Tissue Contents and Subcellular Distribution of Chromium and Other Trace Metals in Experimental Diabetic Rats After Intravenous Injection of Cr 50–Enriched Stable Isotopic Tracer Solution

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In order to study the metabolism of essential trace elements in diabetics, we studied alloxan-diabetic rats for the distribution patterns of chromium (Cr), cobalt (Co), iron (Fe), selenium (Se), and zinc (Zn) in the liver, kidney, pancreas, and testes, as well as in the organ subcellular fractions. Normal rats were used as controls. Cr 50–enriched stable isotopic tracer solution was given by intravenous injection to avoid the difficulties of estimation of Cr status. Our data show that the concentrations of Zn in liver and kidney, of Co, Fe, and Zn in pancreas, and of Fe and Zn in testes of the diabetic rats were significantly higher than in the control rats. Nevertheless, the concentrations of Cr in pancreas, Fe in kidney, and Cr and Se in testes of the diabetic rats were significantly lower than in the controls. Furthermore, we observed significant alterations of element concentrations in subcellular fractions of various organs in the diabetic rats. These results suggest that changing hormone levels may interfere with the accumulation of some trace elements both in the organs and in the subcellular fractions of rats.

DIABETES MELLITUS is a serious and chronic endocrinopathy disease. Previous research has suggested an interrelationship between diabetes and various micronutrients, including chromium (Cr), selenium (Se), zinc (Zn), copper (Cu) etc.[1-3] The significant alteration of these elements in diabetic individuals and animals has been attributed to insulin deficiency.[1,3] In addition, some of these minerals have been shown to modulate directly glucose and lipid homeostasis.[2,3] Patients with diabetes have lower Cr, Zn, and Se, and higher Cu levels, in their whole blood or plasma than those found in normal subjects.[1,4,5] Chromium deficiency in human and laboratory animals can cause impaired glucose tolerance, which can be reversed with Cr supplementation.[6] Zinc enhances the binding of insulin to its receptor and potentiates the effect of insulin.[7] Recent evidence demonstrates that oral selenate can improve glucose homeostasis.[2] Nevertheless, information concerning the influence of diabetes mellitus on the metabolism of trace metals is still limited. Difficulties in the estimation of the concentrations of some of these micronutrients, Cr, might account for the discrepant results.[4,7,8]

Furthermore, much of the data about the concentrations of trace metals in diabetics are based on the values in whole organs.[9,10] Analysis of the subcellular distribution of trace elements in diabetes could provide clues concerning their physiologic and pathologic functions. Unfortunately, up to now, only a few studies have examined such subcellular distribution.[11]

The present research was undertaken to investigate the subcellular distribution of Cr, as well as Co, iron (Fe), Se, and Zn, in the liver, kidney, pancreas, and testes of alloxan-diabetic rats. Simultaneously, normal rats were used as the controls. To avoid the difficulties of estimation of Cr status in rat, Cr 50–enriched stable isotopic tracer solution was given by intravenous injection.

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MATERIALS AND METHODS

Chemicals

All of the chemicals used were of the highest purity and were obtained from Sigma Chemical Co (St Louis, MO), unless otherwise indicated. The enriched stable isotope Cr 50 compound $^{50}Cr_2O_3$, in which the isotopic enrichment degree of Cr 50 is 94.2%, was purchased from the Institute of Atomic Energy, Beijing, China.

Glassware was soaked in 1:1 nitric acid for at least 1 week and rinsed thoroughly with deionized water before use.

Animal and Experimental Design

Twelve male Wistar rats were purchased from the Center of Experimental Animals of Beijing Medical University. The average weight of the rats was approximately 170 ± 20 g. All had similar initial normal fasting plasma glucose levels. Diabetes was induced in half of the rats by intravenous injection of freshly prepared alloxan solution (Sigma) at the dose of 90 mg/kg body weight. The fasting plasma glucose concentration was measured 2 days later by the glucose oxidase method and ranged from 427 to 542 mg/dL. This elevated level was maintained for more than 7 days without any therapy and was associated with decreased growth rate, polydipsia, polyuria, and polyphagia. The average weight loss was 20 g 5 days after the alloxan injection. The other half of the rats were used as controls. The rats were fed a commercial diet and supplied with deionized water during the entire experimental procedure.

The $^{50}Cr(III)$ tracer solution described was injected intravenously every 24 hours at a dose of 50 μg Cr^{3+}/100 g body weight for 3 days. On the fourth day, all of the rats were killed and the organs collected, washed with deionized water, and stored at −70°C until use.

Subcellular Fraction Separation

The rat liver, kidney, pancreas, and testes, which have relatively high Cr content, were selected for the subcellular study. The tissues were completely washed several times with deionized water before storage. Urine retained in the kidney was removed. Before homogenization, the tissue samples were minced into strips and washed 3 times with HEPES/sucrose buffer. The samples were homogenized with 4 vol of

Table 1. Element Concentrations in Standard Reference Materials by INAA (μg/g)

Element	NIST 1577a Bovine Liver		GBW 08551 Pork Liver		IAEA H8 Horse Kidney	
	Present Study*	Certified[12]	Present Study*	Certified[13]	Present Study†	Certified[14]
Cr	0.23 ± 0.07	0.2 ± 0.1	0.16 ± 0.03	0.20‡	—§	0.24 ± 0.24
Co	0.214 ± 0.032	0.224 ± 0.026	0.09 ± 0.01	0.100‡	0.132 ± 0.019	0.1291 ± 0.0154
Fe	167 ± 8	181 ± 9	1079 ± 9	1050 ± 40	298.78 ± 22.53	262.29 ± 44.88
Se	0.65 ± 0.06	0.71 ± 0.03	0.88 ± 0.05	0.94 ± 0.03	4.87 ± 0.56	4.67 ± 0.83
Zn	119 ± 4	122 ± 5	173 ± 2	172 ± 4	174 ± 15	192.89 ± 12.46

*The number of determination is 6.
†The number of determination is 2.
‡Reference values.
§No data because of the high phosphorus and bromine concentrations interference in the sample.

the 10-mmol/L HEPES/0.25-mol/L sucrose buffer (pH 7.5) and centrifuged at 50 × *g* for 7 minutes to remove nondisrupted cells. The supernatant was then centrifuged successively at 800 × *g* for 10 minutes, 9,000 × *g* for 10 minutes, 30,000 × *g* for 25 minutes, and 100,000 × *g* for 2 hours in a Beckman (Palo Alto, CA) model 7L ultracentrifuge to obtain nuclei, mitochondria, lysosome, microsome, and cytosol fractions. Each centrifugation step was performed twice, and the corresponding supernatants were mixed together. All of the above operations were carried out at 4°C.

Determination

Instrumental neutron activation analysis (INAA) was performed to detect Cr and other trace elements in the subcellular fraction samples. The samples and standards were irradiated in the Institute of Atomic Energy's heavy water nuclear reactor at a neutron flux of approximately 6.0×10^{13} n/cm^2 · s for 24 hours. After 2 weeks decay, the ^{51}Cr, ^{60}Co, ^{59}Fe, ^{75}Se, and ^{65}Zn radioactivities were counted by a high-purity germanium detector connected to a PC-based Ortec multi-channel-analyzer (MCA).

Statistical Analysis

Data are presented as means ± SD. Significance of differences in values between the diabetic and the control group rats were determined by the Student's *t* test.

RESULTS

Quality Control and Blanks

To evaluate the accuracy of INAA, several standard reference materials were used for quality control: NIST 1577a Bovine Liver, GBW 08551 Pork Liver, and IAEA H8 Horse Kidney. The analytical results are presented in Table 1.

In the experiments, the blanks of the reagents and all the packed materials for irradiation were also analyzed by INAA. The results indicated that the trace element contamination caused by them was negligible.

Total Concentrations of Elements in Organs of Diabetic and Control Rats

The concentrations of Cr, Co, Fe, Se, and Zn in the organs of the diabetic and the control rats after intravenous injection of Cr 50–enriched stable isotopic tracer solution are presented in Table 2. The concentration of Zn in the liver of the diabetic rats was significantly higher than in the control rats ($P < .001$). The value of Se in the liver of the diabetics was just slightly higher than in the controls ($P > .05$), while the concentrations of Cr, Co, and Fe in the diabetics were slightly lower than in the control rats ($P > .05$). In pancreas, the Cr concentration in the control rats was approximately twice that in the diabetic rats, whereas for Co, Fe, Se, and Zn, their concentrations in the diabetic rats were statistically higher than in the controls ($P < .001$). The concentration of Fe in the kidney of the diabetic rats was significantly lower than in the control rats, while the Zn concentration was significantly higher in the diabetic rats ($P < .05$). The renal concentrations of Cr, Co, and Se were not much different between groups. As in the testes, the concentrations of Cr and Se in the diabetic rats were significantly lower than in the controls ($P < .001$), while Fe and Zn were significantly higher than in the controls ($P < .001$).

Subcellular Distribution Patterns of Cr, Co, Fe, Se, and Zn in the Rat Liver

The subcellular distribution patterns of Cr, Co, Fe, Se, and Zn in the diabetic and the control rat livers are shown in Fig 1. Compared with the other elements in rat hepatic cells, Cr had

Table 2. Element Concentrations in Organs of Diabetic and Control Rats (μg/g dry weight)

Element	Liver		Pancreas		Kidney		Testes	
	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control
Cr	0.93 ± 0.27	1.16 ± 0.22	0.24 ± 0.02	0.45 ± 0.03*	2.18 ± 0.25	2.46 ± 0.34	0.36 ± 0.03	0.63 ± 0.03*
Co	0.039 ± 0.003	0.045 ± 0.003	0.048 ± 0.003	0.036 ± 0.003*	0.074 ± 0.006	0.068 ± 0.004	0.018 ± 0.003	0.015 ± 0.003
Fe	250 ± 8	268 ± 7	77 ± 3	65 ± 4*	211 ± 9	239 ± 10†	120 ± 6	88 ± 4*
Se	0.71 ± 0.21	0.60 ± 0.18	0.54 ± 0.09	0.30 ± 0.09*	1.29 ± 0.03	1.32 ± 0.09	1.47 ± 0.11	2.90 ± 0.15*
Zn	108 ± 4	92 ± 5*	55 ± 1	44 ± 1*	93 ± 5	77 ± 4†	95 ± 2	70 ± 2*

NOTE. Values are the average of 6 rats with the standard deviation.
*$P < .001$.
†$P < .05$.

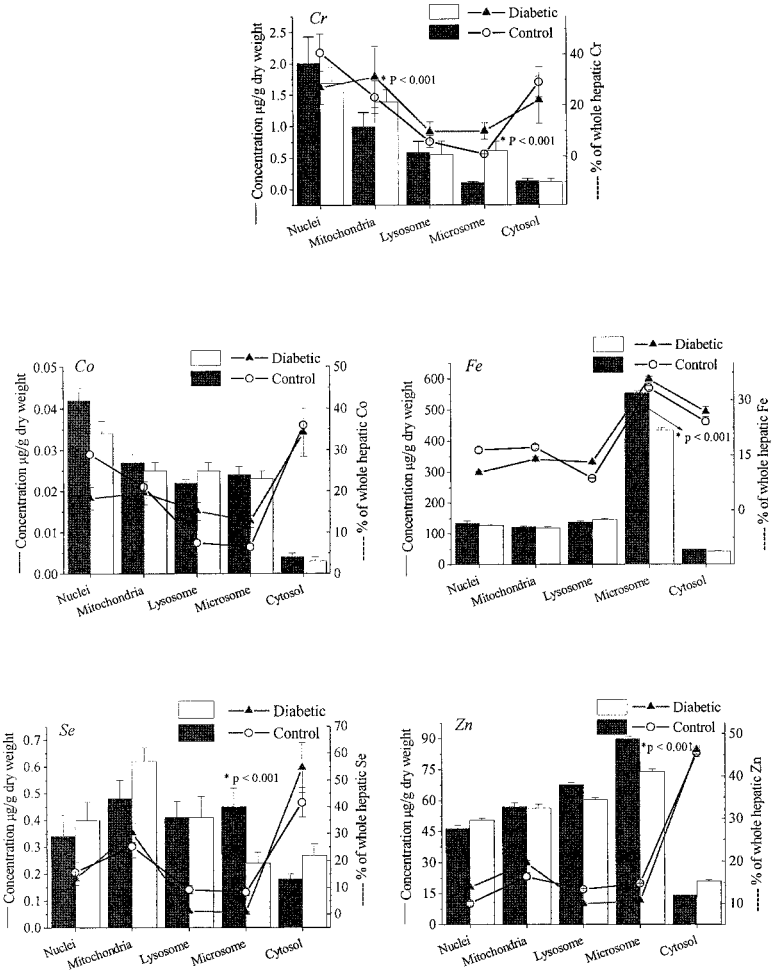

Fig 1. Subcellular distribution patterns of Cr, Co Fe, Se, and Zn in rat liver (error bar represents the standard deviation).

a different subcellular distribution pattern between the 2 groups of rats. The diabetic rats had significantly higher Cr levels in mitochondria and microsome than the controls ($P < .001$).

The hepatic organelle distribution patterns of Co, Fe, and Zn between the diabetic and the normal rats were quite similar. The highest concentration of Co was found in the nuclei, while Fe and Zn were highest in the microsomes in both groups of rats. However, the Fe and Zn concentrations in microsomes were significantly lower in diabetic rats compared with normal ($P < .001$). The Se concentrations in the hepatic microsomes of the control rats were significantly higher than in the diabetics ($P < .001$), while those in the other fractions were not obviously different in the 2 groups.

Subcellular Distribution Patterns of Cr, Co, Fe, Se, and Zn in the Rat Pancreas

Marked differences in the concentrations of the trace elements in the pancreatic subcellular fractions were observed between the 2 groups of rats (Fig 2). The Cr concentrations in all the cell fractions of the diabetic rats except the nuclei were statistically lower than those of the control rats ($P < .001$). In contrast, the concentrations of Co, Fe, Se, and Zn were significantly higher in all of the subcellular fractions except the cytosol in the diabetic rat pancreas compared with the control rats ($P < .001$). More than 45% to 70% of the elements are primarily enriched in the cytosolic fraction of both groups of rats.

Subcellular Distribution Patterns of Cr, Co, Fe, Se, and Zn in the Rat Kidney

The subcellular distribution patterns of the elements in the rat kidney are shown in Fig 3. Cr concentrations in all of the subcellular fractions were significantly higher in the control rats, while Fe concentrations were significantly higher in the nucleic and mitochondrial fractions of the control rats than in the diabetic rats ($P < .001$). In contrast, Se and Zn were significantly higher in most of the renal subcellular fractions of the diabetic rats ($P < .001$ for Se and $P < .05$ for Zn). The Co concentration was higher in the mitochondrial and lysosomal fractions of the diabetic rats ($P < .001$). Approximately 40% to 60% of Cr, Co, Fe, and Zn are stored in the renal cytosolic fraction of the 2 groups of rats. Nevertheless, the renal Se percentage accumulation in the intracellular fractions of both the diabetic and the control rats decreases in the order of microsome > nuclei > lysosome > mitochondria > cytosol.

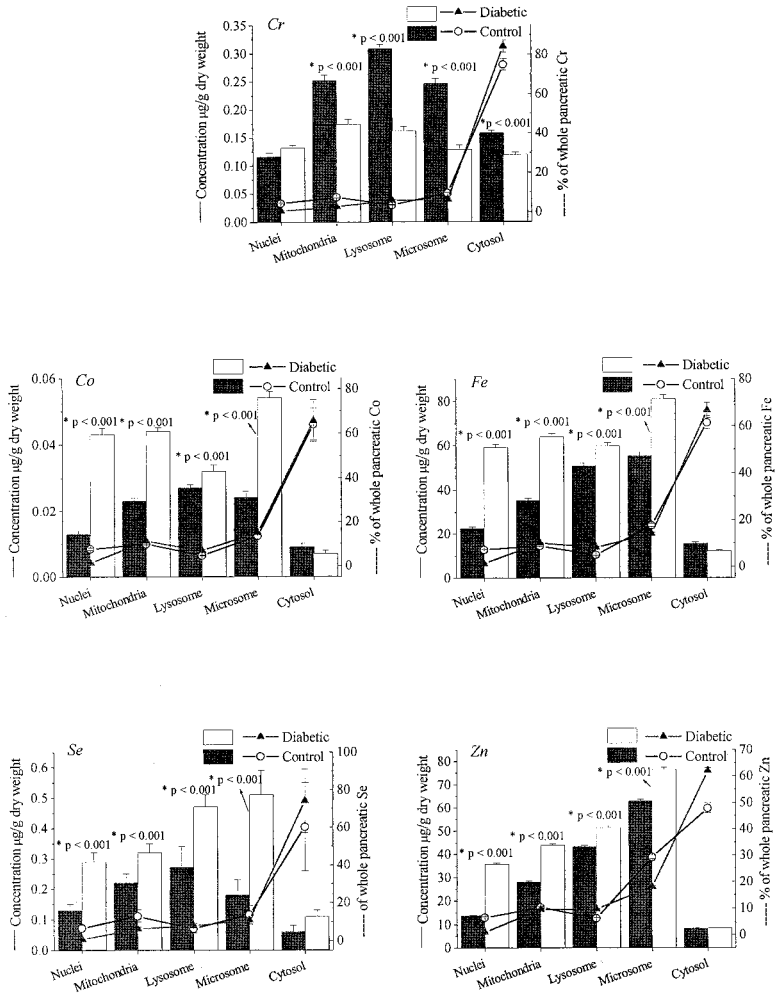

Fig 2. Subcellular distribution patterns of Cr, Co Fe, Se, and Zn in rat pancreas.

Subcellular Distribution Patterns of Cr, Co, Fe, Se, and Zn in the Rat Testes

The distribution of the trace elements in the subcellular fractions of the testes of the diabetic and the control rats is shown in Fig 4. Cr concentrations in all of the diabetic rats testis organelles were significantly lower than in the control rats ($P < .001$). The Co concentrations in all of the intracellular fractions were similar in both groups. The Fe concentrations were significantly higher in the cytosol and lysosome of the testis of the diabetic rats. Se was significantly higher in the nuclei of the control rats ($P < .001$), while it was higher in the diabetic rats in the mitochondria and cytosol ($P < .001$). The Zn level was significantly higher in the nuclei and microsome of the control testes, but higher in the cytosol of the diabetic testes ($P < .001$).

Similar to the accumulation pattern in the other organs, most quantities of Cr, Co, Fe, and Zn are stored in the cytosolic and nucleic fractions of the testis. In contrast, Se exhibits a different accumulation pattern between the 2 groups rats. For diabetic rats, more than 55% and 20% of Se are enriched in the cytosolic and nucleic fractions, respectively, while for control rats, only 29% and nearly 46% of Se is present in the cytosol and nuclei.

DISCUSSION

These studies demonstrated that the concentrations of Cr, Co, Se, Fe, and Zn in the tissues and their subcellular fractions of the normal and the diabetic rats are not uniform and are influenced by the presence of diabetes. Abnormal hormone level is known to affect gastrointestinal function in diabetics that appears as polyphagia and polyuria compared with healthy controls. However, in this study, the concentrations of Cr in pancreas and testes, and Fe in kidney of the control rats were all significantly higher than in the diabetic rats, while Zn concentrations were significantly higher in all of the observed organs of the diabetic rats. Therefore, the experiment demonstrated that the elevated food consumption of diabetics was not a major contribution for those results. This conclusion was also supported by Failla et al[11] and Johnson et al,[15] who pair-fed trace elements to both diabetic and control groups and found that some trace metals accumulated in the organs of the diabetic rats.

To our knowledge, there is little information available concerning trace metal subcellular distribution in various organs of diabetic animals. It is well known that some micronutrients have insulin-like functions and could modulate insulin secre-

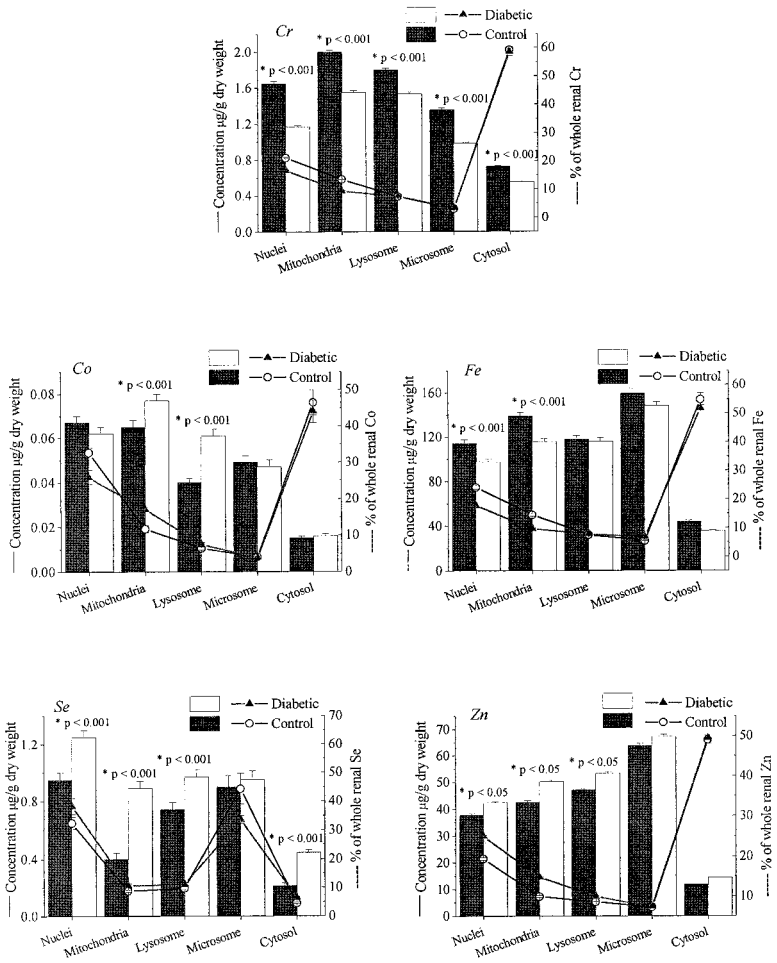

Fig 3. Subcellular distribution patterns of Cr, Co Fe, Se, and Zn in rat kidney.

tion or sensitivity in humans and animals.[1-3] The best known chromium biological role is as a cofactor for the peripheral action of insulin. Its deficiency can cause diabetic-like symptoms.[16] Because the concentration of Cr in humans and animals is so low, few studies have given the result of its distribution in organs. In this research, when Cr 50 isotopic tracer technique was used, the Cr concentrations in all of the observed tissues of the diabetic rats were found to be more or less lower than in the control rats. Our subcellular studies indicated that the diabetic rats exhibited elevated average Cr concentrations in the liver mitochondrial and microsomal fractions, suggesting that Cr might have some unknown physiological functions in glucose or lipid metabolism to compensate for the low level of insulin in diabetic rats. Previous research has shown that the reaction of insulin with mitochondria was enhanced by the presence of Cr(III).[17] Additionally, in this study, Cr(III) did not show special accumulation or combination in the pancreas of the diabetic rats, which implies that Cr did not play its biological function via accumulation in the pancreas and combination with insulin. Furthermore, most Cr accumulated in the cytosolic fractions of various organs, which means that Cr(III) likely combined with proteins, as Cr-protein complexes were present in cytosol.

The average concentrations of Se in most of the observed tissues except in kidney were elevated in the diabetic rats. Furthermore, the diabetic rats had significantly higher Se concentrations in almost all of the pancreatic and renal organelles than the control rats. The distribution of Se in organs of diabetics is still unclear. However, there is some controversy among the studies performed on serum Se levels in diabetic patients. Some researchers found a significant increase in diabetics,[7] while others observed that the levels were similar in patients and controls.[8] Nevertheless, some investigators even found a statistically significant increase in Se concentrations in diabetics.[4] Selenium is an important trace element as a component of the antioxidant enzyme glutathione peroxidase in biological systems. More recently, evidence has also been presented that Se could affect carbohydrate metabolism.[18] Selenium could stimulate glucose transport, cyclic adenosine monophosphate phosphodiesterase activity, and ribosomal S_6 protein phrsphorylation in rat.[18] Our results regarding Se in the diabetic rats appear to provide evidence that Se has an unknown function of spontaneous modulation in diabetes. Taking into consideration the contradictory results and the sparse literature in this field, future research is needed to clarify the role of Se in the diabetic processes, which will benefit Se supplementation guidelines for these patients.

Zn is another noteworthy element in that the total concen-

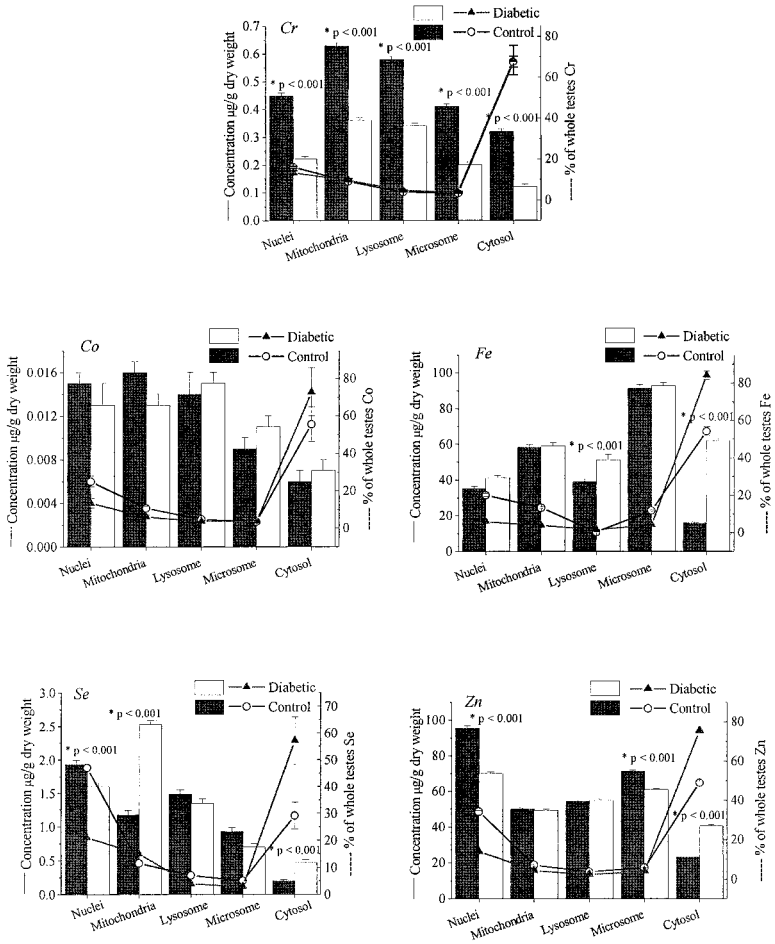

Fig 4. Subcellular distribution patterns of Cr, Co Fe, Se, and Zn in rat testes.

tration in all of the studied tissues was significantly higher in the diabetic rats than in the control rats. In addition, higher Zn levels were observed in the pancreatic nuclei, mitochondria, lysosome, and microsome of the diabetic rats. Similar results were obtained by Failla and Kiser,[9,11] who found that the quantities of Zn in the liver and kidney of streptozotocin (STZ)-diabetic rats were increased compared with control animals. Zn is known to be an activator of fructose 1-6 diphosphate aldolase or as an inhibitor of fructose 1-6 diphosphatase.[3] The addition of Zn to insulin is known to induce conformational changes and to enhance insulin binding to its receptor.[3] Our results and others indicated that the diabetic rats had an abnormal Zn metabolism in their bodies. Furthermore, our subcellular results might be a sign that Zn plays a positive role in diabetic pancreas. This suggestion is supported by the finding of Tobia et al[19] that Zn supplementation preserves pancreatic function in rats.

In this study, the concentrations of Fe in the liver and kidney of the diabetic rats were lower than in the control rats. Contradictory results were obtained by Johnson and Evans,[15] who found higher Fe concentrations in both the liver and kidney of STZ-diabetic rats. Although some differences exist in these results, all of the studies demonstrated that there was an alteration of Fe metabolism in diabetics. Up to now, the role of Fe in glucose homeostasis has been unclear and research into its metabolism in diabetics has been insufficient.

Significantly higher Co concentrations were found in both the homogenate and the subcellular fractions of pancreas in the diabetic rats than in the controls. However, similar to Fe, the relationship between Co and diabetes is still unknown. Saker et al[20] reported that treatment of STZ-diabetic rats with cobalt chloride resulted in a significant decrease in serum glucose concentrations, thus suggesting that Co might have some biological function in diabetic homeostasis.

The testis and its subcellular fractions were found to accumulate significant trace elements except for Co in both diabetic and control rats. We know of no such research on the element distribution in testis of diabetics. Nevertheless, diabetes is usually accompanied by peripheral neuropathy, such as impotence, and therefore decreases fertility and sperm output. The abnormal element distribution in testes of the diabetic rats is suspected to be associated with this sexual dysfunction. Further research needs to be done to clarify this suggestion.

CONCLUSIONS

Our study on Cr, Co, Fe, Se, and Zn distributions in organs and their subcellular fractions in the alloxan-diabetic rat indi-

cate that element metabolism in diabetics is different from that in normal subjects. Furthermore, our study provides evidence that changes in hormone levels may interfere with cellular trace element accumulation as well. Some essential trace elements, such as Cr, Se, and Zn, have demonstrated important physiological roles in diabetic homeostasis, eg, stimulation of insulin excretion, enhancement of insulin activity, etc. Thus, the alteration of trace metal metabolism in diabetics should be noted. More research should be performed with the goal to understand the biological roles of trace elements in diabetes and, therefore, help us to determine the value of micronutrient supplementation in diabetic patients.

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