Effects of High Fat Ketogenic Diet On Renal Functions of Wistar Rats

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Abstract
Background/Objective: The ketogenic diets are special diets characterized by a reduction in carbohydrates and a relative increase in the proportion of fat and protein. They are popularly used in the treatment of epilepsy and weight control. The aim of this study was to investigate the effects of high fat ketogenic diet on the functions of the kidney. **Materials and Methods:** Twenty Wistar rats were randomly divided into two diet groups containing ten (10) rats per group and maintained under standard environmental conditions. Group A was the control group and were fed with a normal chow diet. Group B were fed with the modified high fat ketogenic diet containing 65% saturated fat. At the end of five weeks, the kidneys were harvested and processed histologically and blood samples were taken for biochemical assays. **Results:** The high fat ketogenic diet resulted in a significant increase in serum globulin and total protein of the animals fed with ketogenic diet, compared to control. There was no significant difference in the serum electrolyte levels when both groups were compared, although there was a slight increase in the electrolyte level of animals fed with ketogenic diet. Histological findings revealed marked alteration in the renal structure of animals fed with high fat ketogenic diet including active interstitial congestion, vascular ulceration, stenosis and interstitial infiltrates of inflammatory cells. The result of this study suggests that high fat ketogenic diet may have negative effects on the kidney functions.

Keywords: Ketogenic diet, kidney function, interstitial congestion, vascular ulceration, stenosis.

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Introduction

The ketogenic diet (KD) is a high-fat, adequate-protein, and very-low-carbohydrate diet regiment that mimics the metabolism of the fasting state (1), for the purpose of harnessing its high fat content in the treatment of various diseases (2) and weight reduction (3). The diet was named ketogenic diet because consumptions of this diet resulted in the production of ketone bodies through fatty acid oxidation, which serves as an alternative source of energy for the brain. The body is forced to switch to fatty acid oxidation when the intake of carbohydrates is reduced. Ketogenesis then occurs in the body and a sufficient number of calories are left in the body for normal daily activity (4). The ketogenic diet which was first introduced in the early 1900s has recently experienced a rise in popularity as an adjunctive treatment for patients who either do not respond to or cannot tolerate anticonvulsant medications (5). So far, long term consumption of Ketogenic diet has been successfully used in the treatment of refractory paediatric epilepsy (6), but the use of ketogenic diet for weight loss and the short and long term effects of this diet still remains controversial (**7**).

Studies conducted by Reid and Ugwu showed that electrolytes consumed by most people on a ketogenic diet is insufficient, this was demonstrated by the evidence of fatigue in individuals who were placed on ketogenic diets, which must have resulted from insufficient mineral intake in early ketogenic diet studies (8). A well-documented side effect of the ketogenic diet is a constellation of symptoms known as "keto flu," which includes lightheadedness, fatigue, headaches, nausea, and constipation. These symptoms maybe a result of the body's rapid excretion of sodium and fluids as carbohydrate intake is restricted and glycogen stores are depleted. Increasing sodium by 1-2 g per day may restore electrolyte balance (9). An evidence of electrolyte imbalance is a decrease in blood pressure, which mainly results from decreased body concentration of sodium, a very important electrolyte, which is necessary for a lot of physiological functions. The decrease in sodium concentration occurs mainly because of an increase in water loss and sodium excretion (10). The kidney is the major organ for the homeostasis of electrolytes including sodium. The effect of high fat ketogenic diet on the kidney appears to have conflicting reports. For example, stereotypical results showed that the numerical density of glomerulus and total number of glomeruli in rats fed the high fat ketogenic diet for a prolonged period of time was significantly decreased, indicating glomerular atrophy (11). In another study, the ketogenic diet was proven to reverse diabetic nephropathy (12) by producing prolonged exposure to the ketone 3-beta-hydroxybutyric acid (3-OHB), which blocks the inhibition of agouti-related peptide by glucose (13, 14). The diet reversed blood glucose to normal in akita mice (15) as indicated by urinary albumin/creatinine ratios, and in patients with type 2 diabetes mellitus it greatly improved fasting glucose levels (16) and increased insulin sensitivity (17), thereby alleviating glycosuria and reversing kidney damage due to excessive glucose excretion. The ketogenic diet halted the progression of renal insufficiency in patients with chronic renal failure, as accessed by serial determination of creatinine levels (2). A lot of studies on this topic laid more emphasis on the function of the kidney, without concurrently looking at the structural changes that may take place from consuming high fat ketogenic diet. Arising from the increasing demand for ketogenic diet in the treatment of various diseases such as epilepsy and in the reduction of body weight, it is therefore the aim of this study to examine the effects of high fat ketogenic diets on the functions and simultaneously, the structure of the kidneys of Wistar albino rats.

Materials and methods

Experimental design

Twenty (20) male albino Wistar rats were obtained from the Anatomy animal house, School of basic medical sciences, University of Benin. The rats were also housed in the Anatomy animal house for the duration of the study. The rats were weighed on arrival and then acclimatized for a period of two weeks. During this period they were fed on the normal rat chow and given free access to feeds and water. At the end of acclimatization the animals were grouped into two, control group (10) and experimental group (10). The experimental group was fed 65% high fat ketogenic diet, while the control group was fed the normal rat chow. Both groups were on the diet for 5 weeks and they were allowed free access to feeds and water during the duration of the experiment.

Collection of sample

Blood collection and analysis

At the end of five weeks, the animals were anesthetized with chloroform. Once under anesthesia, the animals were subjected to a median thoracolaparotomy. 5mL of blood were collected via puncture of the inferior vena cava into plain bottles, allowed to clot and spun on a centrifuge after dislodging the clot to get the serum which was used for the analysis. Serum samples were stored in glass vials with stoppers in a freezer for the biochemical determination of serum proteins, urea, creatinine, sodium, potassium, and chloride levels to evaluate glomerular and tubular kidney function. Serum proteins, urea and creatinine were determined by spectrophotometry method. Plasma sodium, potassium, and chloride levels were determined using the ion-selective electrode method.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM) in tables. Student t test was used for the comparison between experimental and control groups. $P<0.05$ was considered statistically significant. The Graph pad prism version 5.0 statistical package was used for the result analysis.

Histological Preparation

At the end of five weeks, the kidney was harvested from sacrificed rats, fixed in 10% formol saline for histological preparation and evaluation.

Table 1: Percentage composition of ketogenic diet.

Constituents	Percentage composition
Coconut Flour	15
Butter	65
Fish Meal	15
Bone Meal	2
Limestone	1
Salt	0.5
Vit-Min Premix	0.5
Lysine	0.3
Meth &Cys	0.3

(6)

Table 2: Percentage composition of standard chow diet

Constituents	Percentage composition
Water	12
Protein	15
Palm oil	7
Fiber	6
Calcium	7
Phosphorus	0.7
Enzyme	0.1
Corns	52.2

(18)

Results

Serum Electrolytes and Serum Proteins

Table 3: Showing the mean values of serum electrolytes and serum proteins in rats of control and test groups fed with normal diet and high fat ketogenic diet respectively

Parameters	Control (Normal diet) (n = 7)	High Fat Ketogenic diet (n = 7)
Sodium	135.2 ± 0.969	135.6 ± 1.122
Potassium	6.060 ± 0.312	6.740 ± 0.408
Bicarbonate	21.00 ± 0.894	21.60 ± 0.927
Chloride	101.4 ± 1.435	102.80 ± 1.319
Urea	34.80 ± 1.393	40.60 ± 1.930
Creatinine	0.800 ± 0.045	0.840 ± 0.040
Total protein	7.600 ± 0.130	10.380 ± 0.660*
Albumin	3.960 ± 0.040	3.820 ± 0.092
Globulin	3.640 ± 0.133	6.420 ± 0.609*

*Values: mean ± SEM (Standard Error of Mean); Number of replicates (n): 7; Statistical tool: Student t test.*P < 0.05 indicates significant difference, when treated groups are compared with control.*

Histological Result

The sections were stained with hemotoxylin and eosin (H&E), and interpreted with the electron microscope.

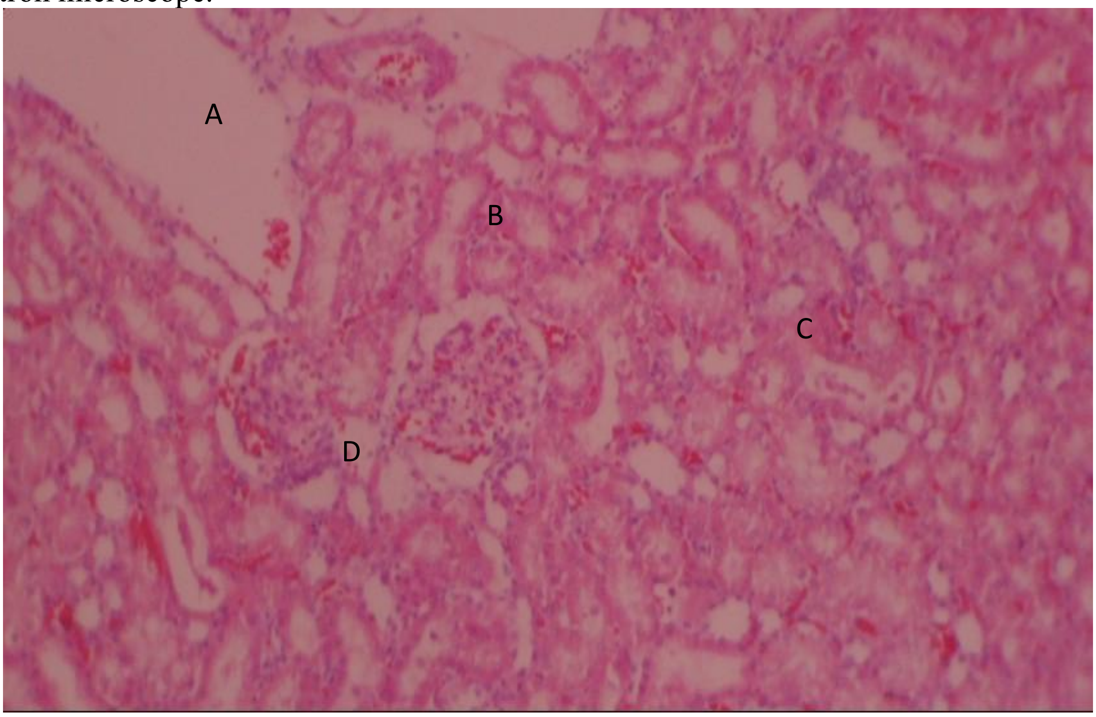

Figure. 10. Section of the kidney of rats fed with normal diet showing A, Arcuate artery, B, tubules, C, glomeruli and D, interstitial space (H&E x 100).

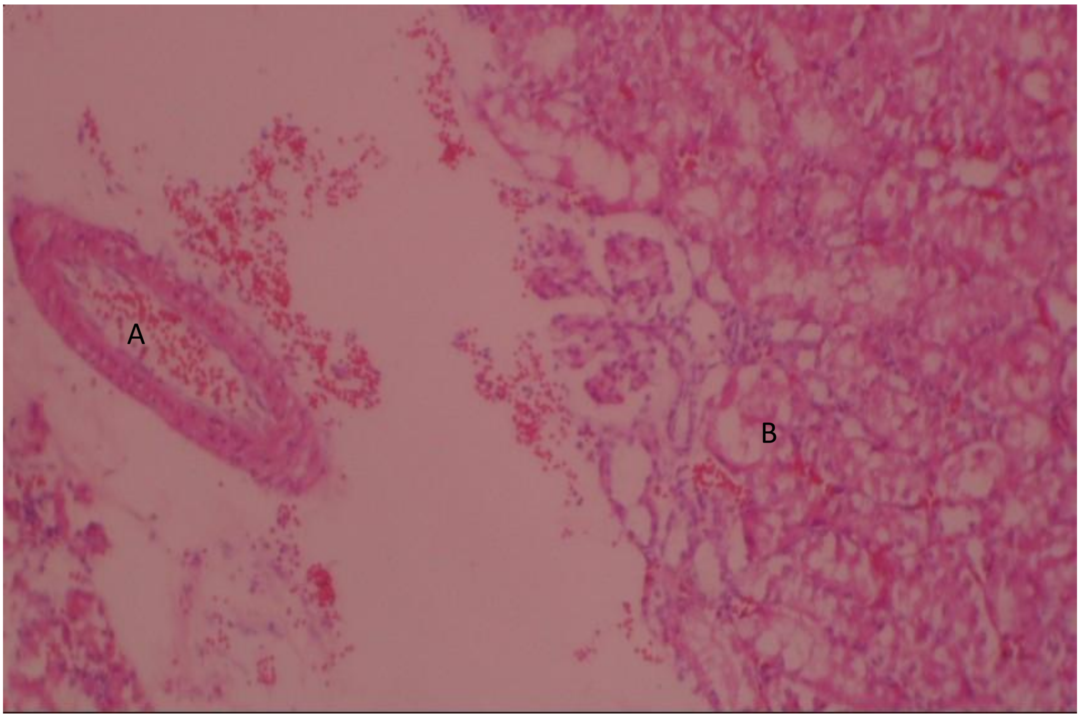

Figure 11. Section of the kidney of rats fed with normal diet showing A, Arcuate artery and B, tubules (H&E x 100).

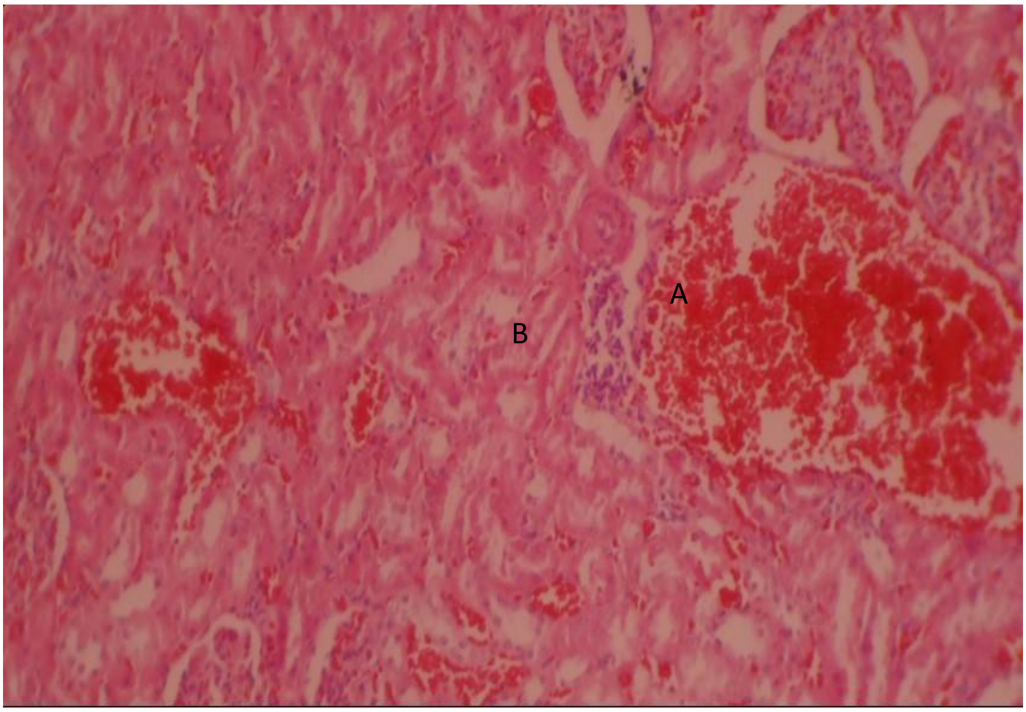

Figure 12. Section of the kidney of rats fed with high fat ketogenic diet showing A, marked active interstitial congestion and B, interstitial infiltrates of inflammatory cells (H&E x 100).

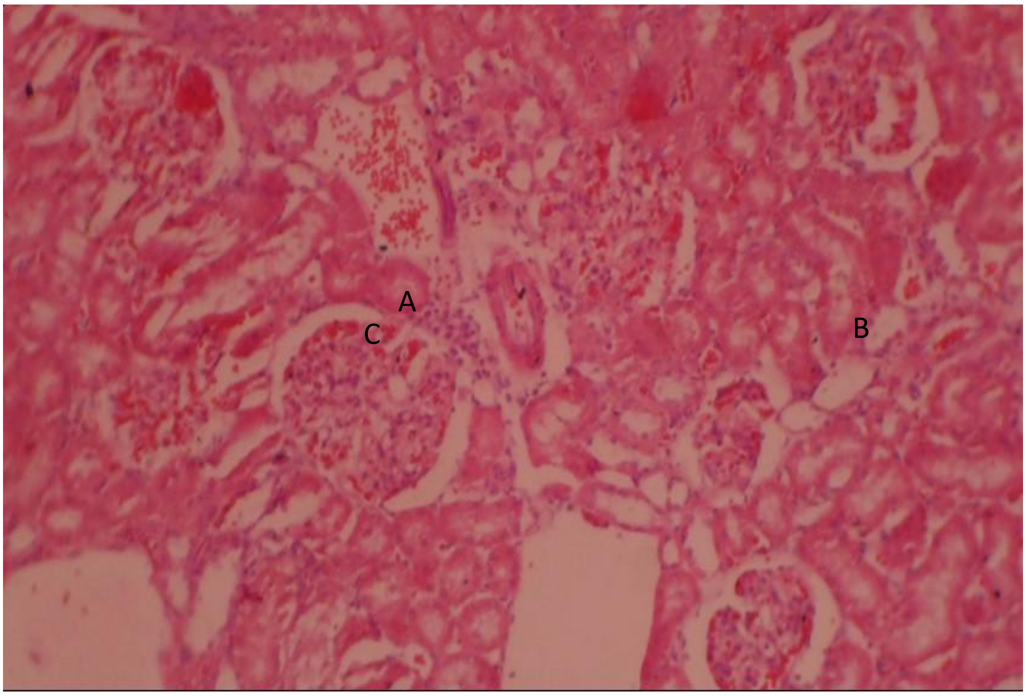

Figure 13. Section of the kidney of rats fed with high fat ketogenic diet showing A, patchy vascular ulceration and stenosis, B, mild interstitial active congestion and C, focal infiltrates of inflammatory cells (H&E x 100).

Discussion

In this study, we also looked at the effect of high fat ketogenic diet on some parameters of the liver, along with the functions and structure of the kidneys, with the aim of relating the selected functions of the liver to any change that may occur in the kidneys function and/or structure. The result of this study showed that there was no significant difference in the serum electrolytes level of animals fed with modified high fat ketogenic diet. Although, the serum sodium, potassium, bicarbonate, chloride, creatinine and urea of the animals fed with ketogenic diets were slightly higher than standard-fed rats, this may suggest a minor effect of the ketogenic diet on renal function. Perhaps this effect would have been more significant in a shorter duration of study, as it could be an acute effect rather than a chronic effect. This is because the kidneys may have adapted to the increased excretory load of fat metabolites over the cause of the duration of study, thereby adjusting itself back to normal function. A similar finding to this study was reported by Arsyad *et al.,* where they did not identify any significant alteration of kidney functions, but noticed slight reduction of serum electrolytes after 60 days administration of ketogenic diets (18). This study is also in agreement with the work of Oboh and Olumese (19), who reported no significant difference in serum electrolyte level, after the intake of ketogenic diet for 8 weeks.

Although there was a slight decrease in the serum albumin level of the animals fed with KD, this difference was not significant. It is documented that a decrease in serum albumin is seen in nephrotic syndrome, a clinical syndrome defined by massive proteinuria responsible for hypoalbuminemia which is primarily caused by intrinsic kidney diseases, such as membranous nephropathy, minimal-change nephropathy, and focal glomerulosclerosis (20). In their study, Eiya and Aikpitanyi-iduitua stated that Albumin levels significantly decreased in ketogenic groups compared with control (2). A decrease in albumin may also be an indication that the liver may have been affected, since the liver is the only site for albumin synthesis (21). Perhaps, a longer study period would have

resulted to a significant decrease in albumin level of the ketogenic diet treated group.
It was also seen that there was a significant increase in serum levels of globulin and total protein of animals fed with modified high fat ketogenic diet. The increase in serum globulin could have been a response to the mild inflammation seen in the kidney of the animals fed with modified high fat ketogenic diet. α_1 antitrypsin, α_2 macroglobulin and α_2 haptoglobulin fractions of serum globulin have been known to increase significantly in acute inflammatory disorders and tissue necrosis (22), a feature seen in this study of kidney tissues in the group of animals fed with high fat ketogenic diet. The increased globulin may have been a direct stimulation of the liver in response to stress, infection, acute inflammation, or tissue necrosis (22). The total protein content in human tissue fluid plays an important role on the state of health and the level of the total serum protein reflects the loss of protein caused by liver function and renal disease (23). Measurement of total serum protein can be used to monitor the nutritional status of the body indirectly, and benefit diagnostics of certain diseases (24, 25). Total serum protein primarily reflects the cumulative sum of albumin and globulins and can be readily calculated by subtracting albumin from total protein and is an approximation of the quantitative globulin (26). Thus, the significant increase in total protein seen in this study may have been a consequential effect of the significant increase in serum globulin levels also found in this study.
The findings of this study showed no histological alterations in the kidneys of control animals. However, in the kidneys of animals fed with modified high fat ketogenic diet, there was marked active interstitial congestion, vascular ulceration, stenosis and interstitial infiltrates of inflammatory cells. These changes could be due to excess excretory load, increased intake of energy, and tissue turnover triggered by the presence of high fat diet (27). The consequence of glomerular hyperperfusion, hyperfiltration and hypertension is glomerulosclerotic damage, which could be the long term effect of the morphological observations in this study. Previous works have shown that a high fat ketogenic diet leads to increased renal plasma flow, glomerular filtration rate (GFR), glomerular pressure, and filtration fraction, with resultant net afferent dilatation (28). In a study of obese and non-obese individuals with normal renal function, renal biopsy features such as increased mesangial matrix, podocyte hypertrophy, mesangial cell proliferation, and glomerulomegaly occurred more often than in their non-obese counterparts (29). Similar structural alterations of renal tissues were also reported by who noticed dilatation of glomerular capillaries, large blood vessels, subcapsular adipocyte accumulation, glomerular atrophy and necrosis in their study. They concluded that increase in the volume of the kidneys of high fat diet fed animals may have resulted from oedema due to mononuclear cell infiltrations among the tubules (11). All these indications suggest a role for high fat KD in the alteration of renal function as well as its morphology as shown in this study.

Conclusion
The result of this study showed that high fat ketogenic diet may be harmful to the kidneys. Although there was only slight increase in the serum electrolyte levels of treated animals, this may be an indication that increases in serum electrolyte are acute responses to high fat ketogenic diet. These acute responses may have been corrected by the kidney as duration of administration continued, and this may have resulted in a reduction of serum electrolytes overtime. In addition, there was a significant change in the serum globulin and serum total protein level of animals that were fed with ketogenic diet compared to the control group; this may have resulted from renal tissue inflammation and necrosis which were also seen in the histological report. Also, the histological findings from this

study clearly showed that the high fat ketogenic diet altered the morphology of the kidney of animals fed with the diet, causing focal tubular necrosis, vascular ulceration, stenosis, moderate and marked active interstitial congestion, and mild interstitial infiltrates of inflammatory cells, all of which are strong indications of a damaged kidney. These alteration of renal morphology and function may be due to increased renal plasma flow, glomerular filtration rate (GFR), glomerular pressure caused by increased renal excretory load of fat metabolic products from the high fat ketogenic diet.

Recommendation

Future studies would be to study the effects of high fat ketogenic diet on renal functions measuring the urine concentrations of electrolytes, proteins and ketosis. In addition, a longitudinal study, where urine samples are collected weekly, should be done to enable acute and chronic effects of KD on the renal functions to be critically observed.

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