and fed the basal diet; and DIAB-CrP, group made diabetic by ip injection of streptozotocin (55 mg/Kg) and fed the basal diet supplemented with chromium picolinate (600 ng/g). Data are presented as means ± standard deviation. * denotes a group mean that is significantly different from the group mean of the CONT group at the same time point.

course of the study. Both groups of diabetic animals maintained this relatively constant body weight despite the fact that they animals consumed approximately three times as much food as the CONT group (*Figure 2*). The amount of chromium ingested appeared to have little effect on body weight since the DIAB-CrP animals consumed approximately four times as much chromium as the DIAB-CONT. Left ventricle wet weight was significantly lower in the two diabetic groups (*Table 1*), but when normalized to body weight, the hearts of these animals were significantly larger than those of the CONT group. These data suggest that cardiac mass was preserved in the diabetic state while body weight was not. Again, neither the dietary content of Cr nor the amount of Cr ingested influenced this measure. Supplementing the diet with Cr did increase the protein content of the heart relative to that of the DIAB-CONT (*Table 1*). However, this observation is difficult to interpret since diabetes alone did not significantly reduce cardiac protein content.

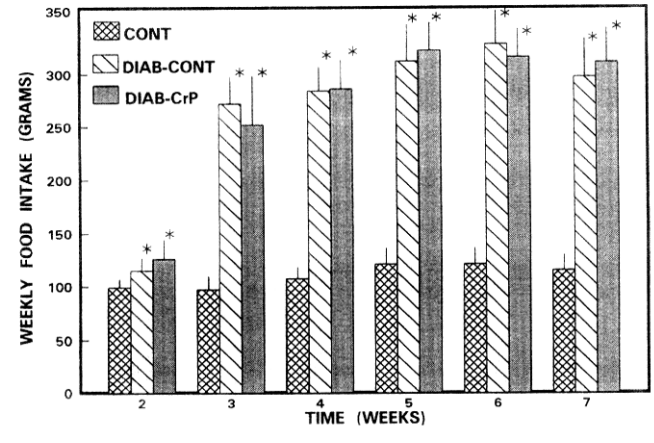

Figure 2 Weekly food intake over the course of this study. Groups are the same as defined in *Figure 1.* Data are presented as means ± standard deviation. * denotes a group mean that is significantly different form the group mean of the CONT group.

Table 1

	BW (g)	LV (mg)	LV/BW (mg/g)	Protein (mg/g)
CONT (n = 10)	240 ± 10.7	600 ± 32.1	2.49 ± 0.119	187 ± 14.1
DIAB-CON (n = 10)	187 ± 20.6*	530 ± 51.0*	2.74 ± 0.123*	176 ± 10.1
DIAB-CRP (n = 10)	179 ± 22.3*	499 ± 63.0*	2.84 ± 0.209*	192 ± 10.8#

Body weight (BW), left ventricle weight (LV), and protein content of the left ventricle of animals from the control (CONT), diabetic (DIAB-CON), and diabetic chromium (DIAB-CRP) treated groups.
Data are presented as means ± standard deviation.
* indicates a group significantly different from the CONT group.
# indicates a group significantly different from the DIAB-CON group.

Maximal enzyme activities

The maximal activities of marker enzymes were determined under optimal conditions. Citrate synthase activity was unaffected by either the induction of diabetes or the Cr content of the diet (*Table 2*). In contrast, diabetes significantly reduced hexokinase activity relative to the activity of the CONT group, while dietary Cr supplementation increased the activity of this glycolytic marker enzyme to that present in the CONT group. Diabetes alone increased the activity of HOAD 42% above that of the CONT group ($P < 0.05$). Dietary chromium supplementation increased cardiac HOAD activity an additional 23% above that of the DIAB-CONT group ($P < 0.05$).

Myosin isoenzyme distribution

Rodent cardiac myosin occurs in three different isoenzymatic forms designated as V1, V2, and V3. These isoen-

Table 2

	CS	HK	HOAD
	(mmol gr wet weight^{-1} min^{-1})		
CONT (n = 10)	183 ± 17	1.63 ± 0.056	44.4 ± 2.2
DIAB-CON (n = 10)	193 ± 21	1.49 ± 0.059*	63.2 ± 4.8*
DIAB-CRP (n = 10)	190 ± 19	1.64 ± 0.128#	77.6 ± 5.2*#

Metabolic enzyme activities of left ventricle homogenates. The enzymes measured include citrate synthase (CS), hexokinase (HK), and beta hydroxyacyl Co A dehydrogenase (HOAD). Data are presented as means ± standard deviation.
* indicates a group significantly different from the CONT group.
# indicates a group significantly different from the DIAB-CON group.

zymes differ in their electrophoretic mobility and ATPase activity.[19] As shown in *Figure 3*, the cardiac myosin of young adult rats occurs predominantly (>85%) in the high ATPase myosin isoenzyme, V1 isoenzyme. The lower ATPase isoenzymes, V2 and V3, constitute only 10 to 15% of the total cardiac myosin. Diabetes dramatically altered the isomyosin profile, reducing the amount of the V1 isoenzyme by approximately 50% while increasing the amount of the V2 and V3 isoenzymes by approximately 4 and 8 fold, respectively. Such myosin isoenzyme shifts typically occur in the diabetic heart.[9,10,15] Supplementing the diet with chromium picolinate further accentuated the impact of diabetes on myosin isoenzyme profile. In the DIAB-CrP group, the V1 isoenzyme constituted only 19% of the total myosin, whereas expression of the V3 isoenzyme increased to 60% of the total myosin present in the heart. Expression of the V2 isoenzyme remained essentially insensitive to the CrP supplementation.

Discussion

The central defect of diabetes, a reduced availability and hence use of glucose for cellular metabolism, secondarily affects many cellular processes of the heart in a deleterious manner.[7] On the other hand, chromium has been found to enhance cellular processes directly responsible for insulin action and glucose transport,[20,21] but no data are available that describe the effects supplementation of this micronutrient might exert on cellular properties secondarily affected by diabetes. Therefore, this study examined the impact chromium picolinate supplementation might have on cardiac myosin isoenzyme distribution and cardiac metabolism, properties of cardiac muscle cells altered by diabetes. As demonstrated in the current study and by others, chemically induced diabetes increases expression of the low ATPase isoenzyme, V3, while decreasing expression of the high ATPase myosin isoenzyme, V1 (*Table 2*).[8,9,15] Both exogenous insulin treatment and pharmacologic blockade of cardiac fatty acid oxidation can partially reverse the effects of diabetes on the distribution contractile protein, leading to the suggestion that glucose utilization for energy provision contributes to the regulation of the cardiac myosin isoenzyme phenotype.[23] Because Cr is thought to potentiate the action of insulin, we hypothesized that dietary chromium picolinate supplementation would increase the amount of the V1 isoenzyme in the diabetic heart, while decreasing the amount of the V3 isoenzyme.

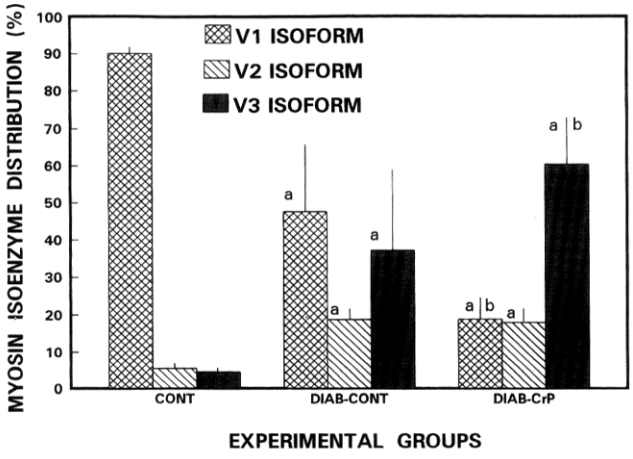

Figure 3 The relative distribution of cardiac myosin among its three isoenzymatic forms in diabetic rats fed a diet with and without chromium picolinate. Groups are the same as defined in *Figure 1*. Data are presented as means ± standard deviation. "a" denotes a group mean that is significantly different from the group mean of the CONT group. "b" denotes a group mean that is different from the group mean of the DIAB-CONT group.

Surprisingly, the current study found that adding chromium picolinate to the diet had the opposite effect on the myosin isoenzyme phenotype (*Figure 3*), further increasing expression of the V3 isoenzyme while decreasing expression of the V1 isoenzyme. Available evidence supports two possible explanations for these findings. First, the observed shift in the isomyosin profile of the DIAB-CrP animals raises the possibility that chromium picolinate supplementation further diminished glucose utilization in the diabetic heart. However, the potential for processing glucose appears to be increased in the DIAB-CrP animals because hexokinase activity equals that found in the CONT group (*Table 2*). Such a change in hexokinase activity is consistent with an increase, not a decrease in glucose utilization by the diabetic heart.[24] Glucose transport is the limiting factor in glucose utilization, but glucose transport was not measured in this study, making it impossible to dismiss the possibility that dietary CrP further reduced glucose transport or insulin action in the diabetic hearts. If this were the case, then glucose availability to cardiac myocytes would be more limited in the DIAB-CrP group, and the suspected improvement in the potential for glucose utilization would be of limited physiological value. This possibility seems highly unlikely as the accepted action of chromium is to improve not depress glucose transport.[1,2]

The second explanation for the cardiac myosin phenotype present in the DIAB-CrP group lies in the possibility that the chromium picolinate treatment acted independently of any changes caused by the diabetic state. Okada et al.[25] have observed that $^{51}CrCl_3$ given to intact animals preferentially binds to the nuclei of liver cells and enhances RNA synthesis in a dose dependent manner. Furthermore, in vitro experiments suggest that trivalent chromium increases RNA synthesis by increasing the number of initiation sites.[26] Alterations in the myosin phenotype, such as seen in the current study, arise from changes in the expression of specific genes. Increased expression of the V3 isoenzyme results from an increased expression of the beta myosin heavy chain gene, the gene that encodes for the heavy chain unique to this isoenzyme. Simultaneously, expression of the alpha myosin heavy chain gene, the gene that encodes for the myosin heavy chain unique to the V1 isoenzyme, is diminished.[27] This redirection of gene expression results in a phenotype characterized by greater amounts of the V3 isoenzyme and lesser amounts of the V1 isoenzyme. Hence, the chromium picolinate may have acted directly on these genes to produce the myosin phenotype observed in the hearts of the DIAB-CrP group. Supporting this suggestion is the observation that dietary supplementation with chromium picolinate (1500 ng/g of diet) significantly decreased ex-

pression of the V1 isoenzyme in hearts of normal animals in the absence of any apparent change in the capacity of the heart to utilize glucose.[28]

The increased amount of the V3 isoenzyme in the diabetic heart is consistent with a decline in intrinsic cardiac contractility and is frequently cited as a cause of the diminished functional capacity of the diabetic heart.[8,9] As such, chromium picolinate supplementation may be perceived as further reducing cardiac functional capacity of the diabetic heart. However, it is important to note that the effect of diabetes on cardiac contractile properties is not limited to changing the myosin isoenzyme profile. A variety of cellular processes which are essential in the maintenance of normal cardiac contractile function, including sarcolemmal Na/K-ATPase activity, sarcoplasmic reticulum Ca^{2+}/Mg2+ activity and mitochondrial activity, are deleteriously affected by diabetes.[7] It is, therefore, difficult to unequivocally identify a single subcellular change as the sole cause of a decline in cardiac functional capacity. Additionally, the in vitro working heart model has been widely used to assess the functional effect of the myosin isoenzyme shift induced by diabetes.[8,9] In contrast to this model, an increased expression of the V3 isoenzyme has been shown to either have no effect or actually improve cardiac functional capacity when measured in intact animals experiencing the elevated cardiovascular stress imposed by exercise.[29,30] Both the cause and the functional ramifications of the increased expression of the V3 myosin isoenzyme in the hearts of the DIAB-CrP remains to be explained.

Chronic diabetes substantially reduces cardiac utilization of glucose for energy provision while increasing the utilization of fatty acids for this purpose.[7] Results of the current study suggest that this shift in substrate utilization is accompanied by adaptive changes in the enzymatic processes responsible for metabolizing these energy substrates (*Table 2*). That is, hexokinase activity decreased while HOAD activity increased, adaptive changes that are consistent with the shift in substrate utilization by the diabetic heart and are consistent with the findings of Chen et al.[31] Dietary chromium supplementation reversed the diabetic effects on hexokinase activity while exacerbating these effects on HOAD. Such an increase in cardiac hexokinase activity has been found in conjunction with an increased cardiac glucose oxidation,[24] suggesting that the dietary chromium supplementation positively affected glucose utilization in the diabetic hearts. However, such an interpretation must be made with caution. First, it remains to be determined if these metabolic changes do result in significant changes in the actual substrate utilization pattern of the diabetic heart. Secondly, phosphofructokinase (PFK) and pyruvate dehydrogenase are generally considered to be the glycolytic regulatory enzymes most affected by diabetes.[7] Measurement of hexokinase activity does not provide insight into the activities of these other enzymes, thus providing limited insight into overall changes in glucose metabolism. Lastly, chromium supplementation elevated cardiac HOAD activity by approximately 23% raising the possibility that fatty acid utilization was greater in these hearts. If this were the case, then citrate, an inhibitor of PFK, might be produced in greater quantities, thereby reducing rather than enhancing glycolytic activity. Regardless of these limitations, these data do demonstrate that chromium picolinate can elicit adaptive changes that potentially benefit the diabetic heart.

In summary, supplementing the diet of a diabetic animal with chromium picolinate positively impacts the metabolic potential of cardiac tissue (*Table 2*). The observed increase in cardiac hexokinase activity suggests that this dietary manipulation might possibly improve the depressed glucose metabolism, which is typical of the diabetic heart and considered a fundamental defect caused by this disease.[7] On the other hand, chromium picolinate exacerbates the effects of diabetes on the expression of cardiac myosin (*Figure 3*), increasing the amount of the V3 isoform and decreasing the amount of the V1 isoform to levels not previously reported. Significant increases in the amount of the V3 isoform has been associated with increased cardiac functional capacity in intact, healthy animals,[29,30] while measurements made using in vitro heart preparations suggest that an increased expression of the V3 isoform reduces cardiac functional capacity.[8,9] The functional effect of such a dramatic change in the myosin isoform profile as observed in the current study remains unknown. For this reason, further investigations into the physiological and functional ramifications of consuming elevated levels of chromium in the context of a diabetic condition must be undertaken. Furthermore, interpretation of the data from this study must be made with great care and prudence.

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