

Nicotinamide Ameliorates Serum C-Peptide and Brain Tryptophan Levels in STZ-Induced Diabetic Sprague- Dawley Rats

O.A.T. Ebuehi¹, C. Fregene², G.O. Ihegboro³

Department of Biochemistry, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria

Abstract: Metabolic complications of Type-1 diabetes mellitus are a significant source of morbidity and mortality. Nicotinamide treatment has been reported to reverse pancreatic beta cell damage and used to prevent Type-1 diabetes mellitus. The effects of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan have not been fully investigated. The objective of the study is to evaluate the effect of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan levels in Streptozotocin (STZ)-induced diabetic rats. Type-1 diabetes was induced by intra-peritoneal injection of streptozotocin (55mg/kg) using Sprague-Dawley rats. After 2 days, the rats were divided into diabetic control and nicotinamide-treatment group. Nicotinamide was orally administered at daily doses of 375mg/kg and 500mg/kg for a period of 4 weeks, while another group of rats without this treatment served as control. The diabetic control group showed significant ($p < 0.05$) increase in fasting blood glucose, but a decrease in serum C-Peptide and brain tryptophan levels compared with the control. Treatments with 375mg/kg and 500mg/kg nicotinamide showed a significant decrease in fasting blood glucose, but an increase in serum C-peptide and brain tryptophan levels compared with diabetic control. Data of the study indicate that nicotinamide may prevent diabetic complications by alleviating its metabolic symptoms of hyperglycemia and polyphagia, which may ameliorates pancreatic islet cell damage in diabetic rats.

Keywords: Nicotinamide, serum C-Peptide, tryptophan, glucose, diabetes, rat.

1. Introduction

Type 1 diabetes mellitus is an auto-immune disease characterized by destruction of the pancreatic islet cells, leading to a decrease in and eventually cessation of insulin secretion (Diabetes, 2014). Clinical overt Type 1 diabetes mellitus is thought to be a late stage of the process of gradual destruction of islet cells. It is possible to modify this process in order to slow down or prevent the development of clinical diabetes. Imperfection in insulin secretion results in increase production of glucose by the liver but decrease removal from the blood, leading to hyperglycemia, a clinical hallmark of diabetes mellitus (Banerjee *et al.*, 2005, Kentaro *et al.*, 1982). Hyperglycemia results in classic symptoms of polyuria, polydipsia and polyphagia. Hyperglycemia seems to initial a hunger signal that triggers excess release of neuropeptide Y, resulting in polyphagia. Hyperglycemia exaggerates polyphagia (Diabetes, 2014). It is estimated that more than 300million people suffer from diabetes mellitus which has huge economic impact. A person with diabetes mellitus can face implication cost ranging from 1,000-15,000 dollars a year, such a serious drain on health resources in developing countries (Diabetes, 2014, Wahieb and Godin, 1987).. Hyperglycemia leads to acute and long-term metabolic complication of retinopathy, nephropathy, neuropathy and atherosclerosis which are significant source of morbidity and mortality. Insulin administration hardly prevent long-term complications of diabetes mellitus because optimal insulin dosage, which reduces complications, increases in risk of episodes of hypoglycemia and is difficult to adjust. In addition, insulin is also lipogenic (Steiner *et al.*, 1967). A clinical call to design a cost-effective, natural agent with hypoglycemia and ameliorating effects on diabetic complications has become medical priority (Welborn *et al.*, 1981). Nicotinamide has been shown to prevent pancreatic islet from inflammation

and to be a component of glucose tolerance factor which enhances insulin sensitivity (Shibata, 1978, Ziegler, 2000). Large-doses of nicotinamide treatment reverse beta cell damage at the "honeymoon period" in diabetes (Trapp and Jung, 2006). The effect of nicotinamide on brain tryptophan in animal models has not been fully investigated (Freeman *et al.*, 2006). The objective of this study was to investigate the effects of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan levels in STZ-induced diabetic rats and to suggest possible mechanism of action.

2. Materials and Methods

Animals

Twenty male Sprague- Dawley rats (116.83 $\pm$ 4.25g) were used for the study. The rats were acclimatized for a period of two weeks to the laboratory conditions in conformity with the international guidelines on ethics of animal experimentation. Rats were housed in well ventilated cages with 5 per cage at room temperature with 12h of light and dark cycle and access to drinking water and rat chow. The rats were fed with commercial rat chow and water *ad libitum*.

Induction of Diabetes

The rats were fasted for 24h before injection of freshly prepared solution of STZ was administered intra-peritoneally at a dosage of 55mg/kg body weight. STZ was freshly prepared in 0.1M sodium citrate buffer, pH 4.5. This dose produced type 1 diabetes having average fasting blood sugar level of 389.20 $\pm$ 2.02mg/dl after 2days of injection.

Experimental Design

The rats were randomly divided into groups 1 and 2. Group 2 was later re-distributed into three groups with 5 animals per group in the whole.

Group 1 : Normal control rats were rat chow and water for 4 weeks

Group 2 : Diabetic rats were fed with rat chow for 4 weeks.

Group 3 : Diabetic rats received oral administration of 375mg/kg nicotinamide for 4 weeks.

Group 4 : Diabetic rats received oral administration of 500mg/kg nicotinamide for 4 weeks.

Four weeks after diabetic injection, the rats from each group were sacrificed after an overnight fast and blood samples collected from the eyes using capillary tubes. The blood samples were centrifuged at 2000rpm for 10min and the sera obtained were used for biochemical assay.

Biochemical Assays

Determination of serum C-peptide

The method is an immuno-enzymometric assay. The principle is based on antibody-antigen reaction. The test involves the linking of a labeled enzyme called conjugate (HRP-conjugate) to the test antigen (C-peptide) which is bound to a monoclonal biotylated antibody immobilized in a streptavidin coated microtiter well. The enzyme develops a yellow colored reaction which is measured spectrophotometrically at 450nm using ELISA reader (Irving *et al.*, 2005).

Determination of brain tryptophan

The method of Nkonge and Balance (1982), modified by Ebuehi and Akinwande (1992) was used to determine brain tryptophan concentration. This method is based on reaction between free tryptophan and ferric chloride solution under acidic conditions to produce a reddish yellow solution that is measured spectrophotometrically at 545nm.

3. Results

The nicotinamide orally administered at 375mg/kg and 500mg/kg respectively decreased glucose levels in STZ-induced diabetic rats (Fig. 1). The free tryptophan concentration in the brain decreased in diabetic control compared with the normal control, but increased at 375mg/kg and 500mg/kg dosage respectively compared with the diabetic control (Fig. 2).

The bound tryptophan level in the brain decreased in the diabetic control but increased at doses of 375mg/kg and 500mg/kg compared with the diabetic control (Fig. 3). The effect of 55mg/kg body weight streptozotocin and treatments with 375mg/kg and 500mg/kg body weight on total tryptophan is presented in Fig. 4. The results show that 55mg/kg body weight significantly ($p < 0.05$) decreased brain tryptophan compared with normal control but treatments with 375mg/kg and 500mg/kg body weight significantly increased brain tryptophan compared with diabetic control.

The effect of 55mg/kg body weight streptozotocin and treatments with 375mg/kg and 500mg/kg body weight on fasting serum C-peptide is reported in Figure 5. The results show that 55mg/kg body weight significantly ($p < 0.05$) decreased fasting serum C-peptide compared with normal control, but treatments with 375mg/kg and 500mg/kg body weight nicotinamide significantly increased fasting serum C-peptide compared with diabetic control (Fig. 5).



Figure 1: Effect of nicotinamide on fasting blood glucose in rat.

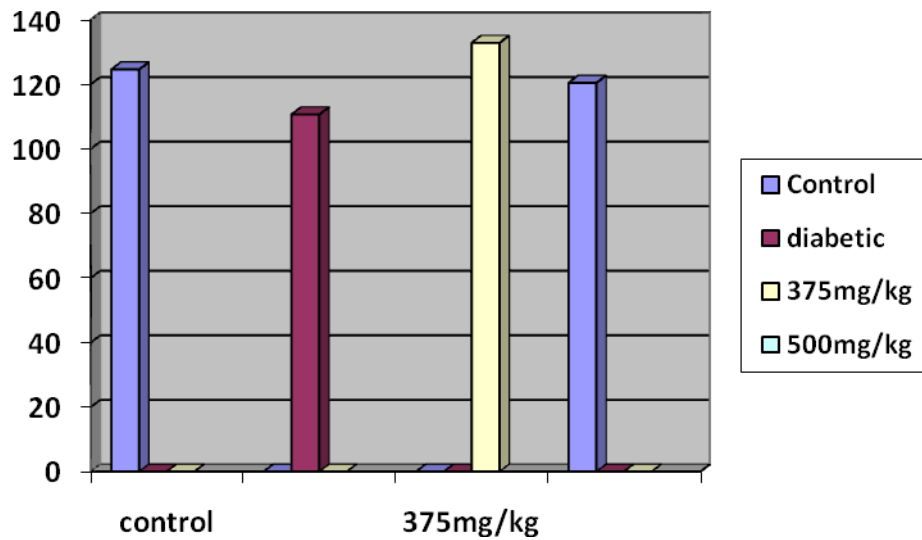


Figure 2: Effect of nicotinamide on free tryptophan in the brain.

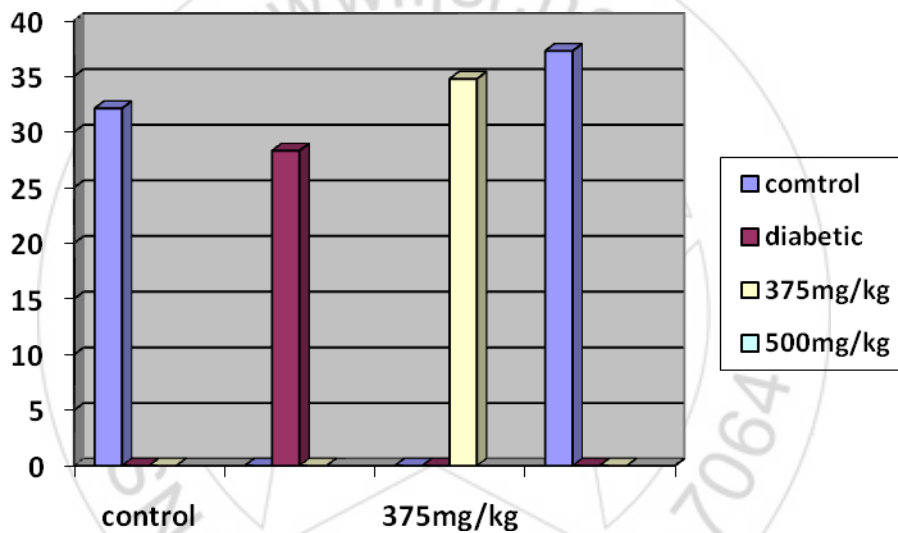


Figure 3: Effect of nicotinamide on bound tryptophan in the brain.

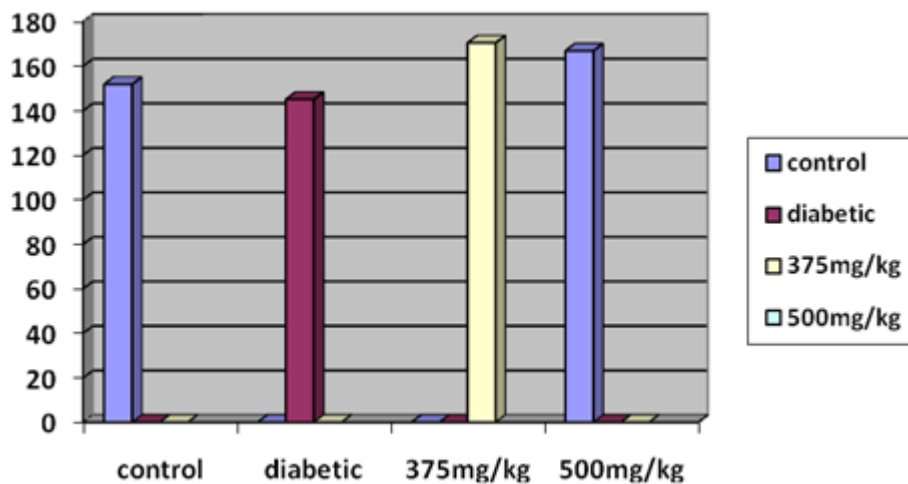


Figure 4: Effect of nicotinamide on total tryptophan in rat brain

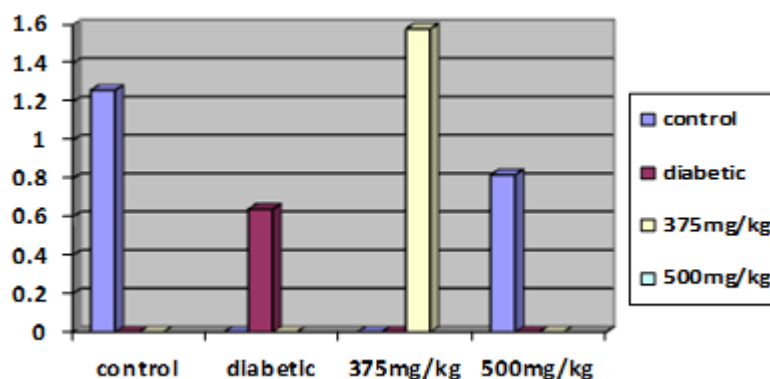


Figure 5: Effect of nicotinamide on serum C-peptide in rat

4. Discussion

The present study shows that nicotinamide possesses diabetic ameliorating potentials as evidenced by increased serum C-peptide and brain tryptophan with decreased fasting blood glucose levels in the treated diabetic rats compared with diabetic control. These parameters are indicative of increased insulin secretion and improved pancreatic islet function. Serum C-peptide has been shown to be bio-maker of insulin secretion. Dosing with C-peptide has been shown to improve diabetic complications. In this study, nicotinamide treatments resulted in increased serum C-peptide which may account for decreased fasting blood glucose and brain tryptophan levels in treated diabetic rats. The mechanism of action may be to repair the damaged pancreatic islet by enhanced poly-ADP- ribose polymerase activity. It has been shown that repair of beta cells by nicotinamide at the "honeymoon stage" restores pancreatic islet function. Studies reveal that nicotinamide increases the activity of poly-ADP- ribose polymerase activity. It is also suggested that nicotinamide upregulates poly-ADP- ribose polymerase level via chromatin remodeling. Studies have shown that nicotinamide activates NAD-dependent histone deacetylase which effect chromatin remodelin. Further studies to elucidate mechanism are required.

The significant increase in brain tryptophan implies "tryptophan sparing" in diabetic rats treated with nicotinamide. It has been shown that improved insulin secretion indication by increased C-peptide level facilitates brain tryptophan uptake by increasing skeletal muscle tissue uptake of competing aromatic amino acids. It also reduces brain tryptophan catabolism for energy production via increased glucose utilization. This "spares" tryptophan and increases its bioavailability for serotonin biosynthesis (Fernstrom and Wurtman, 1971, Ebuehi *et al.*, 2009). Studies have shown that serotonin is satiety-inducing neurotransmitter. In this study, nicotinamide treatment resulted in increase brain tryptophan level which may imply reduced polyphagia. The mechanism for increased brain tryptophan is via increased C-peptide secretion and improved pancreatic islet function (Welborn *et al.*, 1981). Further studies to elucidate mechanism are required. It is suggested that polyphagia exacerbates hyperglycemia which is the harbinger for diabetic complications such as retinopathy, nephropathy, neuropathy and atherosclerosis which are significant sources of morbidity and mortality. Nicotinamide treatment may prevent or delay the

complications of diabetes by improving pancreatic islet function and increasing C-peptide secretion.

5. Conclusion

Data of the study indicate that nicotinamide treatment improved insulin secretion and pancreatic islet function in male rats after 28days. Furthermore, nicotinamide treatment improved brain tryptophan uptake and enhanced tryptophan sparing effect.

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