

# Fe<sup>2+</sup> Coupled with Vitamin c (Vc) Can Enhance Glucose Metabolism and Decrease Blood Glucose Levels of Alloxan-Induced Diabetic Mice

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## ABSTRACT

That FeSO<sub>4</sub> not FeCl<sub>3</sub> can reduce the blood glucose levels of alloxan-induced diabetes mice has been reported in previous research. This study explained the cause of difference in activity of two different iron ions on diabetic mice based on glucose consumption (GC). FeSO<sub>4</sub>, FeCl<sub>3</sub>, Vitamine c (Vc), FeSO<sub>4</sub> + Vc, metformin were administrated to the alloxan induced-diabetic mice, respectively. After administrated, serum glucose, fructosaminr, insulin, triglyceride, total cholesterol, total iron and Fe<sup>2+</sup> levels and GC of liver *in vivo* were analyzed, respectively. *In vitro*, effect of different iron ions coupled with Vc or streptozotocin on GC of liver of diabetic mice of model group were analyzed. The body weights and serum insulin levels of Fe<sup>2+</sup> and Fe<sup>2+</sup> + Vc treated diabetic mice notably increased. The serum glucose, fructosamine, triglyceride and total cholesterol levels were significantly decreased, whereas serum total iron and Fe<sup>2+</sup> levels and GC in liver were increased in the Fe<sup>2+</sup>, Vc and Fe<sup>2+</sup> + Vc groups compared with in the model groups. In addition, hardly change of serum insulin level was caused by Fe<sup>2+</sup>, Vc and Fe<sup>2+</sup> + Vc treatment. However, the similar result did not obtain a Fe<sup>3+</sup> treated. Further, liver's GC of untreated-diabetic mice was lower than of normal mice and significantly increased after Fe<sup>2+</sup> not Fe<sup>3+</sup> added to the reaction resolution *in vitro*, and further increased when Fe<sup>2+</sup> and vitamin c (Vc) synchronously added to the reaction system, however, decreased when Fe<sup>2+</sup> and Streptozotocin were added synchronously to the reaction resolution. It is suggested that iron coupled with reducer can enhance the glucose metabolism to eventually achieve to controlling blood glucose levels.

**Keywords:** Glucose Metabolism; Glucose Consumption; FeSO<sub>4</sub>; Oxidant Stress; Vc

## 1. Introduction

Under normal physiologic conditions, there is a critical balance in generation of oxygen free radicals and anti-oxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity. However, rapid cultural and social changes, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioral patterns has broken the balance and caused abrupt accumulation of free radical *in vivo* [1-4]. It has been generally accepted that free radicals can react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, thereby interfering with cell function. However, whether and how accumulation of free radicals *in vivo* impact on glucose metabolism by other pathway can few be understood.

Iron (Fe), a trace element, is a key membrane component and an essential cofactor required for a number of cell functions. For example, 50% mitochondrial proteins operate smoothly in the present of iron. Iron has involved in many physiological functions as a major electron-carrier, such as, the tricarboxylic acid (TCA) cycle, oxygen transportation and oxidation-phosphorylation process [5]. Iron content and the special ration of Fe<sup>2+</sup> and Fe<sup>3+</sup> be maintained *in vivo* helped for the normal metabolic regulation of sugar, protein and lipid. The inter-conversion of Fe<sup>2+</sup> and Fe<sup>3+</sup> might be a critical factor for maintaining normal metabolism. However, if the inter-conversion rate from oxidized iron (Fe<sup>3+</sup>) to reduced iron (Fe<sup>2+</sup>) becomes slow for some reasons, iron will fail to play its function. There is evidence that falling oxidation stress *in vivo* was accompanied by increased serum iron (Fe<sup>2+</sup>) content [6]. It was found that after oral administration of [Cr<sub>3</sub>O(O<sub>2</sub>CCH<sub>2</sub>CH<sub>3</sub>)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> to healthy Sprague Dawley rats, plasma glucose and insulin levels

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simultaneously lowered [7,8]. Whether [Cr<sub>3</sub>O(O<sub>2</sub>CCH<sub>2</sub>CH<sub>3</sub>)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> weakened oxidant stress *in vivo*, increased the solubility of iron and finally lowered the blood glucose levels is worth further studying.

Diabetes was an epidemic whose critical character was foul-up of glucose metabolism. Among the factors to cause foul-up of metabolism, the Fe<sup>3+</sup>'s content increase, Fe<sup>2+</sup> and total iron's content decrease led by oxidant stress is the critical factor induced diabetes generation. In previous study, we have also reported that supplementation of FeSO<sub>4</sub> can decrease blood glucose level and accelerate glucose metabolism [9].

Glucose consumption (GC), which is the amount of glucose degraded by the organ, tissue or organism in the unit time, reflects the speed of glucose metabolism. In this study, the effects of Fe<sup>2+</sup>, Fe<sup>3+</sup>, Vc, and co-effect of Fe<sup>2+</sup> and Vc on serum glucose, lipid, fructosamine levels and serum Fe<sup>2+</sup> and total iron content alloxan-induced diabetic were compared. Then we analyzed GC in liver of alloxan-induced diabetic mice *in vitro* and the changes of GC after complemented different forms of iron coupled with reducer vitamin c (Vc) and oxidant Stz to find the mechanism of oxidant stress and iron (Fe<sup>2+</sup>) to regulate blood glucose metabolism in alloxan-induced diabetes mice. Finally, we analyzed the mechanism that iron coupled with anti-oxidant controlled blood glucose levels and offered a new strategy for prevention and treatment of diabetes.

## 2. Material and Methods

### 2.1. Chemicals

Alloxan and Streptozotocin (STZ) were purchased from Sigma (St. Louis, MO, USA). Vitamin c (Vc), which content was more than 99%, was purchased from Ying Chen Chemical Co., Ltd. All chemicals used in this study were of analytical grade or higher.

### 2.2. Drug Administration in Experimental Animals

Male ICR mice with body weight of 16.46 ± 2.21 g were purchased from the Central Animal Facility, Chinese Academy of Sciences (Nanjing, China). The animal certification number was SCXK Su20020018.

All the mice were kept in steel cages in the room with controlled temperature at 22°C ± 2°C and a light/dark cycle of 12 h/12 h. They were fed with the standard pellet chow diet, which was comprised of (%) 5 corn oil, 23 casein, 31.9 corn starches, 30.4 sucrose, 5.3 cellulose, 1.2 vitamin complex and 3.7 mineral mixture, and allowed free access to the drinking water. Iron and Fe<sup>2+</sup> content was 48 mg/Kg dry diet and 3.6 mg/Kg dry diet, respectively. The diabetic mice were induced by in-

traperitoneal injection of alloxan at a dose of 200 mg/kg body weight. The alloxan solution was freshly prepared with 0.1 mol/L citrate buffer (pH 4.0), kept in ice and injected immediately. Ten randomly selected mice that were only injected with citrate buffer were used as the normal group. Five days after injection of alloxan, blood was drawn from the tail vein of the fasted mice, The serum glucose level was determined by the glucose assay kit (Jiancheng Biological Institute, Nanjing, Jiangsu). These mice whose serum glucose levels exceeded 13 mmol/L were considered as the diabetic mice. All mice were not treated by insulin in the study. Procedures involving animal handling and care were conducted in conformity with the institutional guidelines of school of Medicine, Jiangsu University.

60 alloxan-induced diabetes mice (divided into six groups) and 10 normal mice as a normal group were used in the experiment. The mice were divided into the following seven treatment groups.

Group 1 (Normal group): Normal mice treated with the citrate buffer only.

Group 2 (Model group): Alloxan-treated diabetes mice intragastrically administrated with distilled water only.

Group 3 (FeSO<sub>4</sub> group): Alloxan-treated diabetes mice daily gavaged with FeSO<sub>4</sub> at a dose of 25 mg/Kg body weight [9].

Group 4 (FeCl<sub>3</sub> group): Alloxan-treated diabetes mice daily gavaged with FeCl<sub>3</sub> at a dose of 25 mg/Kg body weight [9].

Group 5 (Vc group): Alloxan-treated diabetes mice daily gavaged with Vc at a dose of 200 mg/Kg body weight [10].

Group 6 (FeSO<sub>4</sub> + Vc group): Alloxan-treated diabetes mice daily gavaged with FeSO<sub>4</sub> at a dose of 25 mg/Kg body weight and Vc at a dose of 200 mg/Kg body weight.

Group 7 (Metformin group): Alloxan-treated diabetes mice daily gavaged with metformin at a dose of 50 mg/Kg body weight.

The blood glucose levels were checked every seven days. After continuously administrated drugs for 28 days, all mice were fasted overnight (18 - 24 hours), and their blood was drawn from the orbital sinus after anesthetization with ether and collected into the tubes containing potassium oxalate and sodium fluoride. These indexes including the blood insulin, glucose and fructosamine levels, Fe<sup>2+</sup> and total iron content, blood total cholesterol, phospholipids, and triglycerides levels were determined with corresponding assay kit (Jiancheng Biological Institute, Nanjing, Jiangsu) according to their manufacturer's protocols. And then, their liver tissues were excised immediately, washed with ice-cold saline, weighed and homogenized with 3 times 0.9% NaCl solution (1/3 parts (w/v)) in ice bath using a homogenizer.

The homogenates were centrifuged at  $10,000 \times g$  for 15 minutes at 4°C to discard any cell debris. The glucose consumption (GC) of supernatant was analyzed. Simultaneously, the effect of iron and oxidant (STZ) and reducer (vitamine c, Vc) on GC of diabetic mice in the model group was analyzed.

### 2.3. The Design of Experiment *in Vitro*

To disclose the mechanism that iron coupled with oxidant stress regulated GC of diabetic mice, three test systems *in vitro* were designed using diabetes of model group and method and purpose of system was shown in **Table 1**.

### 2.4. Analytical Methods

The glucose consumption (GC) of mice's liver was carried out according to the method described by Zhang *et al.* [11]. The reaction solution was composed of 1 mL supernatant of liver homogenates, 0.3 mL H<sub>2</sub>O and 1 mL pH 7.4 0.2 mol/L phosphate buffer solutions contained 0.2% glucose and 0.2% NaN<sub>3</sub>. Before incubated and after being incubated for 48 h at 37°C, the sample of reaction solution was withdrawn for assaying glucose, respectively. The supernatant of liver exterminated activity homogenates in 80°C for 60 min instead of the supernatant was used as control. Glucose was assayed by blood glucose assay kit according to the manufacturer's protocols. After adding

all of other solutions, the final stable reaction volume was adjusted by H<sub>2</sub>O. GC was calculated as follows:

$$G_c = C_G - S_G \quad (1)$$

$G_c$ : GC (g/48 h);  $C_G$ : the glucose concentration of control tube.  $S_G$ : the glucose concentration of sample tube.

The levels of blood glucose, fructosamine, total cholesterol and triglycerides in the serum samples were determined by their corresponding colorimetric assay kits (Jiancheng Biological Insititute, Nanjing, Jiangsu) according to the manufacturer's protocols. The serum Fe<sup>2+</sup> and total iron levels were determined with the phenanthroline spectrophotometry methods [12].

### 2.5. Statistical Analysis

Data were expressed as the mean  $\pm$  SD for values obtained from ten mice in each group. Differences between groups were calculated by the Student's t-test [13]. The differences between the means were assessed using Duncan multiple range test with  $p < 0.05$  considered statistically significant.

## 3. Results

The animal's body weights were stable and significant lower in FeCl<sub>3</sub> group and diabetic model group than in normal group (**Table 2**) ( $p > 0.05$ ), and the body weight

**Table 1. The methods and purposes of three test systems.**

System	Method	Purpose
1	Adding 0.2 mL of H <sub>2</sub> O and 0.1 mL of FeSO <sub>4</sub> or FeCl <sub>3</sub> with various concentrations in glucose consumption test, respectively.	To compare the effect of FeSO <sub>4</sub> or FeCl <sub>3</sub> on glucose consumption in liver of diabetic mice
2	Adding 0.1 mL of FeSO <sub>4</sub> with various concentrations, 0.1 mL of vitamin C with various concentrations and 0.1 mL of H <sub>2</sub> O in glucose consumption test.	To study the impact of reduced oxidative stress on glucose consumption
3	Adding 0.1 mL of FeSO <sub>4</sub> with various concentrations, 0.1 mL of streptozotocin with various concentrations and 0.1 mL of H <sub>2</sub> O in glucose consumption test.	To study the impact of enhanced oxidative stress on glucose consumption

**Table 2 Effect of tested drugs on body weight of diabetic mice for 28 days.**

Group	Body weight (g)				
	0	7	14	21	28
Normal	18.75 $\pm$ 2.43	20.43 $\pm$ 1.96	22.01 $\pm$ 2.41	23.14 $\pm$ 2.07	23.59 $\pm$ 2.58
Model	16.43 $\pm$ 2.61	16.12 $\pm$ 2.73*	16.76 $\pm$ 2.66*	17.24 $\pm$ 2.58*	17.33 $\pm$ 2.89*
Metformin	16.87 $\pm$ 2.74	16.92 $\pm$ 2.39*	17.93 $\pm$ 2.42*	18.45 $\pm$ 2.80*	19.16 $\pm$ 2.87*
FeSO <sub>4</sub>	16.71 $\pm$ 2.51	17.25 $\pm$ 2.86*	18.77 $\pm$ 2.65*	19.04 $\pm$ 2.49**	20.74 $\pm$ 2.74**
FeCl <sub>3</sub>	16.55 $\pm$ 2.33	16.31 $\pm$ 2.86*	16.25 $\pm$ 3.01*	15.99 $\pm$ 2.76*	15.48 $\pm$ 2.99*
Vc	16.42 $\pm$ 2.08	16.48 $\pm$ 2.74*	16.92 $\pm$ 3.16*	17.48 $\pm$ 2.79*	18.24 $\pm$ 2.94*
FeSO <sub>4</sub> + Vc	16.43 $\pm$ 2.65	17.78 $\pm$ 2.47*	19.16 $\pm$ 2.89	20.51 $\pm$ 2.63**	21.47 $\pm$ 2.77**

Data are presented as Mean  $\pm$  SD for ten mice in each group. A significant difference is considered at  $p < 0.05$  (DMRT). \*: Compared with the normal group; °: Compared with the model group; \*°: Compared with the FeCl<sub>3</sub> group; °°: Compared with the (FeSO<sub>4</sub> + Vc) group.

of animals in FeSO<sub>4</sub>, FeSO<sub>4</sub> + Vc and metformin group were all significantly increased, and higher than not only their initial body weights but also the body weight of model group in the all experimental process (**Table 2**) ( $p < 0.05$ ). Although the body weight of FeSO<sub>4</sub> + Vc were higher than those of FeSO<sub>4</sub> group, but no significant difference between two group's body weight was found after administered drugs ( $p > 0.05$ ). The levels of serum glucose in diabetic mice were summarized in **Table 3**. At the beginning of the experiment, all tested groups of mice had nearly the same blood glucose levels ( $p > 0.05$ ) and their blood glucose levels were higher than those of normal group. The constant blood glucose levels of diabetic model group during all experimental process demonstrated that the model mice could be used to study. The results serum glucose levels of the mice in the FeSO<sub>4</sub>, FeSO<sub>4</sub> + Vc and metformin groups decreased, and were significantly lower than those in the model group and FeCl<sub>3</sub> group ( $p < 0.05$ ). The difference of serum glucose levels was also found between FeSO<sub>4</sub> + Vc and Vc group ( $p < 0.05$ ).

The serum insulin levels of diabetes mice were lower

than normal mice (**Table 4**). After administrated drugs, metformin remarkably elevated the serum insulin level of model animal and the similar effect was not found in other drugs group. The fructosamine and total triglyceride, cholesterol levels of model and FeCl<sub>3</sub> group were significantly higher than those of normal group. No significant difference between FeCl<sub>3</sub> and model group were observed in these indexes ( $p > 0.05$ ) (**Table 4**). Compared those in the model group and FeCl<sub>3</sub> treated mice, the serum fructosamine, triglyceride and total cholesterol levels in the FeSO<sub>4</sub>, Vc, FeSO<sub>4</sub> + Vc and metformin groups were significantly decreased ( $p < 0.05$ ). Moreover, these indexes of FeSO<sub>4</sub> + Vc group were lower than of FeSO<sub>4</sub> and Vc group (**Table 4**). Because of the alloxan induced effect, total iron and iron (II) were remarkably decreased in diabetic mice (**Table 5**). Supplying FeSO<sub>4</sub>, Vc and FeSO<sub>4</sub> + Vc could increase the total iron and iron (II) levels. Especially, increase amount of the total iron and iron (II) of the FeSO<sub>4</sub> + Vc group was biggest in three group. But the similar effect could be found in FeCl<sub>3</sub> group.

The GC test *in vitro* (**Figure 1**) showed the GC was

**Table 3. Effect of test drugs on serum glucose level in diabetic mice for 28 days.**

Group	Serum glucose level (mmol/L)				
	0	7	14	21	28
Normal	5.34 ± 1.35	5.46 ± 1.28	5.68 ± 1.10	5.70 ± 1.38	5.68 ± 1.57
Model	20.26 ± 2.24*	20.57 ± 2.33*	20.78 ± 2.96*	21.43 ± 2.83*	22.01 ± 2.71*
Metformin	20.24 ± 2.35*	18.79 ± 2.68*	16.91 ± 3.12* <sup>◊</sup>	16.38 ± 2.94* <sup>◊</sup>	16.48 ± 2.84* <sup>◊</sup>
FeSO <sub>4</sub>	20.31 ± 2.01*	19.74 ± 2.90*	18.61 ± 2.64* <sup>•</sup>	17.17 ± 2.33* <sup>◊</sup>	16.29 ± 2.99* <sup>◊</sup>
FeCl <sub>3</sub>	20.25 ± 2.19*	21.37 ± 2.55*	20.97 ± 2.94*	21.64 ± 3.02*	22.97 ± 2.89*
Vc	20.23 ± 2.41*	19.64 ± 2.63*	19.12 ± 2.78*	18.75 ± 2.38* <sup>◊</sup>	17.88 ± 2.57* <sup>◊</sup>
FeSO <sub>4</sub> +Vc	20.25 ± 2.12*	18.14 ± 2.56* <sup>•</sup>	17.03 ± 2.93* <sup>◊</sup>	15.76 ± 2.83* <sup>◊</sup>	14.02 ± 2.75* <sup>◊</sup>

Data are presented as Mean ± SD for ten mice in each group. A significant difference is considered at  $p < 0.05$  (DMRT). \*: Compared with the normal group; ◊: Compared with the model group; •: Compared with the FeCl<sub>3</sub> group; ◊: Compared with the (FeSO<sub>4</sub> + Vc) group.

**Table 4. Effect of test drugs on serum insulin, fructosamine, triglyceride and total cholesterol levels in diabetic mice for 28 days.**

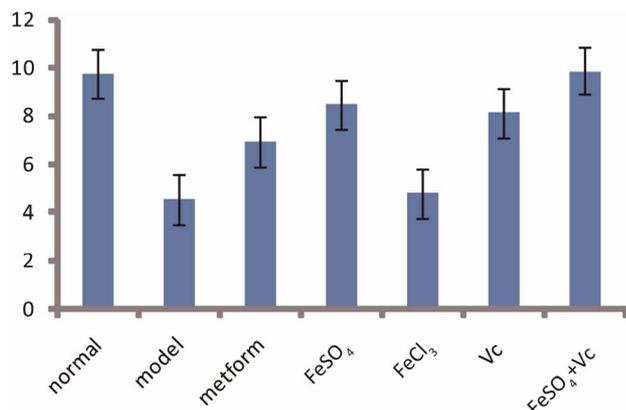
Group	Insulin (μU/L)	Fructosamine (mmol/L)	Triglyceride (mmol/L)	Total cholesterol (mmol/L)
Normal	71.34 ± 9.08	2.53 ± 0.19	3.62 ± 0.48	4.09 ± 0.38
Model	52.83 ± 8.32*	4.71 ± 0.24*	7.03 ± 0.31*	7.54 ± 0.36*
Metformin	66.59 ± 7.58* <sup>◊</sup>	3.94 ± 0.17* <sup>◊</sup>	6.02 ± 0.42* <sup>◊</sup>	4.73 ± 0.32* <sup>◊</sup>
FeSO <sub>4</sub>	53.14 ± 8.06*	3.06 ± 0.20* <sup>◊</sup>	5.57 ± 0.42* <sup>◊</sup>	4.57 ± 0.36* <sup>◊</sup>
FeCl <sub>3</sub>	52.19 ± 8.62*	4.67 ± 0.23*	6.85 ± 0.33*	7.43 ± 0.42*
Vc	55.61 ± 8.91	4.07 ± 0.18* <sup>◊</sup>	6.36 ± 0.37* <sup>◊</sup>	5.63 ± 0.51* <sup>◊</sup>
FeSO <sub>4</sub> + Vc	54.86 ± 7.77	2.84 ± 0.22* <sup>◊</sup>	5.44 ± 0.34* <sup>◊</sup>	4.38 ± 0.42* <sup>◊</sup>

Data are presented as Mean ± SD for ten mice in each group. A significant difference is considered at  $p < 0.05$  (DMRT). \*: Compared with the normal group; ◊: Compared with the model group; •: Compared with the FeCl<sub>3</sub> group; ◊: Compared with the (FeSO<sub>4</sub> + Vc) group.

**Table 5. Effect of FeSO<sub>4</sub>, FeCl<sub>3</sub> and metformin on serum total iron and iron (II) contents in diabetic mice for 28 days.**

Group	Total iron content (μmol/L)	Iron (II) content (μmol/L)
Normal	21.45 ± 1.67	7.46 ± 1.18
Model	16.31 ± 2.31*	4.03 ± 2.06*
Metformin	18.27 ± 2.07 <sup>o</sup>	4.25 ± 1.83*
FeSO <sub>4</sub>	21.44 ± 1.88 <sup>oo</sup>	6.84 ± 1.75 <sup>††</sup>
FeCl <sub>3</sub>	17.61 ± 1.94 <sup>o</sup>	3.79 ± 1.66*
Vc	19.63 ± 1.83 <sup>oo</sup>	5.82 ± 1.49
FeSO <sub>4</sub> + Vc	23.67 ± 2.05 <sup>oo*</sup>	7.52 ± 1.95

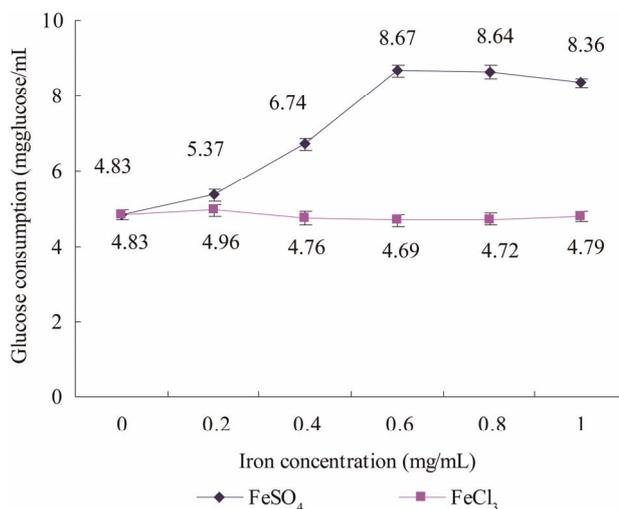
Data are presented as Mean ± SD for ten mice in each group. A significant difference is considered at  $p < 0.05$  (DMRT). \*: Compared with the normal group; <sup>o</sup>: Compared with the model group; <sup>†</sup>: Compared with the FeCl<sub>3</sub> group, <sup>oo</sup>: Compared with the (FeSO<sub>4</sub> + Vc) group.

**Figure 1. Effect of FeSO<sub>4</sub>, FeCl<sub>3</sub>, Vc, (FeSO<sub>4</sub> + Vc) or metformin on glucose consumption in liver of diabetic mice for 28 days. Data are presented as Mean ± SD from ten mice in each group.**

significantly lower in model group than in normal group. After administrated drugs, the GC of the animal liver in FeSO<sub>4</sub>, Vc, FeSO<sub>4</sub> + Vc and metformin group increased significantly, especially, the GC in FeSO<sub>4</sub> + Vc was nearly equal to the one in normal group. No significant difference GC between FeCl<sub>3</sub> and diabetic model groups was found.

After different concentration of FeSO<sub>4</sub> and FeCl<sub>3</sub> was added to the react solution, the GC of liver of diabetic mice showed different change (Figure 2). With the concentration of FeSO<sub>4</sub> increasing from 0 to 0.6 mg/mL, the GC of diabetic mice's liver gradually increased, when concentration of FeSO<sub>4</sub> was between 0.6 - 0.8 mg/mL, the GC kept stable and when concentration of FeSO<sub>4</sub> was over 0.8 mg/mL, the GC gradually reduced. However, with FeCl<sub>3</sub> concentration increasing from 0 to 1 mg/mL, no significant change in GC was observed.

Effect of the different concentration of FeSO<sub>4</sub> coupled

**Figure 2. Effect of FeSO<sub>4</sub> or FeCl<sub>3</sub> on glucose consumption in liver of diabetic mice. ♦:FeSO<sub>4</sub>; ■:FeCl<sub>3</sub>.**

with varied amount of Vc on GC diabetes mice was studied (Figure 3). When Vc concentration increased from 0 to 0.6 mg/mL without FeSO<sub>4</sub>, the GC increased gradually and over 0.6 mg/mL, the GC stabilized all the time; with the Vc concentration increased from 0 to 0.6 mg/mL at 0.2 mg/mL FeSO<sub>4</sub>, the GC gradually increased and achieved the maximum (8.13 mg/mL) at the 0.6 mg/mL Vc; with the Vc concentration from 0 to 0.4 mg/mL at the 0.4 mg/mL FeSO<sub>4</sub>, the GC significantly enhanced, then stabilized when Vc concentration was between 0.4 - 0.8 mg/mL and the concentration of FeSO<sub>4</sub> was over 0.8 mg/mL, GC sharply declined; GC has markedly increased with the concentration Vc rising from 0 to 0.2 mg/mL at 0.6 mg/mL FeSO<sub>4</sub>, but when the concentration Vc exceeded 0.2 mg/mL, GC significantly reduced. However, no matter how much FeSO<sub>4</sub> was added to reactive solution, GC sharply declined in the present of streptozotocin (Figure 4). These results also showed that proper control of oxidative stress, supplying Fe<sup>2+</sup> contributes to the regulation of glucose metabolism in animals.

#### 4. Discussion

Many human diseases are associated with increased oxidative stress in body. Studies have observed that middle-aged and elderly patients with type 2 diabetes mellitus have higher levels of oxidative stress markers compared to a control population [14-20]. The damage effects of increased oxidative stress on all tissue were extensively studied [21,22]. However, impact of increased oxidative stress on trace-elements have few been reported. In the body, many trace-elements (such as iron) exist in different valence. When these elements participate in live activities as coenzyme or prosthetic

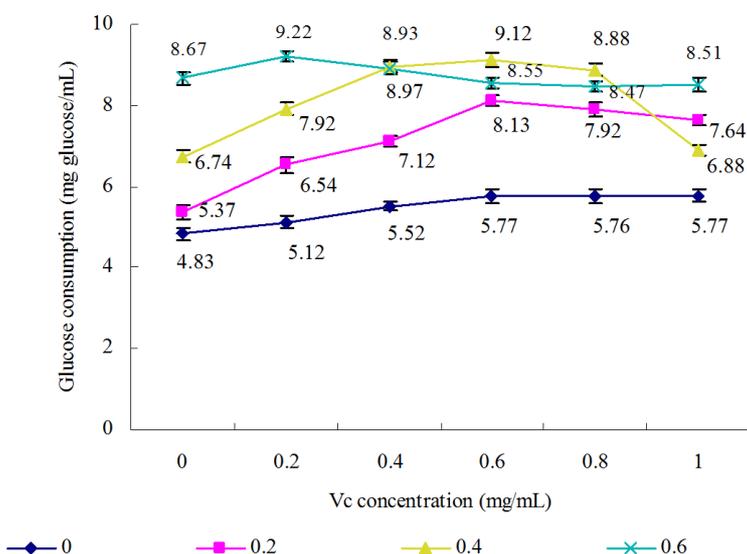


Figure 3. Synergistic effect of combinatorial supplementation of FeSO<sub>4</sub> and Vc on glucose consumption in liver of diabetic mice. ◆: 0 mg/ mL FeSO<sub>4</sub>; ■: 2 mg/mL FeSO<sub>4</sub>; ▲: 4 mg/mL FeSO<sub>4</sub>; ✕: 6 mg/mL FeSO<sub>4</sub>.

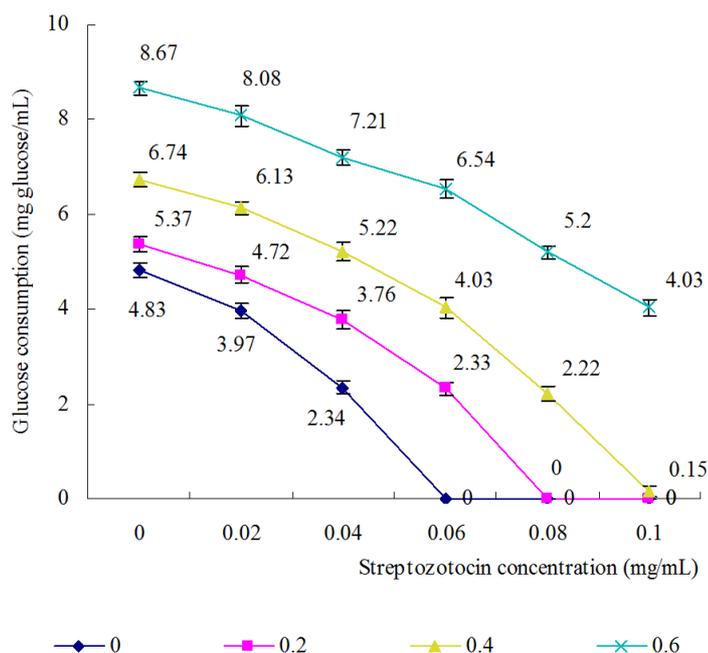


Figure 4. Antagonistic effect of combinatorial supplementation of FeSO<sub>4</sub> and STZ on glucose consumption in liver of diabetic mice. ◆: 0 mg/mL FeSO<sub>4</sub>; ■: 2 mg/mL FeSO<sub>4</sub>; ▲: 4 mg/mL FeSO<sub>4</sub>; ✕: 6 mg/mL FeSO<sub>4</sub>.

group, different valences of these elements common transformed each other and form a micro-cycle. Iron is a most typical example.

Iron presences in the human body as a prosthetic group of those enzymes related with glucose metabolism, such as cytochrome oxidase, catalase, aldehyde oxidase, xanthine oxidase, succinate dehydrogenase,  $\alpha$ -glycerol phosphate oxidase, lipid peroxidase, proline hydroxylase and lysine hydroxylase. Tricarboxylic acid cycle (TCA) is the aerobic oxidation of glucose *in vivo*, an important path-

way to supply energy. More than half of the enzymes or cofactors of TCA cycle and electronic transmission chain contain iron or play a physiological function only in the present of iron. Thus, its deficiency and excess will impact on glucose metabolism and electron transmission of the body. Iron exists as Fe<sup>2+</sup> and Fe<sup>3+</sup>. When iron plays their physiological role, two different valence of iron continuously transforms each other and form iron cycle

Increased oxidative stress has been reported in diabetes mellitus, elder and other chronic state. Previous

studies showed this hyper-oxidation can damage tissue [23-25,18]. However, the studies on that the hyper-oxidation interdicts reduction of Fe<sup>3+</sup> and blocks the glucose metabolism in body have few reported. GC is degraded in unit time can directly reflect the relative strength of glucose metabolism. In our test, the GC of normal and diabetic rat's liver was first compared. It was found that the GC of diabetic rat's liver was significantly lower than of normal rat's liver (**Figure 1**) and elevated by added Fe<sup>2+</sup> instead of Fe<sup>3+</sup> (**Figure 2**). If some reducer (such as Vc) and Fe<sup>2+</sup> simultaneously were added, GC could be elevated further (**Figure 3**) and if some oxidant (such as STZ) and Fe<sup>2+</sup> simultaneous were added, GC markedly decreased (**Figure 4**). Under higher oxidant stress state, supplying Fe<sup>3+</sup> could not facilitate glucose metabolism. Therefore, to control appropriate oxidative stress redounded to glucose metabolism.

The only different in glucose metabolism between *in vivo* and *in vitro* is each cell is surrounded by cell membrane which separates it from the outside world *in vivo*, interior of cell is separated into many special circumscriptions by membrane and glucose metabolism is fulfilled in these special circumscriptions, such as Embden-Meyerhof-Parnas (EMP) takes place in cytoplasm, the tri-carboxylic acid cycle (TCA) pathways and the electron transfer do within mitochondrion and in membrane of mitochondrion, respectively. Biological membranes separates cell from the outside circumstance and also becomes physical barriers which limit glucose to enter into cell. The pathways that glucose enters into cellular primarily includes two type: Na<sup>+</sup>-glucose transporter (active transporter) and facilitate diffusion. Facilitate diffusion was the dominating factor [26]. The flux for glucose to enter into cell may be described with following formula:

$$j = (\alpha D/l)(C^* - C) = p(C^* - C) \quad (2)$$

where  $j$  are the flux for glucose to enter into cell,  $C^*$  the extracellular glucose concentration (bulk concentration);  $C$  the intracellular glucose concentration,  $D$  is the (constant) diffusion coefficient,  $\alpha$  is the glucose distribution coefficient between the extra- and intra-cellular phase, and  $l$  the membrane thickness.  $p$  is called permeability [27].

It is clear that the impetus to increase diffusion flux of glucose depends on permeability and the glucose levels' difference of both sides of the membrane. Insulin heightens the flux of glucose to enter into cell mostly by increasing the affinity of glucose for carrier protein. The high glucose level of intracellular under hyper-oxidation lessens the glucose level' difference of extra- and intra-cellular, the flux for glucose to enter into cell and elevates the blood glucose level. Therefore, reducing

oxidation stress *in vivo* helps to elevate GC of body, accelerate glucose metabolism, facilitate glucose in blood entering into cellular and finally decrease blood glucose levels.

To prove the hypothesis, the alloxan induced diabetic mice were treated with FeSO<sub>4</sub>, FeCl<sub>3</sub>, Vc and FeSO<sub>4</sub> + Vc, respectively. Alloxan is a kind of strange oxidant and not only damages islet cell, induce diabetes and but also elevated oxidant stress of test animal *in vivo*. Therefore, many papers have reported that higher oxidant stress than normal test animals exists in alloxan induced diabetic animals. Hyperoxidation in diabetic mice blocked Fe<sup>3+</sup> reduced to Fe<sup>2+</sup> and contributed to serum iron deficiency of diabetic mice (**Figure 1**). After oral administration of FeSO<sub>4</sub>, Vc and FeSO<sub>4</sub> + Vc, serum glucose, fructosamine and lipid levels would decrease (**Table 4**) and serum total iron and Fe<sup>2+</sup> content and GC of liver would be significantly increase. Specially, the effect of FeSO<sub>4</sub> + Vc was more significant. These results demonstrated that supplied Fe<sup>2+</sup> coupled with controlling oxidative stress helps to enhance glucose metabolism and decrease the blood glucose levels of diabetes.

## 5. Conclusion

The study showed that supplying Fe<sup>2+</sup> coupled with Vc can elevate the GC of diabetic mice's liver *in vitro*. The same result was also obtained *in vivo*. Supplementation of Fe<sup>2+</sup> and Vc to alloxan induced diabetic mice could renew the iron cycle, improve oxygen transportation and oxygen utilization efficiency and decrease serum glucose and lipid levels in the alloxan-induced diabetes mice or even in the diabetes mellitus patients. If Vc and Fe<sup>2+</sup> supplement at the same time, these activity of Fe<sup>2+</sup> in diabetes would be better. In all, it is helpful to monitor Fe<sup>2+</sup> content instead of total iron content and control sufferer oxidant stress to maintain Fe<sup>2+</sup> at a special content *in vivo* for prevention and treatment of diabetes.

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## REFERENCES

- [1] V. B. Junqueira, S. B. Barros, S. S. Chan, L. Rodrigues, L. Giavarotti, R. L. Abud and G. P. Deucher, "Aging and Oxidative Stress," *Molecular Aspects of Medicine*, Vol. 25, No. 1-2, 2004, pp. 5-16.  
[doi:10.1016/j.mam.2004.02.003](https://doi.org/10.1016/j.mam.2004.02.003)

- [2] J. Karolkiewicz, E. Michalak, B. Pospieszna, E. Deskur-Smielecka, A. Nowak and L. Pilaczyńska-Szcześniak, "Response of Oxidative Stress Markers and Antioxidant Parameters to an 8-Week Aerobic Physical Activity Program in Healthy, Postmenopausal Women," *Archives of Gerontology and Geriatrics*, Vol. 49, No. 1, 2009, pp. e67-e71. [doi:10.1016/j.archger.2008.09.001](https://doi.org/10.1016/j.archger.2008.09.001)
- [3] P. Moller, H. Wallin and L. E. Knudsen, "Oxidative Stress Associated with Exercise, Psychological Stress and Life-Style Factors," *Chemico-Biology Interactions*, Vol. 102, No. 1, 1996, pp. 17-36. [doi:10.1016/0009-2797\(96\)03729-5](https://doi.org/10.1016/0009-2797(96)03729-5)
- [4] C. Nasuti, R. Gabbianelli, M. L. Falcioni, A. Di Stefano, P. Sozio and F. Cantalamessa, "Dopaminergic System Modulation, Behavioral Changes, and Oxidative Stress after Neonatal Administration of Pyrethroids," *Toxicology*, Vol. 229, No. 3, 2007, pp. 194-205. [doi:10.1016/j.tox.2006.10.015](https://doi.org/10.1016/j.tox.2006.10.015)
- [5] A. Peter, "Bioenergetics and the Metabolism of Carbohydrates and Lipids," In: K. Robert, K. Daryl and W. Victor, Eds., *Harper's Biochemistry*, 25th Edition, Sience Press, 2000, pp. 182-208.
- [6] S. Filiz, Ö. Gülyüz, E. Deniz and H. Alev, "Antioxidant Effect of Vitamin E in the Treatment of Nutritional Iron Deficiency Anemia," *Turkish Journal of Hematology*, Vol. 23, No. 1, 2006, pp. 15-24.
- [7] B. J. Clodfelder, B. M. Gullick, H. C. Lukaski, Y. Neggers and J. B. Vincent, "Oral Administration of the Biomimetic [Cr<sub>3</sub>O(O<sub>2</sub>CCH<sub>2</sub>CH<sub>3</sub>)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> Increases Insulin Sensitivity and Improves Blood Plasma Variables in Healthy and Type 2 Diabetic Rats," *Journal of Biological Inorganic Chemistry*, Vol. 10, No. 2, 2005, pp. 119-130. [doi:10.1007/s00775-004-0618-0](https://doi.org/10.1007/s00775-004-0618-0)
- [8] Y. Sun, K. Mallya, J. Ramirez and J. B. Vincent, "The Biomimetic [Cr<sub>3</sub>O(O<sub>2</sub>CCH<sub>2</sub>CH<sub>3</sub>)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> Decreases Plasma Cholesterol and Triglycerides in rats: Towards Chromium-Containing Therapeutics," *Journal of Biological Inorganic Chemistry*, Vol. 4, No. 6, 1999, pp. 838-845. [doi:10.1007/s007750050357](https://doi.org/10.1007/s007750050357)
- [9] Z. C. Zhang, B. Lian and F. J. Cui, "Effect of FeSO<sub>4</sub> Treatment on Glucose Metabolism in Diabetic Rats," *Biometals*, Vol. 21, No. 6, 2008, pp. 685-691. [doi:10.1007/s10534-008-9153-8](https://doi.org/10.1007/s10534-008-9153-8)
- [10] N. Aksoya, H. Vural, T. Sabuncub, O. Arslan and S. Aksoy, "Beneficial Effects of Vitamins C and E against Oxidative Stress in Diabetic Rats," *Nutrition Research*, Vol. 25, No. 6, 2005, pp. 625-630. [doi:10.1016/j.nutres.2005.05.005](https://doi.org/10.1016/j.nutres.2005.05.005)
- [11] Z. C. Zhang, X. Li, M. X. Chen and W. L. Shen, "Assay of the Glucose Consumption Rate in Liver with a Novel Colorimetric Method," *Current Pharmaceutical Analysis*, Vol. 7, No. 3, 2011, pp. 195-201. [doi:10.2174/1573412111796353336](https://doi.org/10.2174/1573412111796353336)
- [12] J. Lin and S. H. Ji, "The Determination of Dietary Fe (II) and (III)," *Guangdong Microelement Science*, Vol. 3, No. 8, 1996, pp. 148-152.
- [13] D. Duncan, "Multiple Range Tests for Correlated and Heteroscedastic Means," *Biometrics*, Vol. 13, No. 2, 1957, pp. 164-176. [doi:10.2307/2527799](https://doi.org/10.2307/2527799)
- [14] M. Brownlee, "Biochemistry and Molecular Cell Biology of Diabetic Complications," *Nature*, Vol. 414, No. 6865, 2001, pp. 813-820. [doi:10.1038/414813a](https://doi.org/10.1038/414813a)
- [15] M. Brownlee, "The Pathobiology of Diabetic Complications: A Unifying Mechanism," *Diabetes*, Vol. 54, No. 6, 2005, pp.1615-1625. [doi:10.2337/diabetes.54.6.1615](https://doi.org/10.2337/diabetes.54.6.1615)
- [16] J. L. Evans, I. D. Goldfine, B. A. Maddux and G. M. Grodsky, "Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes," *Endocrine Reviews*, Vol. 23, No. 5, 2002, pp. 599-622. [doi:10.1210/er.2001-0039](https://doi.org/10.1210/er.2001-0039)
- [17] D. Jay, H. Hitomi and K. K. Griendling, "Oxidative Stress and Diabetic Cardiovascular Complications," *Free Radical Biology & Medicine*, Vol. 40, No. 2, 2006, pp. 183-192. [doi:10.1016/j.freeradbiomed.2005.06.018](https://doi.org/10.1016/j.freeradbiomed.2005.06.018)
- [18] P. Rosen, P. P. Nawroth, G. King, W. Möller, H. J. Tritschler and L. Packer, "The Role of Oxidative Stress in the Onset and Progression of Diabetes and Its Complications: A Summary of a Congress Series Sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society," *Diabetes/Metabolism Research and Reviews*, Vol. 17, No. 3, 2001, pp. 189-212. [doi:10.1002/dmrr.196](https://doi.org/10.1002/dmrr.196)
- [19] J. A. Scott and G. L. King, "Oxidative Stress and Antioxidant Treatment in Diabetes," *Annals of the New York Academy Science*, Vol. 1031, No. 12, 2004. pp. 204-213. [doi:10.1196/annals.1331.020](https://doi.org/10.1196/annals.1331.020)
- [20] I. C. West, "Radicals and Oxidative Stress in Diabetes," *Diabetic Medicine*, Vol. 17, No. 3, 2000, pp. 171-180. [doi:10.1046/j.1464-5491.2000.00259.x](https://doi.org/10.1046/j.1464-5491.2000.00259.x)
- [21] P. Anabela and M. P. Carlos, "Diabetes and Mitochondrial Function: Role of Hyperglycemia and Oxidative Stress," *Toxicology and Applied Pharmacology*, Vol. 212, No. 2, 2006, pp. 167-178. [doi:10.1016/j.taap.2006.01.003](https://doi.org/10.1016/j.taap.2006.01.003)
- [22] A. C. Maritim, R. A. Sanders and J. B. Watkins, "Diabetes, Oxidative Stress, and Antioxidants: A Review," *Journal of Biochemical and Molecular Toxicology*, Vol. 17, No. 1, 2003, pp. 24-38. [doi:10.1002/jbt.10058](https://doi.org/10.1002/jbt.10058)
- [23] Z. C. Feng, T. J. Sick, M. Rosenthal, "Oxygen Sensitivity of Mitochondrial Redox Status and Evoked Potential Recovery Early during Reperfusion in Post-Ischemic Rat Brain," *Resuscitation*, Vol. 37, No. 1, 1998, pp. 33-41. [doi:10.1016/S0300-9572\(98\)00031-8](https://doi.org/10.1016/S0300-9572(98)00031-8)
- [24] M. A. Perez-Pinzon, P. L. Mumford, M. Rosenthal and T. J. Sick, "Antioxidants, Mitochondrial Hyperoxidation and Electrical Recovery after Anoxia in Hippocampal Slices," *Brain Research*, Vol. 754, No. 1-2, 1997, pp. 163-170. [doi:10.1016/S0006-8993\(97\)00066-8](https://doi.org/10.1016/S0006-8993(97)00066-8)
- [25] M. Rosenthal, Z. C. Feng, C. N. Raffin, M. Harrison and T. J. Sick, "Mitochondrial Hyperoxidation Signals Residual Intracellular Dysfunction after Global Ischemia in Rat Neocortex," *Journal of Cerebral Blood Flow & Metabolism*, Vol. 15, No. 4, 1995, pp. 655-665. [doi:10.1038/jcbfm.1995.81](https://doi.org/10.1038/jcbfm.1995.81)
- [26] T. Shen and J. Wang, "Biochemistry," Higher Education

Publisher, Beijing, 2000.

[27] W. Ebel, "Carrier Facilitated Diffusion," *Journal of*

*Mathematical Biology*, Vol. 21, No. 3, 1985, pp. 243-271.  
[doi:10.1007/BF00276225](https://doi.org/10.1007/BF00276225)