The Effect of High Dietary Salt Consumption on Renal Function in Streptozotocin-Induced Diabetic Male Wistar Rats

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ABSTRACT

Background: Salt consumption has been linked to increased risk of development of type 2 diabetes mellitus and hypertension due to its glucose resistance and body weight promoting effects. This study investigated the effect of high dietary salt intake on renal function in diabetic male Wistar rats.

Methods: Animals were divided into 4 groups (n=7). Group 1 (control group) were fed with normal rat chow, group 2 (Diabetic) were received streptozotocin (STZ, 60 mg/kg), group 3 (high Salt) were given 8% salt diet, and group 4 had both STZ (60 mg/kg) and feeding with 8% salt diet. Fasting blood glucose was measured weekly and after 28 days prior to sacrifice, blood pressure measurements and 24 h urine samples were collected. After sacrifice, blood was collected and serum was separated for biochemical analysis. The kidneys were removed and preserved in 10% formalin for histological examination.

Results: Serum urea, creatinine and urine urea increased significantly (p<0.05) across group, when compared with control, while urine creatinine reduced (p<0.05) in all groups. There was a significant (p<0.05) increase in superoxide dismutase and catalase in high salt and salt/diabetes groups, when compared with diabetic group. Glutathione peroxidase significantly increased (p<0.05) across groups. Histologically, kidneys showed signs of inflammation in diabetic group, hemorrhagic lesions in high salt group and both hemorrhagic lesions and inflammation in salt/diabetes group.

Conclusion: High dietary salt consumption was shown to affect tubular and glomerular functions by altering kidney histoarchitecture and antioxidant defense system.

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countries in the world (2, 3), and this increase in salt consumption can be an implicating factor in elevated renal fluid retention resulting in elevated blood pressure and other complications. Salt consumption has been linked to increased risk of development of type 2 diabetes mellitus in adults, notably through a direct effect on insulin resistance and a tendency to promote weight gain. Lately, there has been considerable controversy concerning appropriate dietary intake of salt and this has triggered several studies in type 1 and type 2 diabetes, with reports of an association between low dietary salt intake and increased morbidity and mortality (4, 5).

Kidney, an important homeostatic organ has been implicated in both hypertension and diabetic conditions (5, 6). Excess salt loading does not only cause water retention, but also burdens the kidney on its removal, thereby increasing the risk of cardiovascular diseases (CVDs) such as stroke, heart attack, heart failure (6, 7), as well as renal diseases (8-11). The symptoms of failing kidney functions are not specific, and often, chronic kidney disease is diagnosed as a result of examining people believed to be at risk of kidney problems, such as those with high blood pressure or diabetes (12, 13). There are very few studies using dietary salt interventions that target sodium intake and sodium reduction as part of managing protocols in patients with diabetes, however, the studies investigating the relationship between salt intake and the development of diabetes have been particularly sparse. Some follow-up studies recently reported an association between dietary salt intake and diabetes complications in patients with type 1 and type 2 diabetes (4, 5, 14). Consistent reduction in dietary salt intake has been encouraged for patients at high risk of diabetes, dyslipidemia or renal diseases, because these patients are more susceptible to developing CVDs, and CVDs are more prevalent among those with concurrent hypertension (15, 16).

It was reported that 20-60% incidence cases of type 2 diabetes also have high blood pressure (17) and this has resulted in the development of guideline for treatment of diabetes throughout the world to prevent or at least reduce the development of diabetic complications (18-21). Increased consumption of highly salted foods and drinks has also been linked to obesity, which is an essential factor in the development of high blood pressure and type 2 diabetes mellitus. Hypertension and diabetes are both factors in metabolic syndrome, but their link with renal function has not been well-defined. Here, the effect of high salt consumption on renal functions in diabetic Wistar rats were investigated.

### Materials and Methods

Twenty-eight male Wistar rats weighing between 110 and 150 g were used in this study. The animals were procured from Central Animal House, College of Medicine, University of Ibadan, Nigeria, and were fed on standard grower’s mesh (Top Feed) during an initial 14 days acclimatization period, with access to drinking water ad libitum. The animals were kept in polyethylene cages throughout the duration of the experiment and housed at the Department of Physiology Animal House, College of Medicine, University of Ibadan under conventional laboratory conditions according to the University animal handling guidelines and ethics.

To formulate of high salt diet, for every 100 g of feed, 8 g of table salt (Mr Chef®) was mixed properly with 92 g granulated rat chow (Top Feed®) to reach an 8% salt diet according to the methods of Asiwe et al. (22). To induce diabetes mellitus, 0.5% Streptozotocin (STZ) solution in freshly prepared sodium citrate buffer (0.1 M, pH 4.5, 10mg/ml) was administered intraperitoneally to overnight-fasted male Wistar rats at a dose of 60 mg/kg (23). Animals with blood glucose levels above 200 mg/dL after 72 h post-induction were recognized as diabetic rats (23). Blood samples were obtained from the tail vein for blood glucose level determination and the blood glucose level was estimated using an Accu-Check Glucometer.

For experimental design, two weeks after acclimatization, the animals were randomly divided into 4 groups (n=7). Group 1 [Normal control group (CTRL)] were fed with standard rat chow and water ad libitum for the duration of the study. Group 2 [Diabetic group (DM)] were nourished with standard rat chow and water ad libitum after the induction of diabetes for the duration of the study. Group 3 [High salt group (HS)] received 8% salt in their food and water ad libitum for the duration of the study. Group 4 [Test group (HS/DM)] were given 8% salt in their food and water ad libitum after induction of diabetes for the duration of the study.

Initial body weight and blood glucose level were measured, followed by measurements at intervals of seven days. After 4 weeks, the animals were transferred to metabolic cages to collect 24 h urine samples, and then sacrificed. Blood was collected by cardiac puncture and centrifuged at 10,000 revolutions per minute at 4 °C for 15 minutes for serum separation. Kidneys were removed, weighed and half of them preserved in 10% formalin for histological evaluation. The remaining kidney samples were harvested and homogenized for oxidative enzyme activity measurement.

Blood pressure was recorded in conscious state at week 4th 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, while the room temperature was maintained at or above 20°C for accurate blood pressure measurements. VPR used a specially designed differential pressure transducer to non-invasively assess the blood volume in the tail.

Serum and urine samples were used to evaluate the concentrations of creatinine, urea and sodium using their respective kits. They were measured by the molecular device product of microplate reader (SpectraMAX PLUS). The right kidneys were homogenized with phosphate buffered saline and the supernatant was separated. The catalase (CAT), glutathione peroxidase (GPx), superoxide Dismutase (SOD) and malondialdehyde (MDA) were determined using various kits according to the methods of Beer and Sizer (24), McCord and Fridovich (25) and Ohkawa et al. (26), respectively. The absorbance was read with spectrophotometer according to their various protocols.

Kidneys were fixed in 10% neutral-buffered formalin, dehydrated in increasing concentration of ethanol, cleared with xylene and embedded in paraffin. Two micrometer (2 µm) sections were prepared from kidney paraffin blocks and stained with hematoxylin and eosin (H&E). Data were expressed as mean±standard error of mean. All data were analysed using one way analysis of variance (ANOVA) and Post hoc test to compare the groups together with Newman-Keuls test and GraphPad prism 7.0 (GraphPad software, San Diego, CA, USA). p<0.05 was considered statistically significant.

**Results**

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) as shown in Figure 1. The weight of the kidney significantly (p<0.05) 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) as shown in Figure 2. The fasting blood glucose level increased significantly (p<0.05) after induction with STZ in DM (327.0±48.2) and DM/HS (206.0±4.4) groups. However, there was a decrease in the glucose level of DM/HS [wk2 (51.8±19.3), wk3 (48.6±10.2) and wk4 (68.4±10.6)] group after week 1, while the DM group remained elevated above 200mg/dl throughout the study (Figure 3). There was a significant increase in serum creatinine, and urea and in urine urea, when compared with control in all groups (Table 1); but the serum and urine urea increased, when compared with DM group. The serum creatinine decreased in comparison to DM group, and the urine creatinine level decreased across the groups, when compared with CTRL group.

**Figure 1:** Effect of high dietary salt intake on mean arterial blood pressure (mmHg) at 4th week of the study. Values were expressed as mean±SEM. *p<0.05 is significant when compared with CTRL group. CTRL=Control group, DM=Diabetes mellitus group, HS=High salt diet group, HS/DM=Diabetes mellitus group fed with high salt diet.

**Figure 2:** Effect of high dietary salt intake on kidney weight. Values were expressed as mean±SEM. *p<0.05 is significant compared with CTRL group. CTRL=Control group, DM=Diabetes mellitus group, HS=High salt diet group, HS/DM=Diabetes mellitus group fed with high salt diet.

**Figure 3:** Effect of high dietary salt intake on fasting blood glucose. Values were expressed as mean±SEM. *p<0.05 is significant when compared with CTRL group and *p<0.05 is significant when compared with DM group. CTRL=Control group, DM=Diabetes mellitus group, HS=High salt diet group, HS/DM=Diabetes mellitus group fed with high salt diet.
The urine creatinine level decreased in DM/HS group, while in HS group increased in comparison to DM group. As shown in Table 2, the MDA, CAT and SOD in DM group decreased significantly; but GPx level increased when compared with the CTRL group. In HS group, there was an increase for MDA, SOD, 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 CNTRL group. In DM/HS group, MDA, SOD decreased significantly, while there was an increase in CAT and GPx levels.

The photomicrograph of renal tissue shows normal glomeruli, bowman capsule and tubules. No significant lesion was seen. The histology of diabetic group revealed mild peritubular and periglomerular inflammation. The pictures of renal tissue of high salt group illustrated mild hemorrhagic lesions. Histological evaluation of renal tissue in experimental group demonstrated mild hemorrhagic lesions, mild peritubular and periglomerular inflammation (Figure 4).

Table 1: Effect of high dietary salt intake on serum and urine concentrations of urea and creatinine.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Urea (mg/dL)</th>
<th>Serum creatinine (mg/dL)</th>
<th>Urine urea (mg/dL)</th>
<th>Urine creatinine (mg/dL)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>9.78±0.69</td>
<td>0.054±0.003</td>
<td>20.2±0.74</td>
<td>1.71±0.010</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>14.8±0.68*</td>
<td>0.09±0.0041*</td>
<td>28.6±0.91*</td>
<td>0.51±0.024*</td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>19.1±0.45*</td>
<td>0.073±0.0005*</td>
<td>30.5±0.94*</td>
<td>0.65±0.018*</td>
<td></td>
</tr>
<tr>
<td>HS/DM</td>
<td>16.3±0.38*</td>
<td>0.077±0.0098*</td>
<td>31.5±0.53*</td>
<td>0.32±0.037*</td>
<td></td>
</tr>
</tbody>
</table>

Values were expressed as mean±SEM. *p<0.05 is significant when compared with control CTRL: Control group, DM: Diabetes mellitus group, HS: High salt diet group, HS/DM: Diabetes mellitus group fed with high salt diet.

Table 2: Effect of high dietary salt intake on antioxidant enzymes.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (µM)</th>
<th>SOD (µ/mL)</th>
<th>CAT (µmol/min/mL)</th>
<th>GPx (µ/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>41.0±0.59</td>
<td>1.19±0.037</td>
<td>2.4±0.0879</td>
<td>102±4.26</td>
</tr>
<tr>
<td>DM</td>
<td>20.9±1.04*</td>
<td>0.426±0.0327*</td>
<td>1.92±0.0939*</td>
<td>178±5.07*</td>
</tr>
<tr>
<td>HS</td>
<td>20.9±1.04*</td>
<td>1.05±0.066*</td>
<td>1.97±0.0444*</td>
<td>198±4.95*</td>
</tr>
<tr>
<td>HS/DM</td>
<td>23.7±1.08*</td>
<td>0.61±0.0393*</td>
<td>2.25±0.0695*</td>
<td>285±4.9*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. *p<0.05 is significant when compared with the control group. and (p<0.05) is significant when compared with the diabetic group. CTRL: Control group, DM: Diabetes mellitus group, HS: High salt diet group, HS/DM: Diabetes mellitus group fed with high salt diet.

The symptoms of worsening kidney function are not specific, and might include feeling of a generally ill condition and experiencing a decrease in appetite. For decades, high salt intake has been known to have adverse effects on blood pressure, cardiovascular risk and survival. Often, chronic kidney disease is diagnosed by examining people known to be at risk of kidney problems, such as those with high blood pressure and diabetes mellitus (12, 13). This study was carried out to investigate the effect of high salt intake on tubular and glomerular functions in diabetic male Wistar rats. The significant weight loss was associated with the loss of kidney functions as shown both histologically and the urine and serum concentrations of urea and creatinine, respectively. This was consistent with the study of Eiichiro et al. (27) who reported that the weight loss is associated with kidney functions and also depend on the body mass index in male Wistar rats.

The significant decrease in glucose level of HS and HS/DM groups was due to mechanism not fully understood. However, the group with normal diet and

Discussion

The symptoms of worsening kidney function are not specific, and might include feeling of a generally ill condition and experiencing a decrease in appetite. For decades, high salt intake has been known to have adverse effects on blood pressure, cardiovascular risk and survival. Often, chronic kidney disease is diagnosed by examining people known to be at risk of kidney problems, such as those with high blood pressure and diabetes mellitus (12, 13). This study was carried out to investigate the effect of high salt intake on tubular and glomerular functions in diabetic male Wistar rats. The significant weight loss was associated with the loss of kidney functions as shown both histologically and the urine and serum concentrations of urea and creatinine, respectively. This was consistent with the study of Eiichiro et al. (27) who reported that the weight loss is associated with kidney functions and also depend on the body mass index in male Wistar rats.

The significant decrease in glucose level of HS and HS/DM groups was due to mechanism not fully understood. However, the group with normal diet and

![Figure 4: Effect of high dietary salt intake on histology of the kidney. The Photomicrographs showed mild peritubular and periglomerular inflammation in plate 2 (Diabetic group), mild hemorrhagic lesions in plate 3 (High salt group), mild hemorrhagic lesions, mild peritubular and periglomerular inflammation in plate 3 (Test group).](image-url)
STZ had glucose levels above 200 mg/dL throughout the study. This data contradicts the study of Evert et al. that suggested dietary salt restriction should be the recommended to prevent or at least to slow the development of diabetes mellitus (21). Yoshichici and colleagues reported similar data in genetically modified type 2 diabetic rats, though attributed the decrease to increase in plasma adiponectin level (28).

The mean arterial blood pressure increased in groups HS and HS/DM as a result of high dietary salt intake; which can be traced to renin-aldosterone mechanism. However, the increase was slightly higher in HS/DM group. This was consistent with the works of Adrogue and Medias who reported that the combined effect of diabetes mellitus and high salt intake may have a more potent effect on blood pressure and risk of CVDs (29). Also, the National High Blood Pressure Education Program Working Group in 1994 reported that hypertension is twice as common in diabetes as in those who are not; because both diseases have common underlying causes and common risk factors. This could suggest the increase of blood pressure in the diabetic group (29).

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 was supported by Mitchell and Kline (30) and Banfi and Del (31) who reported that creatinine and urea are the common markers of renal functions and significant increase in these markers indicated kidney dysfunction. The HS group showed higher serum urea level when compared with the DM group. This observation could suggest adverse effect of high salt intake on the kidney when 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 (32).

Urea, a major nitrogenous end product of protein and amino acid catabolism produced by liver and distributed throughout intracellular and extracellular fluids is filtered out of blood and partially reabsorbed with water (33). This suggests the increase in urine urea, when compared with the control group. 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 (34). Also, insulin resistance or protein caloric malnutrition according to Sinkeler et al. (35) could suggest the decreased level of urine creatinine in HS/DM group, when compared with the DM group.

In our study, SOD level reduced in all the groups when compared with the control group. This reflected the depletion of endogenous antioxidant enzyme activities in tissues which suggested that kidney oxidative stress was activated. In HS group, the MDA and SOD were observed to significantly increase, when compared with the DM group. The production of peroxides and free radicals that damage all components of the cell is believed to arise from toxic effects of the disturbances of normal redox state of cells. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defences such as glutathione (36). The glutathione peroxidase and catalase levels were increased significantly, when compared with the control group. The observed GPx increase was higher in HS/DM group. This increase suggests the joint effect of salt intake and diabetic nephropathy as contradicted by the works of Sedighi and colleagues who reported a lower glutathione peroxidase level in type 2 diabetes mellitus (37).

The photomicrograph of renal tissue of the diabetic group denoted to a mild hemorrhagic lesion, and mild peritubular and periglomerular inflammation. This suggested the histoarchitectural phenotype of early glomerular and tubular hypertrophy as reported by An et al. (38). However, the mild hemorrhagic lesions observed in high salt only group could result from the increased glomerular enlargement and induced mild glomerular damage that was postulated by Ruta and colleagues too (39), while the test group demonstrated hemorrhagic lesions, as well as inflammation. This suggests the joint adverse effect of salt intake and a diabetic nephropathy.

**Conclusion**

In conclusion, high salt consumption affects the tubular and glomerular functions by altering the histoarchitecture of the kidney, as well as inducing the production of free radicals that damage all components of the cell as a result of the disturbances of normal redox state of the cell. Salt restriction should be encouraged to reduce the prevalence of renal failure, as well as cardiovascular complications both in non-diabetic and most especially in diabetic patients.

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Conflict of Interest
None declared.

References


33 Corbett JV. Laboratory tests and diagnostic procedures with nursing diagnoses. 7th Ed. 2008;90-107.


