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Effect of $FeSO_4$ treatment on glucose metabolism in diabetic rats

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Abstract Iron, the prosthetic group of haemoglobin, was found to lower serum glucose levels of diabetic rats. Its regulative mechanism and effects on enzymatic activities of glucose metabolism are still unknown. In this study, the correlation between iron supply and enzymatic activities of glucose metabolism and respiratory chain were evaluated in liver and kidney tissues of alloxan induced-diabetic rats. After $FeSO_4$ and metformin administration, serum samples were collected for serum glucose and fructosamine level measurements. Kidney and liver tissues were excised at the end of the study for assaying enzymatic activities of isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and cytochrome-*c*-oxidase. Results showed significantly decreased serum glucose and fructosamine levels in treatment groups and enhanced enzymatic activities of several proteins as compared with the diabetic control group. Therefore, these data suggested that $FeSO_4$ administration could increase the supply of oxygen, enhance enzymatic activities of glucose metabolism and the respiratory chain, accelerate glucose metabolism and consequently decrease serum glucose levels.

Keywords Glucose metabolism · Respiratory chain · Fructosamine · $FeSO_4$ · Haemoglobin

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Introduction

Trace elements or metals, which cannot be synthesized by the body and must be absorbed from outside sources, play key roles in biological processes in organisms. Some of these roles are the activation/inhibition of enzymatic reactions through competition at the binding site of the metalloproteins and modifications of the permeability of cell membranes. Imbalances of such elements have been associated with abnormal metabolisms and can cause diseases such as diabetes. It has been reported that the urinary excretion of calcium, zinc and magnesium is increased in two types of diabetes mellitus (DM), resulting in an insufficiency of these elements in the blood of these patients (Brown et al. 1999; Cunningham et al. 1994).

Diabetes mellitus (DM) has become a major and growing health problem in most countries. Glucose metabolism is easy to monitor in diabetes but the disease also heavily involves abnormal lipid metabolism. Insulin, a hormone that is produced in the β-cells of the pancreatic islets of Langerhans, is the major regulator of blood glucose levels in the body. Diabetic

sufferers, however, have completely disturbed glucose metabolic process due to either lack of insulin or dysfunctional insulin. Consequently, glucose or sugar builds up in the blood stream. Numerous studies have demonstrated that some trace elements are involved in the regulation or management of insulin secretion and function (Scott and Fischer 1938; Mertz 1969; Toepfer, Mertz et al. 1974; Kimura 1996; Anderson 1997; Gurson and Saner 1971; Underwood and Mertz 1986). Significant alterations in the concentrations of trace elements in human bodies are correlated with the occurrence of diabetic mellitus. Therefore, regulation of trace element concentrations has been proposed as a potential preventive measure and a treatment strategy for diabetic mellitus.

Iron (Fe) is an essential cation in many enzymes, such as hemoglobin and myoglobin, and it plays a crucial role in many physiological functions. Its role in metabolic pathways, especially the tricarboxylic acid (TCA) cycle, has been well recognized. Fe is important for both O_2 transportation and electron flow (Peter 2000). Decreasing the Fe level inhibits the metabolism of glucose and leads to elevated blood glucose. There have been more reports on the effects of Fe on blood glucose regulation in diabetes focused on excessive Fe supply (Jatoba et al. 2008; Nankivell et al. 1994) than lower dosages of Fe (Qian et al. 2003, Facchini and Saylor 2003). In this study, the effects and regulation mechanism of supplemental $FeSO_4$ on activities of enzymes in glucose metabolism and respiration pathway were investigated in rats with diabetic mellitus.

Materials and methods

Chemicals

Alloxan, $FeSO_4$ and all other chemicals used in this experiment were purchased from Sigma (St. Louis, MO, USA). All chemicals were of analytical grade or better.

Drug administration in animals

Male SD rats of body weight of 187.5 ± 18.43 g were purchased from the Central Animal Facility, Chinese Academy of Sciences (Nanjing, China). Animals were housed in steel cages in a temperature (22 ± 2°C) and light/dark (12/12 h) controlled room. All the rats were fed a standard pellet chow diet and water was freely available. Diabetic rats were induced by intraperitoneal injection of alloxan at a dose of 200 mg/kg body weight. The alloxan solution was freshly prepared with 0.1 mol/l pH 4.0 citrate buffer, kept on ice and injected immediately. Ten randomly selected rats injected with only the same citrate buffer were used as the normal group. Five days after injection of alloxan, fasted blood was drawn from the tail vein and used for measurement of serum glucose levels with the glucose assay kits (Jiancheng Biological Insititute, Nanjing, Jiangsu). These rats were confirmed as the diabetic model when their serum glucose levels exceeded 13 mmol/dl. No insulin was given to the rats in the study.

The alloxan-induced diabetic rats (30) were randomly and averagely divided into three groups: the diabetic control group, $FeSO_4$ treatment group and metformin treatment group (positive drug group). $FeSO_4$ group was intragastrically administered 100 mg/Kg body weight $FeSO_4$ and positive drug group was given equal amount of metformin. Normal group and diabetic control group were administered equal amounts of distilled water.

Serum glucose levels were monitored periodically for 28 days. These levels represent the uncontrolled hyperglycemic condition. For the fasting condition, at the end of 28 days all rats were fasted for 6–8 h before blood was collected into potassium oxalate and sodium fluoride tubes for analysis of serum glucose and fructosamine. After the animals were sacrificed, liver and kidney tissues were excised immediately and stored at −70°C until use. Procedures involving animal handling and care were conducted in conformity with the institutional guidelines of School of Jiangsu Medicine University (Jiangsu, China).

Estimation of serum glucose and fructosamine

Fasting serum glucose was determined by the method of Glucose Oxidase-Peroxidase (GOD-PAP) with the glucose assay kit manufactured by Rongsheng Biotechnology Co. Ltd. (Shanghai, China).

Fructosamine level was determined according to the method described by Xu et al. (2002). Briefly, serum (50 µl) was suspended in 0.1 M sodium phosphate buffer (100 µl, pH 8.0) containing 3.2 mM IAM (iodoacetamide), and allowed to stand

in a thermal bath at 37°C for 30 min before 2 ml of 0.25 mM NBT (nitroblue tetrazolium) was added. NBT was freshly prepared in sodium carbonate (0.2 M, pH 10.3). The mixture was kept for another 30 min in the thermal bath before reaction was terminated by putting the test tube into ice. Finally, the absorbance was measured at 530 nm. Distilled water instead of serum was used as blank and 50 μl 2 mM BSA (bovine serum albumin) was used as control. Fructosamine level was calculated using the following equation:

$$I = \frac{X}{X - Y} \times 2mM$$

where I is fructosamine level; $X =$ absorbency of control and $Y =$ absorbency of sample.

Activity assays for enzymes in glucose metabolism and respiratory pathway

Hexokinase and pyruvate dehydrogenase activities in livers and kidneys of diabetic rats

Whole liver tissue was homogenized in 0.1 M Tris–HCl buffer (pH 7.4) and the particle free homogenate was used for enzyme activity assay. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard. Hexokinase and pyruvate dehydrogenase were assayed using kits from Jiancheng Biotechnology Co. Ltd. (Nanjing, China) following the manufacturer's instructions.

Enzymatic activity determination for proteins in the tri-carboxylic acid cycle in liver and kidney mitochondrias

Mitochondria were isolated from fresh liver and kidney tissues following previously published protocols (Johnson and Lordy 1967). Protein concentration was estimated by the method of Lowry et al. (1951). Purified mitochondria were assessed by examining the activities of isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase, and cytochrome-*c*-oxidase. The activity of isocitrate dehydrogenase was assayed by the method from King (1965). Succinate dehydrogenase and malate dehydrogenase activities were assayed using kits purchased from Jiancheng Biotechnology Co. Ltd. (Nanjing, China). The method of Minakami et al. (1962) was followed for the determination of reduced nicotinamide adenine dinucleotide (NADH)-dehydrogenase activity. The activity of cytochrome-*c*-oxidase was assayed by the method of (Wharton and Tzagoloff 1967). All measurements were performed with a UV-2100 spectrophotometer (Unic, Shanghai, China).

Statistical analysis

All data for various biochemical parameters were analyzed using a one-way analysis of variance (ANOVA) and the differences between means were compared by Duncan's Multiple Range Test (DMRT) (Duncan 1957). Results were presented as mean ± s.d. from ≥10 rats in each group. The significant level of 5% ($P < 0.05$) was used as the minimum acceptable probability for the difference between the means.

Results

Body weights and serum glucose levels of rats receiving treatment

Body weights of the hyperglycermic rats induced by alloxan were tracked during the study and presented in Fig. 1. Body weights of rats in the metformin treated group and the $FeSO_4$ treated group gradually increased during the experimental period. The diabetic control group, on the other hand, showed decreased body weights at the same period.

Results of serum glucose in diabetic rats were summarized in Fig. 2. As compared to the diabetic control group, serum glucose levels in the $FeSO_4$ and the metformin treatment groups decreased gradually ($P < 0.05$) and fructosamine in plasma of diabetic rats was found to be significantly reduced 28 days after treatments ($P < 0.05$), but extent of the serum glucose levels dropped was small (Table 1).

Carbohydrate metabolism enzyme activities

When enzymatic activities of carbohydrate metabolism and respiratory pathway were examined in livers and kidneys of normal, diabetic control, $FeSO_4$ and metformin treatment groups, it was found that activities of hexokinase, pyruvate dehydrogenase,

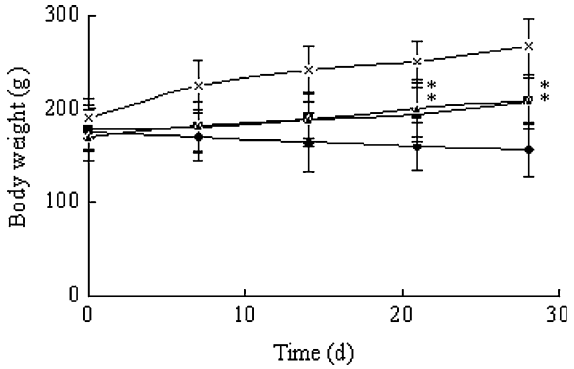

Fig. 1 Effect of $FeSO_4$ and metformin on body weights of diabetic rats. (◆) represents the diabetic control group with no treatment; (■) represents the diabetic group treated with 10 mL, 10 mg/mL metformin; (▲) represents the diabetic group treated with 10 ml, 10 mg/ml $FeSO_4$; (×) represents the normal rats group. All these data were expressed as mean ± S.E ($P < 0.05$) * Denotes $P < 0.05$ as compared to the diabetic control group

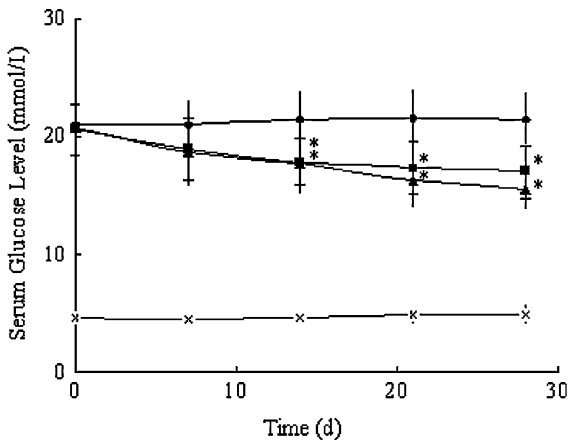

Fig. 2 Effect of $FeSO_4$ and metformin on serum glucose levels of diabetic rats. (◆) represents the diabetic control group with no treatment; (■) represents the diabetic group treated with 10 mL, 10 mg/mL metformin; (▲) represents the diabetic group treated with 10 ml, 10 mg/ml $FeSO_4$; (×) represents the normal rats group. All these data were expressed as mean ± S.E ($P < 0.05$). * Denotes $P < 0.05$ as compared to the diabetic control group

isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and Cytochrome-*c*-oxidase were significantly decreased in the control diabetic rats ($P < 0.05$) as compared with normal rats (Normal group; Table 2). However, $FeSO_4$ and metformin administration ($FeSO_4$ group and Metformin group) completely reversed this effect of control diabetic rats ($P < 0.05$).

Table 1 Effect of $FeSO_4$ and metformin on blood fructosamine levels of diabetic rats

Group	Fructosamine levels (mmol/l)
Normal	2.321 ± 0.193
Diabetic control	3.476 ± 0.342
$FeSO_4$	2.645 ± 0.276*
Metformin	3.105 ± 0.290*

All these data were expressed as mean ± S.E. Fructosamine levels were determined 28 days after drug administration

* Denotes $P < 0.05$ as compared to the diabetic control group

Discussion and conclusion

During the evolution of human being, geographic environment has played a lasting significant role on the health of mankind. Models for this impact on life quality and life span also evolved as human being progressed. The messenger between the geographic environment and health of human being is trace elements or metals. However, while moderate dose of trace elements or metals are beneficial to health, either over or under dose of such elements could contribute to diseases like diabetic mellitus. Iron shows the same property. Excessive level of iron previously documented could either impair mitochondrial function (Masini et al. 1994) or increase oxidant stress (Knutson et al. 2000, Jansson et al. 1985). Excess iron also would cause overload of iron in pancreas, further inhibit excreting the insulin from the pancreas and impair the glucose of tolerance, and finally lead to increase the serum glucose levels (Zhou et al. 2000; Jiang et al. 2004). However, effect of moderate dose of iron on diabetes has not been reported till now.

Many trace elements or metals have been found to effectively reduce serum glucose levels in diabetes. Becker et al. (1996) and Kimura (1996) provided evidence that selenium was a trace element that exerted certain insulin-like actions on the glucose homeostasis of diabetic rats. Nomura et al. (2005) reported the effect of cobalton on the liver glycogen content in streptozotocin-induced diabetic rats. When cobalt was administered to model rats, the level of glycogen was adjusted to normal and the serum glucose was also decreased to the lower level. Iron is another nutritional trace metals that has been found to play important roles in the genesis and progression of several diseases. Over 100 kinds of enzymes (such as hydrogen peroxidase, monoamine oxidase) with their

Table 2 Effects of $FeSO_4$ and metformin on activities of enzymes in carbohydrate metabolism and respiratory chain in livers and kidneys of diabetic rats

Parameters			Normal	Diabetic control	$FeSO_4$	Metformin
EMP pathway	Hexokinase	Liver	151.87 ± 9.50	81.68 ± 8.77*	147.09 ± 11.48*, ***	136.10 ± 9.92*, **
		Kidney	109.39 ± 9.18	53.06 ± 8.69*	90.53 ± 7.69*, **, ***	83.17 ± 8.53*, **
	Pyruvate dehydrogenase	Liver	92.88 ± 10.46	62.81 ± 9.52*	83.93 ± 9.03**, ***	77.86 ± 12.10*, **
		Kidney	69.15 ± 6.86	32.66 ± 5.80*	64.96 ± 9.11**, ***	49.40 ± 4.77*, **
TCA pathway	Isocitrate dehydrogenase	Liver	740.56 ± 60.08	496.81 ± 52.36*	722.15 ± 54.50**, ***	559.21 ± 52.93*, **
		Kidney	646.44 ± 53.75	476.44 ± 54.59*	598.18 ± 56.08**	536.15 ± 52.17*, **
	Succinate dehydrogenase	Liver	23.81 ± 2.88	12.56 ± 1.20*	19.81 ± 1.97*, **, ***	14.69 ± 2.04*, **
		Kidney	12.46 ± 0.89	7.99 ± 0.71*	11.90 ± 0.81**, ***	8.46 ± 0.60*
	Malate dehydrogenase	Liver	351.91 ± 38.22	239.57 ± 19.72*	331.27 ± 33.11**, ***	244.776 ± 40.46*
		Kidney	254.26 ± 22.88	193.07 ± 21.95*	236.78 ± 24.46**	191.02 ± 22.97*
Respiratory chain	NADH-dehydrogenase	Liver	26.60 ± 2.55	14.23 ± 1.69*	25.58 ± 2.06**, ***	17.39 ± 1.94*
		Kidney	19.25 ± 2.99	12.28 ± 1.22*	18.32 ± 1.78**, ***	16.09 ± 2.87*
	Cytochrome-*c*-oxidase	Liver	6.44 ± 0.59	3.03 ± 0.339*	6.08 ± 0.53**, ***	3.62 ± 0.58*
		Kidney	6.00 ± 0.62	3.19 ± 0.29*	5.79 ± 0.55**, ***	3.97 ± 0.34*

Note: EMP, Glycolytic Pathway; TCA, Tri-carboxylic acid cycle. All these values are expressed as mean ± sd for 10 rats in each group. Units: Hexokinase, μmol of glucose phosphorylated/min/g protein; pyruvate dehydrogenase, μmoles of NADH /min/mg protein; isocitrate dehydrogenase, nmoles of α-ketoglutarate liberated/h/mg protein; succinate dehydrogenase, μmoles of succinate/min/mg protein; malate dehydrogenase, μmoles of NADH /min/mg protein; NADH-dehydrogenase, μmoles of NADH oxidized/min/mg protein and Cytochrome-*c*-oxidase, O.D × 10 min/mg protein

* Denotes $P < 0.05$ as compared with the normal group

** Denotes $P < 0.05$ as compared to the diabetic control group

*** Denotes $P < 0.05$ as compared with the metformin group

related activities contain Fe or rely on Fe in the body. Above 50% enzymes and cofactors in tri-carboxylic acid cycle contain iron or require iron for maintaining their activities. Iron deficiency of the body may affect the trafficking of O_2 between lung and other tissues, inhibit electron flow along the mitochondrial respiratory chain, prevent the decomposition of glucose and finally result in elevated serum glucose. In this sense, the diabetic mellitus has also been related with alterations in the homeostasis of iron. Increased urinary iron excretion has been reported in diabetes, causing a decrease in serum iron level from these sufferers (Howard et al. 1991).

In the present study, obtained these data suggested that the activities of various enzymes taking part in glucose metabolism and bio-oxidation, such as hexokinase, pyruvate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and Cytochrome-*c*-oxidase, had decreased in livers and kidneys of diabetic rats. However, following with $FeSO_4$ and metformin treatments, the activities of these enzymes significantly increased (Table 2). Though $FeSO_4$ and metformin could both contribute to serum glucose regulation, differences in mechanism of regulation between $FeSO_4$ and metformin existed. Metformin reduced fasting plasma glucose concentration by enhancing glucose transport (Matthaei et al. 1991; Bailey and Turner 1996), inhibiting the hepatic glucose production rate (Cusi and DeFronzo 1998; Cusi et al. 1996) and promoting hepatic glycogenolysis (Christiansen et al. 1997; Stumvoll et al. 1995) and gluconeogenesis (Pierce and Dhalla 1985). On the other hand, there were no reports that iron could promote insulin secretion or glucose transport. The loss of iron, a prosthetic group of the haemoglobin, would directly result in the deactivation of Haemoglobin as an O_2 carrier. Deficiency of Fe was frequently observed in diabetes (Cui et al. 2007; Christophe et al. 2000). Under normal physiological conditions, organism oxygen supply and demand were well balanced. However, under diabetic conditions oxygen supply significantly dropped, which could impair organism functions and if severe enough caused irreversible tissue damage. Oxygen transport efficiency was the critical factor that affects oxygen supply and utilization. Previous studies demonstrated an oxygen supply decreased in diabetic sufferer with impaired energy metabolism (Pierce and Dhalla 1985; Stanley et al. 1997) and diabetic heart (Malhotra and Sanghi 1997). Hemoglobin and myoglobin acted as carriers for oxygen from lung to tissues and for carbon dioxide and proton to travel from tissues to lung. Since Fe was the key prosthetic group in hemoglobin and myoglobin, responsible for their carrier functions, Fe deficiency could directly cause their deactivation which led to lower oxygen supply, weaken bio-oxidation, accumulate NADH and inhibit the enzymes in glucose metabolism (Table 2).

In conclusion, moderate dose of iron in the diet could increase oxygen supply, elevate activities of enzymes related with the glucose metabolic pathway, accelerate the decomposition of serum glucose and subsequently decrease serum glucose levels of alloxan-induced diabetic rats and diabetic mellitus sufferers. However, because of the complexities of Fe metabolism and the fact that the excessive Fe may cause many diseases including diabetes (Jiang et al. 2004), the long term use of Fe as a treatment for diabetes is unlikely.

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