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Effects of Folic Acid and Magnesium Co-Administration on the Activity of Hepatic Glucokinase (GCK) in Streptozotocin – Induced Type I Diabetic Wistar Rats

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Abstract: Hyperglycemia is a common feature of diabetes mellitus. It results from a decrease in glucose utilization by the liver and peripheral tissues and an increase in hepatic glucose production. Glucose phosphorylation by glucokinase is an initial event in glucose metabolism by the liver. However, glucokinase gene expression is very low in diabetic animals. Hepatic GCK is a key enzyme in glucose homeostasis and, as such, is a potential target for treatment strategies of diabetes. The present day study investigated the effect of co- administration of folic acid and magnesium on GCK activity. Thirty wistar male rats were divided into six groups of five each. Diabetic groups received 20 and 500 mg/kg folic acid and /or magnesium chloride, separarately or in combination. Diabetic control and normal control received 0.9% saline for 4 weeks. It was found that during combined exposure of folic acid and magnesium, the adverse effects of the diabetes induced by STZ was less pronounced in the group that had FA+ Mg than their individual effects. This suggests the synergistic beneficial effects of folic acid and Magnesium against STZ-induced diabetes in Wistar Rats. Investigations of the hepatic glucokinase concentration by Real-Time PCR showed a decreased in GCK concentration in diabetic control rats. GCK activity increased significantly ($p<0.05$) in group treated with FA+Mg. These results indicated that FA+Mg co- administration may probably exhibit a significant potential as a hypoglycemic agent perhaps via its ability to enhance GCK gene expression and its activity.

Keywords: Glucokinse, Folic Acid, Magnesium, Diabetes Mellitus

INTRODUCTION

Diabetes mellitus is found in universal of the world and is rapidly increasing in most parts of the world. As diabetes aggravates and *β*-cell function deteriorates, the insulin level begins to fall below the body's requirements and causes prolonged and more severe hyperglycemia (Gerich, 2003).

Liver is an insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis by regulating the interaction between the glucose utilization and production. Thus, a suitable antidiabetic agent should improve glucose-induced insulin secretion, hepatic glucose me- tabolism, and peripheral insulin sensitivity (Ferre, *et al.*, 1996)

To stimulate pancreatic insulin secretion, glucose must be metabolized in pancreatic *β*-cell. Glucose must be first be phosphorylated before being utilized by cells. This reaction is catalyzed by a family of enzymes called hexo- kinases, which are found in different organisms diverse from bacteria to humans (Cardenas *et al.*, 1998).

Mammalian hexokinase IV, also known as GCK (glucokinase), plays a key role in maintaining glucose homoeostasis (Matschinsky, *et al.*, 2006) , and is the major glucose-phosphorylating enzyme expressed in hepato- cytes and pancreatic *β*-cells. GCK is a monomeric pro- tein of 465 amino acids and a molecular weight of about 50 kD. There are at least two clefts, one for the active site, binding glucose and MgATP, and the other for a putative allosteric activator that has not yet been identi-fied (Kamata *et al.*, 2004). GCK is unique amongst hexokinases in that it displays a sigmoidal substrate dose-response curve, demonstrates low affinity and positive cooperativity for substrate glucose, and is not susceptible to product (glucose-6-phosphate) inhibition. (Mehdi *et al*., 2013)

These properties are critical to the role GCK plays as the glucose sensor. Also, GCK mutations have been

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associated with maturity onset diabetes of the young (Froguel, *et al*., 1993). Given its pivotal role in regulating glucose homeostasis, there has been significant interest in GCK as a target for treating diabetes mellitus. GCK is one of the essential factors for the glucose- stimulated insulin secretion (Bourbonais, *et al*., 2011)

Long-term regulation of hepatic GCK activity is controlled by its mRNA level. Transcription of GCK is regulated differentially by an upstream promoter in pancreatic *β*-cells and a down- stream promoter in hepatocytes. Activation of either one of them leads to the generation of a GCK mRNA and produces active form of GCK (Iynedjian *et al*., 1989; Magnuson and Shelton, 1989).

In the liver, expression of GCK is very closely dependent on the presence of insulin. Stimulation of transcription of genes encoding GCK, leads to a decreased glucose level (Celik and Erdogan, 2008).

Fang *et al*., 2014, assessed the effect of FA treatment on T1DM by analyzing the gene expression profile of Endothelial progenitor cells (EPCs) from T1DM patients treated with FA and comparing it to that from healthy controls, and showed that FA normalized a majority of changes in gene expression induced by T1DM (99.6%), suggesting the therapeutic potential of FA in the treatment of T1DM. Magnesium is an essential ion involved in glucose homeostasis (Nadler and Rude 2005). It is a co-factor in the glucose transport system of plasma membrane, has an important role in the activity of various enzymes involved in glucose oxidation, may play a role in the release of insulin, and can modulate the mechanism of energy transfer from high energy phosphate bond (Yajnik *et al*., 1984).

MATERIALS AND METHODS

Streptozoxocin (Sigma Aldrich Inc.st Louis, MO, USA), Folic acid (Sigma), Magnesium, (Sigma Aldrich Inc.st Louis, MO, USA), Glucose. All other chemicals and drugs were obtained commercially and were of analytical grade.

Experimental Animals

Thirty Wistar rats male weighing between 200 to 250g (aged six to eight weeks) were obtained and housed in the animal house unit of the Department of Human Physiology, Ahmadu Bello University, Zaria. The normal standard rat chow and tap water were provided *ad libitum* during the experiment. Animals were stabilized to acclimatize to animal house environment for one week before commencement of the experiment. The study protocol was approved by the Institutional Animal Ethnic Committee of the University, Ahmadu Bello University, Zaria.

Methodology

Induction of Diabetes Mellitus

Diabetes was chemically induced by intraperitoneal (*i.p*) injection of freshly prepared in 0.1 mol/L citrate buffered solution (pH 4.5) of streptozotocin (Sigma Aldrich, St. Louis, MO, USA) at a dose of 60 mg/kg body weight. Control (vehicle) rats were injected with equal volume of 0.1 mol/L citrate buffer. Four days after STZ injection, diabetes induction was confirmed by measuring fasting blood glucose level in a tail vein blood samples using ACCU-CHEK compact plus glucometer (Roche, France). Rats with glucose level of 200 mg/dl or higher were considered as diabetic (Stanley *et al.,* 2001). Glucose levels of diabetic rats were checked before starting of treatment, so that animals could be homogenously and randomly distributed between the groups.

Experimental Design

Normal healthy rats were used as normal control and diabetic-induced rats and were randomly randomly alloted into six groups (n=5):

GROUP 1- Normal untrated were given normal saline 1 ml/kg daily orally for 4 weeks

GROUP 2- Diabetic untreated were given Normal saline 1ml/kg daily orally for 4 weeks.

GROUP 3- Diabetic were treated with magnesium 500 mg/kg daily orally for 4 weeks (Williams and Wilkin, 1909).

GROUP 4- Diabetic were treated with folic acid 20 mg/kg daily orally 4 weeks (Paget and Barnes, 1964).

GROUP 5- Diabetic were treated with magnesium 500 mg/kg + folic 20 mg/kg acid daily orally for 4 weeks

GROUP 6- Diabetic were treated with Insulin 6 U.I/kg *i.p*. daily orally for 4 weeks. (Stanley *et al.*, 2001).

Determination of Blood Glucose Levels

Blood samples were collected from the rat tail vein weekly for a period of four weeks. Determination of the blood glucose levels will be carried out using ACCU-CHEK compact plus glucometer (Roche, France).

Determination of Hepatic Enzyme Activity

Glucokinase activity was determined from liver samples homogenized in an ice cold buffer containing 50 mmol/L Tris-HCl, pH 7.4, 100 mmol/L KCl, 10 mmol/L mercaptoethanol, and 1 mmol/L EDTA. Homogenates were centrifuged at 100,000 *g* for 1 h; the postmicrosomal supernatants were used for the spectrophotometric continuous assay as described previously [Davidson and Arion,1987], with a slight modification, whereby the formation of glucose-6- phosphate from glucose at 27°C was coupled to its oxidation by glucose-6-phosphate

dehydrogenase and nicotinamide adenine dinucleotide (NAD). Briefly, the assays were carried out in a solution 50 mmol/L Tris-HCl, pH 7.4, 100 mmol/L KCl, 10 mmol/L mercaptoethanol, and 1 mmol/L EDTA. The reactions were initiated by adding 5U/mL of glucose-6-phosphate dehydrogenase (G6PDH) from *Leuconostoc mesenteroides*(Roche) and the rate of increase in absorbance at 340nm was measured. The GCK activity was calculated as the difference between the kinase activities measured at different glucose concentrations and 0.5mM glucose (hexokinase activity), and was expressed as mUnits/mg liver. One unit of enzyme activity is defined as that activity catalyzing the formation of 1 mmol of product per minute at 30°C [Seo yoon *et al*., 2000].

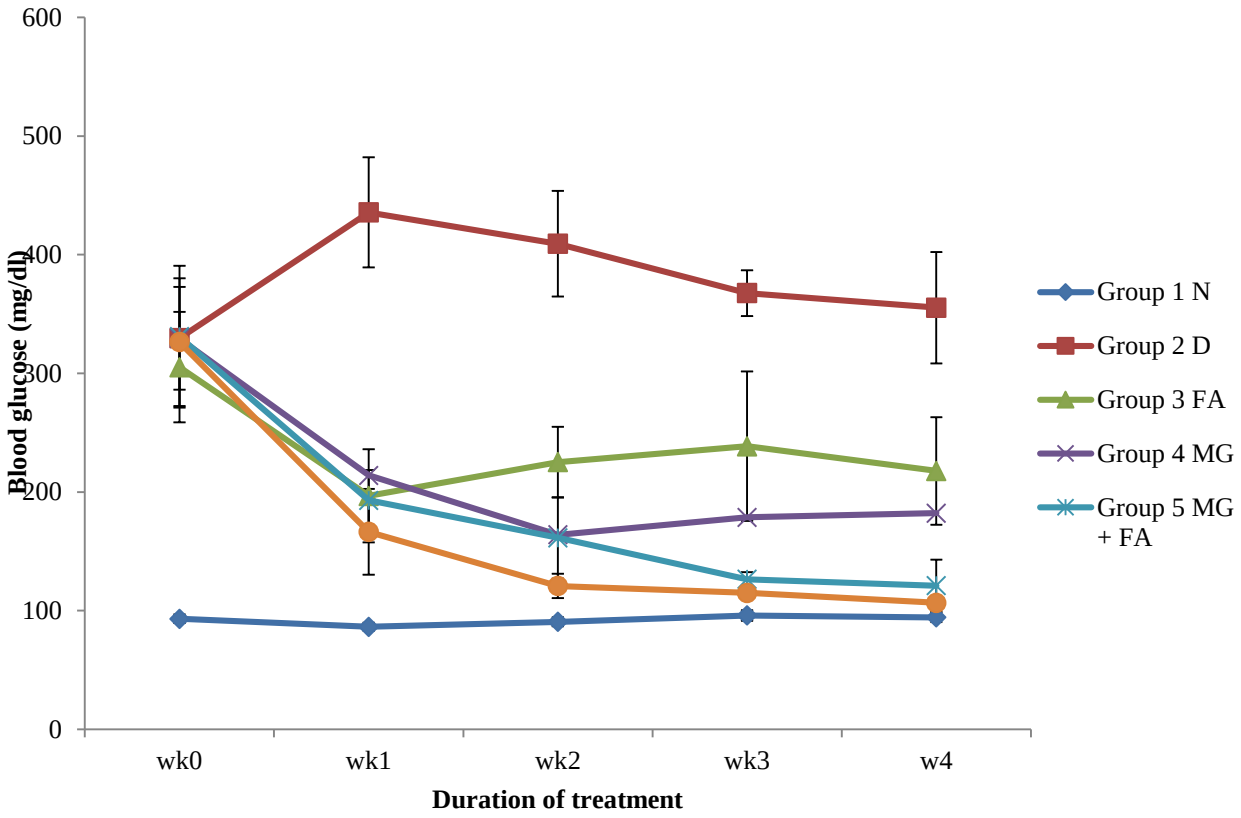
Statistical Analysis

All data were expressed as Mean±. SEM and data were entered and analyzed using statistical package SPSS (version 20) followed by one way analysis of variance (ANOVA) with multiple comparisons. The Tukey's post-hoc test was used to determine difference between groups. Values of $p<0.05$ was considered as statistically significant (Duncan *et al.*, 1977).

RESULTS

Hepatic glucokinase concentration (GCK)

The recorded values of diabetic control rats showed a notable ($P < 0.05$) decrease in GCK activity (9.40±0.53 IU/L) as compared with the normal control rats (19.60 ± 0.51 IU/L) (fig 1). In the diabetic treated group, a significant increase ($p < 0.05$) was recorded in the GCK concentration in the FA+ Mg group (18.36 ± 0.40 IU/L) compared to that of diabetic control group 14.78 ± 2.33 IU/L). Both treatment groups (FA+ diabetes and Mg+ diabetes) showed a detectable amelioration of GCK of diabetic rats; the recorded values were 15.06 ± 0.32 IU/L) and 12.12 ± 0.73 (IU/L) for FA and Mg groups respectively (Fig 1).



Note: Values are mean ± SEM, n=5
P< 0.05 versus diabetic group
P< 0.05 versus Normal group

Figure 1. **Effect of Normal saline, Folic acid and/ or Magnesium on Blood Glucose Concentration in STZ Induced diabetic Wistar rats.**

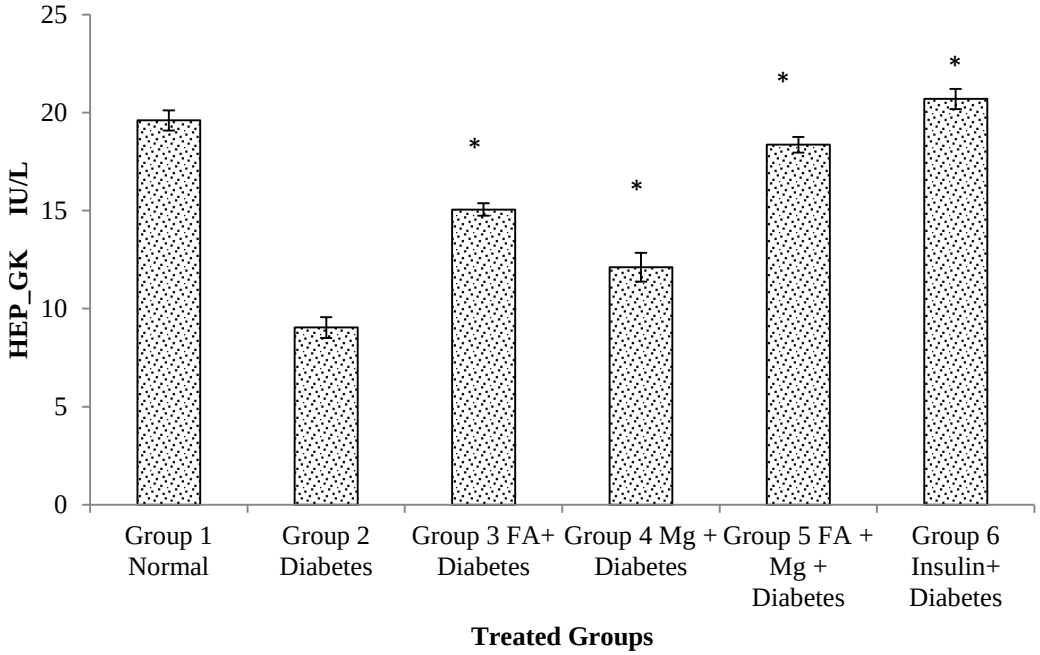


Figure 1. The effect of administration of FA and /or magnesium on GCK concentration in STZ induced diabetic rats (IU/L) (Mean ± SD) ($P < 0.05$). Group 1: Normal rats received 1 ml/kg normal saline. Group 2: diabetic rats received 1ml/kg normal saline. Group 3: diabetic rats received 20 mg/kg Folic acid(FA). Group 4: Diabeticl rats received 500 mg/kg(Mg) Magnesium chloride . Group 5 : Diabetic recieved 20 mg/kg + 500 mg/kg (FA+Mg)*Significant differences with Group 2 ($P < 0.05$).

DISCUSSION

The effect of STZ on glucose and insulin homeostasis reflects toxin-induced abnormalities in pancreatic beta cell function. Initially an inhibition of insulin biosynthesis and glucose- induced secretion as well as an impairment of glucose metabolism both glucose oxidation and glucose consumption (Bedoya et al., 1996), becomes evidence. On the other hand, STZ has no immediate and direct inhibitory effect upon glucose transport (Elsner et al., 2000) or upon glucose phosphorylation through glucokinase (lenzen et al., 1987b). Howevre, at later stages of functional beta cell impairment, deficiencies at the level of gene and protein expression and function of these structures becomes apparent (Wang and Gleichmann, 1998).

The present day study demonstrated that FA and magnesium co-administration significantly increased GCK activity in diabetic rats ($P < 0.05$). The decreased in the concentration of blood glucose in FA+Mg treated diabetic rats may be associated with enhancement of GCK mRNA expression in the liver, thus increasing the level of glycolysis. In the present study, Folic acid combination with magnesium may have induced mRNA expression levels of hepatic GCK and increased significantly GCK activity in the liver. This data is in agreement with fang *et al*. 2014 finding who reported the effect of FA treatment on T1DM by analyzing the gene expression profile of Endothelial progenitor cells (EPCs) from T1DM patients treated with FA and comparing it to that from healthy controls, and showed that FA normalized a majority of changes in gene expression induced by T1DM (99.6%), suggesting the therapeutic potential of FA in the treatment of T1DM. The present day finding suggest that the FA+Mg combination could restore the altered glucose homeostsis; It is probable that the combination has the ability to acceler ate the hepatic glucose metabolism may be via regulating the expression of the functional genes of GCK. .In fact, this antihyperglycemic action FA+Mg is likely to be associated with a marked enhancement of the GCK mRNA expression in the liver. Current result is consistent with previous studies that showed GCK mRNA expression increase in Persian Shallot (*Allium hirtifolium* Boiss) Extract (Mehdi *et al*., 2013) and 1-Deoxynojirimycin (Li, 2011) treated rats.

Conclusion

In conclusion, the data obtained in this study suggest

that FA+Mg combination may be an effective hypoglycemic agent via its ability to increased level of GCK activity in FA+Mg treated diabetic rats. So it may be useful for preventing or delaying the development of diabetes and its complications.

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