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High dietary salt intake alleviates fasting blood glucose in streptozotocin-induced diabetic male Wistar rats

Jerome Ndudi Asiwe[1], Kenneth Kelechi Anachuna[2], Emuesiri Goodies Moke[3], Kamaldeen Olalekan Sanusi[1], David Ehikhuemen Okonofua[1], Oghenerukevwe Omeru[2], Adesoji Adedipe Fasanmade[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria, [2]Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria, [3]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria

Corresponding Author:
Jerome Ndudi Asiwe, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Oyo, Nigeria.
Tel: +234 8163727468.
E-mail: asiwejerome@yahoo.com

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ABSTRACT

Objectives: Hypertension and type 2 diabetes are common comorbidities. Studies have shown that hypertension is twofold as common in diabetic patients compared with non-diabetics. Excessive intake of dietary salt has reportedly caused increase in blood pressure. This study investigated the effects of high dietary salt intake on diabetic rats. **Materials and Methods:** Twenty-eight animals were randomly selected into four groups ($n = 7$). Group 1 (normal control), fed with standard rats' feed; Group 2 (diabetic control), streptozotocin-induced diabetic rats fed with normal diet; Group 3 (salt control), fed with 8% salt (high salt) diet; Group 4 (test group), streptozotocin-induced diabetic rats fed with high salt diet. Fasting blood glucose was monitored every 7 days. After 28 days of the study, blood pressure measurement was taken using tail cuff non-invasive method (CODA). Histology of the pancreas and kidney was done using hematoxylin and eosin staining. $P < 0.05$ was considered statistically significant. **Results and Conclusion:** Glucose levels of the test group reduced significantly when compared with the diabetic control group. This study suggests that high dietary salt intake plays a role, not only in reducing fasting blood glucose in a manner that is not yet understood but also causes distortion in the histomorphology of the pancreas and renal tissues.

Keywords: Diabetes mellitus, fasting glucose, high salt intake, hypertension, pancreas

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and/or glucose intolerance due to insufficient insulin secretion or insensitivity of tissues to insulin.[1] Occurrence of diabetes mellitus is on the increase and it is now found almost in every population in the world.[1] Epidemiological evidence suggests that without proper and effective prevention and control programs, the world diabetes mellitus incidence will continue to increase.[2] Its global prevalence was about 8% in 2011 and is predicted to rise to 10% by 2030.[3] Nearly 80% of people with diabetes live in low and middle-income countries.[1,3-5]

Diabetes mellitus has several implications in terms of long-term vascular effects and their associated cost. This effect reduces both expectancy and quality of life.[6-8] It poses a big threat and economic burden in respect to health system costs, and indirect costs arising from losses due to patient's disability and premature mortality. In the African Region, the World Health Organization estimated the prevalence of diabetes mellitus in 2000 to be about 7.02 million people, about 0.702% of this population had type 1 diabetes mellitus and 6.32 million had type 2 diabetes mellitus. Furthermore, about 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year.[9,10]

Hypertension and type 2 diabetes are common comorbidities. Essential hypertension accounts for the majority of hypertension in type 2 diabetic patients.[11,12] Studies have shown that hypertension is twofold as common in diabetic patients compared with non-diabetics.[13,14] The occurrence

of diabetes is very much and independently associated with hypertension.[15] The complications with diabetic patients such as cardiovascular diseases, stroke, kidney disease, and retinopathy are exacerbated by elevated blood pressure.[16] Elevation of salt sensitivity of blood pressure (the response of blood pressure to a changing salt intake) has been reported in diabetic patients and can be affected by race, age, sex, and renal disease.[17,18]

Sodium chloride (NaCl) is important in human foods as it adds flavor and also acts as an ingredient for preservation. In spite of the health benefits of salt, such as stimulation of digestion and other metabolic processes,[19] excessive intake reportedly causes increase in blood pressure.[20] Reduction in dietary salt intake is encouraged throughout the world for reducing blood pressure which is a major factor in cardiovascular diseases. However, there have been conflicting reports on dietary salt intake and diabetes and this has initiated several studies on type 1 and type 2 diabetes mellitus. Ekinci *et al.*[21] and Thomas *et al.*[22] reported that low dietary salt intake is associated with higher morbidity and mortality in patients with diabetes mellitus.[21,22] Reports from studies on salt reduction in diabetes mellitus have been limited and hence this study investigates the effect of high dietary salt intake on fasting glucose level and histomorphological changes in streptozotocin-induced diabetic male Wistar rats.

MATERIALS AND METHODS

Formulation of High Salt Diet

Eight percent (8%) of table salt (Mr Chef®) was mixed properly with normal granulated rat chow (Top Feed®) according to the methods of Obiefuna and others.[23-25]

Induction of Diabetes Mellitus

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) in sterile citrate buffer (0.1 M, pH 4.5) to fasted male Wistar rats.[26] Diabetic state of the rats was checked after 72 h by means of a glucometer (ACCU-CHEK® Active) and compatible blood glucose test strips, and animals with fasting blood glucose level of ≥200 mg/dl were selected for the study.[26]

Experimental Design

Twenty-eight male Wistar rats were used for this study, with weights ranging between 160g and 210 g. The animals were procured from Central Animal House, College of Medicine, University of Ibadan, Nigeria and were kept under standard laboratory conditions (12 h light-dark cycle at 18–26°C and relative humidity of 30–70%). They were fed standard rat pellets and water *ad libitum*, while allowing them to acclimatize for 2 weeks before commencement of the experiment at the Central Animal House of the College of Medicine, University of Ibadan, Nigeria. Ethical approval was given by the College of Medicine Ethics Committee (Ethic No. UI-ACUREC/18/0114). Animal handling was done in accordance to established guidelines by the National Institute of Health for care and use of laboratory animals.[27]

The animals were grouped into four groups of seven animals each as follows:

- Group 1 (Normal control – CTRL): Fed with normal feed and water *ad libitum*
- Group 2 (Diabetic control – DM): Fed with normal diet and water *ad libitum* after the induction of diabetes
- Group 3 (Salt control – HS): Fed with 8% salt in their feed with water *ad libitum*
- Group 4 (Test group – HS/DM): Fed with 8% salt in their feed with water *ad libitum* after induction of diabetes.

Measurement of Blood Pressure

Blood pressure was recorded in conscious animals at week 4 (the end of the experiment) before the animals were sacrificed. The values of systolic and diastolic blood pressures were measured by the tail-cuff method using the CODA non-invasive method (Kent Scientific Co., USA). The CODA tail-cuff system uses volume-pressure recording (VPR) to measure the blood pressure by determining the tail blood volume. Room temperature was maintained at or above 20^{0}C for accurate blood pressure measurements. VPR uses a specially designed differential pressure transducer to measure the blood volume in the tail non-invasively.

Sample Collection

Before the start of the study, the animals' baseline blood glucose level was determined and during the course of the study, blood glucose level was measured weekly. After 4 weeks, the animals were sacrificed, the pancreas and kidneys were harvested, weighed, and preserved in 10% formalin for histological processing. Serum and urine samples were used to measure the concentration of creatinine and urea using their respective kits. They were measured using the microplate reader SpectraMAX PLUS (a molecular Device product). The right kidneys were homogenized with phosphate buffered saline and the supernatant was separated. Catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were determined using various kits according to the methods of Beer and Sizer,[28] McCord and Fridovich,[29] and Ohkawa *et al.*,[30] respectively.

Histology

The organs (pancreas and kidney) was fixed in 10% neutral-buffered formalin, dehydrated in increasing concentration of ethanol, cleared with xylene, and then embedded in paraffin. Two micrometer sections were prepared from pancreas and kidney paraffin blocks, respectively, and stained with hematoxylin and eosin.

Statistical Analysis

Data are expressed as mean ± standard error of mean. All data were analyzed using one-way analysis of variance and comparison of the groups were performed using post-hoc Newman–Keuls test using GraphPad prism 7.0 (GraphPad software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of High Salt Intake on Mean Arterial Blood Pressure (mmHg) and Weight (g) of Pancreas

There was significant ($P < 0.05$) increase in mean arterial pressure in the diabetic control DM (130 ± 5.19), salt control HS (134 ± 6.92) and test group HS/DM (134 ± 3.95) when compared with the control group (109 ± 3.50) [Figure 1]. The weight of the pancreas significantly reduced in DM (0.273 ± 0.04), HS (0.294 ± 0.02) and HS/DM (0.312 ± 0.04) groups when compared with the CTRL (0.430 ± 0.04) [Figure 2].

Effect of High Salt Diet on fasting Blood Glucose

Table 1 presents the effect of high salt intake on fasting blood glucose. There was a significant increase in fasting blood glucose level in the DM group throughout the study period. However, HS/DM group had increased fasting blood glucose level only in the 1st week and decreased significantly in subsequent weeks when compared with the DM group.

Effect of High Salt Diet on Renal Markers and Antioxidants

There were significant increase in serum creatinine, serum urea, and urine urea when compared with CTRL in all groups as shown in Table 2, although the serum creatinine in the test group (HS/DM) decreased when compared with DM. The urine creatinine level decreased across all groups when compared with CTRL. The urine creatinine decreased in HS/DM group, while HS group increased when compared with DM group. Table 3 shows that MDA, CAT, and SOD levels in DM group decreased significantly but GPx level increased when compared with the CTRL group. In HS group, there were increases in MDA, SOD, and GPx when compared with the CTRL group, though there was also an increase when compared with DM group. However, the CAT level decreased in HS group when compared with the CTRL group. In HS/DM group, MDA and SOD decreased significantly, while there was an increased in CAT and GPx levels.

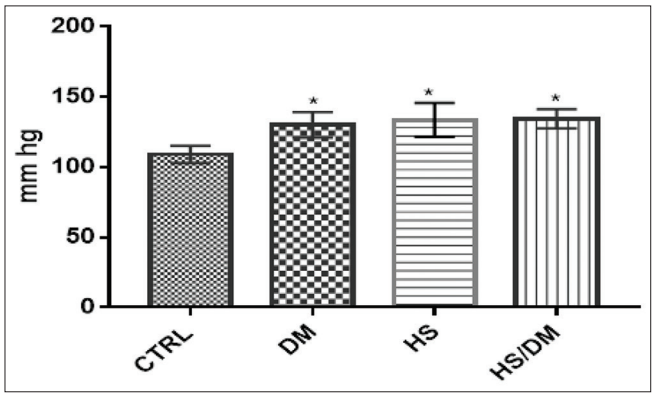

Figure 1: Effect of high salt diet on mean arterial blood pressure (mm Hg) at 4 weeks of the study

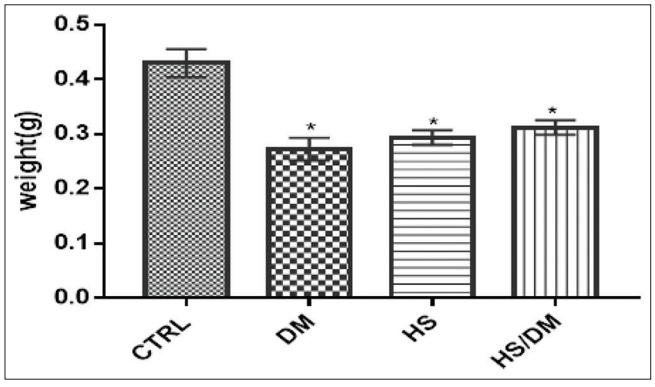

Figure 2: Effect of high salt diet on the weight (g) of pancreas

Effect of High Dietary Salt Intake on the Histology of Pancreas and Kidney

Figure 3 presents that the photomicrograph of the normal control group (plate 1) shows no significant lesion, the diabetic group (plate 2) shows mild vascular congestion, focal area of hyperplasia, and marked absence of Islets of Langerhans. The high salt group (plate 3) shows mild vascular congestion, focal areas of necrosis, and replacement with inflammatory cells. The test group (plate 4) shows moderate vascular congestion and an increase in the connective tissue. The photomicrograph of the renal tissue showed normal glomeruli, bowman capsule, and tubules with normal control group. Mild peritubular and periglomerular inflammation was observed in the diabetic group, mild hemorrhagic lesions in the high salt group, while the test group showed mild hemorrhagic lesions, mild peritubular, and periglomerular inflammation [Figure 4].

DISCUSSION

Dietary and lifestyle changes are positioned as important constituent therapies in managing diabetes mellitus and its many complications. High dietary salt intake is an important risk factor in the etiology of hypertension but the role it plays in glucose metabolism remains controversial. In this study, we report a decrease in the weight of the pancreas. This can be explained by reports that the loss of the trophic effects of locally-produced insulin on the surrounding acinar tissues would result in decrease in pancreatic weight.[31] This study also reports that majority of insulin-staining beta cells disappeared and the resulting pseudo-atrophic islets were characterized by abundant glucagon-secreting alpha cells and minor fibrosis of the islets interstitium. Duct centric lobular atrophy related obstructive pancreatitis is commonly encountered in slowly-progressive insulin dependent diabetes with markedly reduced pancreatic weight[32] though this implicating factor has no significant correlation with the duration of diabetes.[33]

It was reported that the combined effects of diabetes mellitus and high salt intake have stronger effects on blood pressure and cardiovascular risks.[20] These effects may be responsible for the significant increase in blood pressure observed in this study. The National High Blood Pressure Education Programme Working Group in 1994,[13] also reported that hypertension is twice as common in diabetes as in those who are not diabetic because both disease have common underlying causes and co-existing risk factors. Diabetes

Table 1: The effect of high salt diet on fasting blood glucose level (mg/dl)

Groups	CTRL	DM	HS	HS/DM
Baseline (post-induction)	61.0±5.76	327.0±48.2*	73.5±3.4	206.0±4.4*
Week 1	60.0±5.8	327.0±48.2*	73.5±3.4	206.0±4.4*
Week 2	68.7±7.4	197.0±37.2*	57.6±9.4	51.8±19.3#
Week 3	73.4±6.2	237.0±45.6*	61.6±6.0	48.6±10.2#
Week 4	79.2±6.5	263.0±50.8*	66.5±2.3	68.4±10.6#

Values are expressed as mean±SEM. *P<0.05 was significant when compared with CTRL and #P<0.05 was significant when compared with the DM. CTRL=Normal control, DM=Diabetic control, HS=High salt control, and HS/DM=Test group

Table 2: Effect of high dietary salt intake on serum and urine concentrations of urea and creatinine

Groups	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Urine urea (mg/dl)	Urine creatinine (mg/dl)
CTRL	9.8±0.69	0.05±0.00	20.2±0.74	1.71±0.01
DM	14.8±0.68*	0.09±0.00*	28.6±0.91*	0.51±0.02*
HS	19.1±0.45*	0.07±0.00*	30.5±0.94*	0.65±0.02*
HS/DM	16.3±0.38*	0.08±0.01*	31.5±0.53*	0.32±0.04*

Values are expressed as mean±SEM. *P<0.05 was significant when compared with CTRL

Table 3: Effect of high dietary salt intake on antioxidant enzymes

Groups	MDA (μM)	SOD (μ/ml)	CAT (μmol/min/ml)	GPx (μ/l)
CTRL	41.0±0.59	1.19±0.04	2.40±0.09	102±4.26
DM	20.9±1.04*	0.426±0.03*	1.92±0.09*	178±5.07*
HS	20.9±1.04*	1.05±0.07#	1.97±0.04*	198±4.95*
HS/DM	23.7±1.08*	0.61±0.04*#	2.25±0.07#	285±4.90*

Values are expressed as mean±SEM. *P<0.05 was significant when compared with CTRL and #P<0.05 was significant when compared with the DM. CTRL=Normal control, DM=Diabetic control, HS=High salt control, and HS/DM=Test group

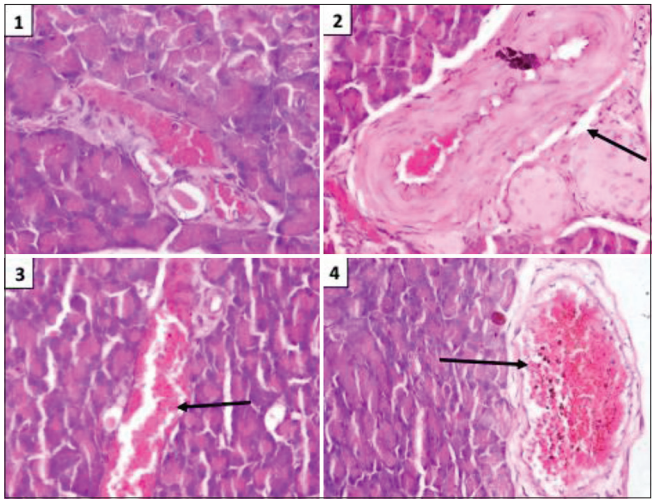

Figure 3: Effect of high dietary salt intake on histology of pancreas. The photomicrograph of the control group (plate 1) shows no significant lesion, the diabetic group (plate 2) shows mild vascular congestion, focal area of hyperplasia, and marked absence of Islets of Langerhans. The high salt group (plate 3) shows mild vascular congestion, focal areas of necrosis, and replacement with inflammatory cells. The test group (plate 4) shows moderate vascular congestion and an increase in the connective tissue (hematoxylin and eosin × 400)

damages arteries and makes them target for atherosclerosis which eventually lead to high blood pressure.

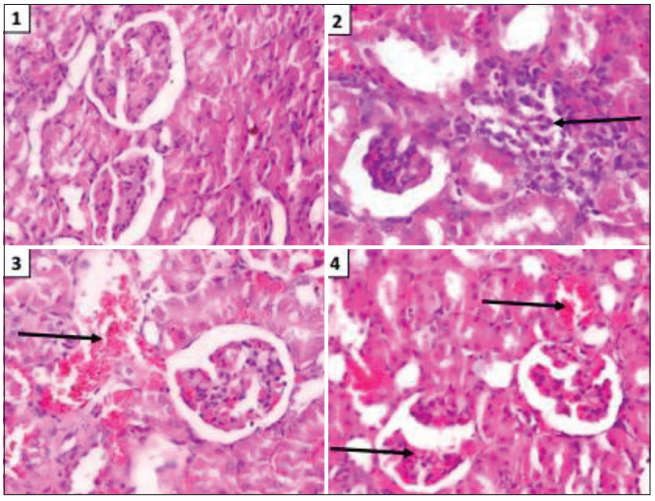

Figure 4: Effect of high dietary salt intake on histology of kidney. The photomicrographs showed normal glomeruli, bowman capsule, and tubules in plate 1 (normal control group), mild peritubular and periglomerular inflammation in plate 2 (diabetic group), mild hemorrhagic lesion in plate 3 (high salt group), and mild hemorrhagic lesion, mild peritubular, and periglomerular inflammation in plate 4 (test group) (hematoxylin and eosin × 400)

The observed data in diabetic group were a clear indication that 60 mg/kg dose of STZ was able to induce diabetic condition in rats.[26] However, we observe a significant decrease in glucose

level of test group. This observation was due to mechanism not yet understood but believed to result from the hyperactivity of the remaining beta cells. Takagi *et al.*[34] demonstrated in a study with Lepr$^{fa/fa}$ rats, a model of type 2 diabetes mellitus and concluded that the high salt intake reduction effects could be as a result of plasma adiponectin. In addition, Ekinci *et al*.[21] and Thomas *et al*.[22] had earlier reported that low dietary salt intake was associated with higher morbidity and mortality in type1 and 2 diabetes mellitus. On the contrary, CDA[35] and Evert *et al*.[36] suggested in their study that dietary salt restriction should be the recommended guideline to prevent or at least slow the development of diabetes mellitus.

The kidneys play an important role in the excretion of waste products and toxins such as urea and creatinine. The significant increase in serum creatinine and urea in this study suggests the inability of the glomerulus to filter creatinine and urea making the serum concentration of these waste products high. This is consistent with reports by Mitchell and Kline[37] and Banfi and Del.[38] The HS group had higher serum urea when compared with the DM group. This observation could suggest adverse effect of high salt intake on the kidney compared to the independent effect of diabetes mellitus. The role of urea in maximal conservation of water by the kidney involves increased urea reabsorption and consequent tendency to increase serum urea.[39] The decrease in urine creatinine may suggest bad muscle health or inflammation and is also believed to result from high salt intake-induced kidney disease as reported by Edmund and David.[40] Furthermore, insulin resistant or protein caloric malnutrition according to Sinkeler *et al.*[41] could suggest the decreased level of urine creatinine in HS/DM group when compared with the CTRL group.

MDA level reduced in all the groups when compared with the control group. However, the DM group had a significant decrease in SOD, CAT, and GPx when compared with the normal control group. This reflects the depletion of endogenous antioxidant mechanism[42] which indicated that kidney oxidative stress was activated. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses such as glutathione.[43,44] There was significant increase in SOD and CAT levels in the test group (HS/DM) when compared to the DM group, as well as a non-significantly increase in GPx. It suggests that the high salt diet possibly restored the antioxidant defense mechanism toward normal.

The observed mild vascular congestion, focal area of hyperplasia, thickened artery, and marked absence of islet cells in diabetic group is as a result of the effects of STZ on the pancreas as reported in a study demonstrating that a single dose of 60 mg/kg of STZ is capable of inducing pancreatic β cell destruction in rats and subsequent reduction in insulin secretion.[26,45] Focal area of necrosis and replacement with inflammatory cells and mild vascular congestion was also observed in high salt group. This observation could be due to high dietary salt intake as supported by reports of Satoh *et al*.[46] that increase in the size of pancreatic islet, β cell hyperactivity, and blood vessel damage are hypertension-induced histomorphological changes in pancreas. However, we observed moderate vascular congestion and increase in the connective tissue in test group. This was believed to result from increased deposition and disorganization of proteins in extracellular matrix.[47]

The photomicrograph of renal tissue of the diabetic group was observed to have mild hemorrhagic lesion, mild peritubular, and periglomerular inflammations. This suggested the histoarchitectural phenotype of early glomerular and tubular hypertrophy as reported by An *et al.*[48] However, the mild hemorrhagic lesion observed in high salt only group could result from the increased glomerular enlargement and induced mild glomerular damage as postulated by Ruta *et al*.[49] The test groups showed hemorrhagic lesions as well as inflammations, indicating the joint adverse effect of salt intake and diabetic nephropathy.

From the results of this study, we conclude that high dietary salt intake though is one of the major risk factor of sustained high blood pressure, it plays a role not only in reducing fasting blood glucose in a manner that is not yet understood but also causes distortion in the histomorphology of the pancreas. High salt intake affects the tubular and glomerular functions by altering the histoarchitecture of the kidney as well as inducing the production of free radicals that damages cellular components.

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