



Biochemical Effects of Some Traditional Nigerian Diets in Experimental Diabetic Rat Models

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ABSTRACT

Diet, besides its simplicity and affordability, has been clinically recommended as the first line of intervention in type 2 diabetes. However, compliance with the so-called diabetic diet has been notoriously poor, mostly because the social and cultural aspects of eating as well as the degree of difficulty involved in entrenching permanent changes in diet have greatly been underestimated. The present study therefore evaluated the suitability of some traditional Nigerian diets namely *Garri* with *afang* soup (GAS), pounded yam with *edikang ikong* soup (PYES) and *ekpank nkukwo* (EN) in alloxan diabetic rats. 15-day feeding with GAS and PYES respectively reduced ($P<0.05$) fasting blood glucose by 25.61% and 25.19%, compared to the reference diet, plantain with beans (37.22%). The glucose lowering effect of EN was however, not significant. Except GAS, the effect of the test diets on serum lipid profile was similar and comparable to the reference diet. Further, the impact on serum biochemical indices - β -carotene, total protein, albumin, urea and creatinine, though not dramatic, was not different from the reference diet, except serum β -carotene that was higher in the reference diet-fed animals than others ($P<0.05$). Taken together, these results suggest that the studied diets, can potentially offer the patients the needed dietary diversification in diabetes management.

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1. INTRODUCTION

Diabetes mellitus, according to the American Diabetes Association is “a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both” (ADA, 2011). It is not a single disease, but a collection of several genetic diseases with different etiologies, but having a common phenotype of abnormal glucose metabolism due to an inappropriate glucose-insulin relationship (Rodrigues et al., 1999; Berdanier, 2003), requiring a multi-dimensional therapeutic approach. The chronic hyperglycemia precipitates overtime, several complications and poses huge social and financial burden on the individuals, families and the society. The reflection of this burden is more in the developing countries that are usually not financially prepared to meet challenge, yet these countries are expected to record the highest number of patients by year 2030 (Diabetes Atlas, 2007; WHO, 2008). In fact, between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010).

Dietary modification is the simplest and cheapest form of diabetes treatment and is even the clinically recommended primary therapy in type 2 diabetes (Mshelia et al., 2005). Many specific interventions have been carried out in the management and perhaps prevention of diabetes and one of the integral components is medical nutrition. Whether for management or prevention of diabetes and its complications the purpose of nutrition recommendation is the underlying concern for optimal nutrition through healthy food choices and an active lifestyle (Ntui et al., 2006). However, compliance with the so-called diabetic diet has been notoriously poor and this is mostly because the social and cultural importance of eating behaviors have often been neglected and the degree of difficulty involved in making permanent changes to entrenched eating habits is still greatly underestimated. Hence, the art of nutrition is as important as the science of nutrition. For these reasons, Africa and other developing countries have no standard dietary therapy in diabetes management (Mshelia et al., 2005).

Patients with diabetes need nutrition recommendations that are not just supported by scientific evidence, but that can be easily understood and translated into everyday life. Just as changes have occurred over the past decade in conventional medications used for treating diabetes, changes have also occurred in medical nutrition therapy for diabetes (Franz et al., 2003). To achieve the goals and objectives of dietary therapy, it is important that diabetic patients are provided with dietary guidelines appropriate to their cultural situations, also considering availability and affordability of the foods. Therefore in our earlier study we demonstrated that some typical Nigerian diets namely *Garri* with *afang* soup, pounded yam with *edikang ikong* soup and *ekpong nkukwo* could be beneficial as well as the regimented diets in glycaemic control in experimental diabetic subjects (Ani et al., 2011a,b).

In the present study, the effect of these diets on the overall serum chemistry of the diabetic subjects was investigated. Alloxan diabetes subjects have been reported to show altered serum lipids, and indices of liver and kidney functions (Atangwho et al., 2007a,b,c). The

impact of a therapeutic intervention on these indices would strongly contribute to the validation or otherwise of same.

2. MATERIALS AND METHODS

2.1 Preparation and Processing of the Diets

The ingredients used for the preparation of the various traditional diets were bought at Ika Ika Oqua Market in Calabar, Cross River State, Nigeria. After initial washing and draining, these were used to prepare the diets according to the traditional/indigenous methods, including *garri* with *afang* soup, pounded yam with *edikang ikong* soup, *ekpang nkukwo* and plantain porridge with beans. The proportions of the condiments used were modified from "A taste of Calabar" selected recipes by Ana (2000). Each of the diets was oven dried at 60°C to constant weight and thereafter homogenized with an electric blender. The homogenized diets were wrapped in aluminum foils and stored in microwaves from where aliquots were withdrawn daily for animal feeding.

2.2 Animals and Experimental Design

Male Wistar rats (140-240g) obtained from the animal house, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria, were used for the study. The animals were kept in wooden cages with stainless wire mesh top in well ventilated animal house. Diabetes was induced by a single dose intra-peritoneal injection of 150mg/kg body weight of alloxan after an overnight fast. A week after injection of alloxan, diabetes was confirmed in alloxan-treated rats showing fasting blood glucose levels > 200mg/dl. The experimental animals were then divided into six (6) sub-groups and treated accordingly:

1. Normal rats fed normal rat pellets (normal control)
2. Diabetic rats fed normal rat pellets (diabetic control)
3. Diabetic rats fed *garri* with *afang* soup (test diet 1).
4. Diabetic rats fed *pounded yam* with *edikang ikong* soup (test diet 2).
5. Diabetic rats fed *ekpang nkukwo* (test diet 3).
6. Diabetic rats fed plantain porridge with beans (diet 4 - reference diet).

The diets and water were both given *ad libitum* for fifteen (15) days after which the animals were sacrificed and liver tissues collected for evaluation. Fasting blood glucose (FBG) was measured using One Touch Glucometer analyzer prior to the commencement of dietary feeding and at end of the 15- day treatment. Similarly, initial and final body weights were measured with an electronic weighing balance. From these, %FBG reduction was calculated thus:

$$\% \text{ Change in FBG} = \frac{\text{Initial FBG level} - \text{Final FBG level}}{\text{Initial FBG level}} \times 100$$

The use of animals in this research and the protocol were duly approved by the College of Medical Sciences' Animal Ethics Committee, University of Calabar, Calabar (UNICAL).

2.3 Biochemical Analysis

At the end of the 15 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapor and

sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain tubes, allowed to clot for about two hours. The clotted blood was thereafter centrifuged (B. Bran Scientific and Instrument Company, England) at 3,000 rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used for biochemical analysis. Serum β -carotene was determined spectrophotometrically (Visible spectrophotometer, SP-300 Optima, Japan), using the method by Bessey et al. (1946). The serum total protein, albumin and urea were estimated using analytical assay kits obtained from DIALAB Production and Vertrice Von Chemisch, Germany. Assay kits obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, were used to estimate serum lipids (total cholesterol, triacylglycerol and HDL- cholesterol) and creatinine.

2.4 Statistical Analysis

The results were analysed for statistical significance by one way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as the mean \pm SD; P values < 0.05 were considered significant.

3. RESULTS

Effect of 15-day feeding of the study diets on diabetic rats is shown on Table 1. There was observed significant reduction ($P < 0.05$) in FBG of animals fed *garri* with *afang* soup (25.61%) and pounded yam with *edikang ikong* soup (25.19%) relative to the diabetic control (5.19%). This effect was similar and compared well with the reference diet, although the extent of reduction was higher with the reference diet (37.22%). However, the glucose reduction effect of *Ekpang nkukwo* was not significant. The effect of the diets on serum lipid profile (Table 2) indicated a significant increase ($P < 0.05$) in serum triglyceride (TG) of untreated diabetic rats relative to the non diabetic control. Within the diabetic test diets, serum triglyceride levels ranged closely with the reference diet (except the group fed *garri* with *afang* soup), although these were significantly high ($P < 0.05$) compared to the normal control value (73.66 ± 11.37).

Table 1. Fasting blood glucose of alloxan diabetic rats before and after 15-day feeding with the traditional and reference diets

Treatment	FBG, before dieting (mg/dl)	FBG, after 15-day dieting (mg/dl)	Decrease in FBG (%)
Normal control (rat pellets)	43.85 \pm 8.27	37.42 \pm 3.77	14.66
Diabetic control (rat pellets)	242.14 \pm 61.02	229.57 \pm 26.69	5.19
Garri with <i>afang</i> soup	282.85 \pm 45.16	210.42 \pm 32.64	25.61*
Pounded yam with <i>edikang ikong</i> soup	265.42 \pm 51.48	198.57 \pm 40.69	25.19*
<i>Ekpang nkukwo</i>	292.71 \pm 97.36	279.35 \pm 50.13	4.56
Plantain with beans(reference)	261.00 \pm 73.00	163.85 \pm 51.73	37.22*

Values are expressed as the mean \pm SD, n= 7; *P<0.05 vs. diabetic control; FBG = Fasting Blood Glucose

There was no significant difference ($P > 0.05$) in total cholesterol (TC) concentration between diabetic control and non diabetic control rats. The effect of the traditional diets (except *garri* with *afang* soup) on TC was similar to that of the reference diet and both compared well with the normal control. LDL-cholesterol concentration decreased significantly in all test groups

and the extent of decrease (except *garri* with *afang* soup) compared well with the reference diet. No significant changes were observed in HDL and VLDL cholesterol following diet treatments.

Table 2. Effect of 15-day feeding with traditional and reference diets on serum lipid profile of alloxan diabetic rats

Parameter	Normal control	Diabetic control	<i>Garri</i> with <i>afang</i> soup	Pounded yam with <i>edikang ikong</i> soup	<i>Ekpang nkukwo</i>	Plantain with beans (reference)
Triglyceride (mg/dl)	73.66 ±11.37	114.50 ±7.47 ^a	207.25 ±27.80	111.88 ±19.03	160.76 ±9.03	139.87 ±38.65
Total cholesterol (mg/dl)	63.35 ±5.31	70.01 ±8.71	81.71 ±20.64	66.42 ±10.24	66.48 ±13.80	63.21 ±4.89
HDL-cholesterol (mg/dl)	21.97 ±3.7	21.79 ±2.54	23.12 ±4.97	26.36 ±9.78	24.17 ±4.32	21.23 ±3.9
VLDL-cholesterol (mg/dl)	14.69 ±2.27	17.78 ±3.25	35.7 ±14.81	21.45 ±4.81	26.52 ±10.15	23.67 ±7.12
LDL-cholesterol (mg/dl)	26.68 ±5.29	30.42 ±7.59	22.92 ±10.30	18.61 ±5.15 [*]	15.83 ±7.74 [*]	18.43 ±1.99 [*]

Values are expressed as the mean ± SD, n = 7; ^aP<0.05 vs. normal control; ^{*}P<0.05 vs. diabetic control

However VLDL cholesterol was significantly increased in rats fed *garri* with *afang* soup, compared to diabetic control and other diets. The effect of the test diets on other serum biochemical indices including α -carotene, total protein, albumin, urea and creatinine (Table 3) were not as dramatic. However, of interest, is the fact that the impact of the reference diet (plantain with beans) on the diabetic rats was not different from the three test diets, except in serum α -carotene content that was significantly high in this group (P<0.05) compared to both controls.

Table 3. Effect of 15-day feeding with traditional and reference diet on selected serum biochemistry of alloxan diabetic rats

Treatment	Serum α -carotene (mg/dl)	Serum albumin (mg/dl)	Serum protein (mg/dl)	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Normal control	155.6±23.37	3.45±0.35	5.89±0.97	36.36±9.4	1.94±0.15
Diabetic control	147.6±29.56	3.57±0.41	6.08±0.75	81.95±15.96 ^a	1.95±0.53
<i>Garri</i> with <i>afang</i> soup	141.04±18.27	2.94±0.67	6.63±0.71	84.44±13.57	1.86±0.76
Pounded yam with <i>edikang ikong</i> soup	145.4±7.3	3.54±0.50	5.9±0.57	82.91±17.36	2.16±0.41
<i>Ekpang nkukwo</i>	186.5±4.79	3.53±0.30	6.53±0.70	80.22±18.49	2.95±0.97
Plantain with beans (reference)	237.06±17.90 ^{a,*}	3.47±0.20	6.12±0.54	73.51±15.03	1.99±0.30

Values are expressed as the mean ± SD, n = 7; ^aP<0.05 vs. normal control; ^{*}P<0.05 vs. diabetic control

4. DISCUSSION

Diet is a very important factor in the prevention and management of diabetes. Accordingly, the World Health Organization (WHO) monitors the diet recommendation for diabetes every five years and reviews the approved diet in accordance with new research and advances in medicine. Today a lot of people are still misinformed, and are following a low carbohydrate diet or a monotonous diet. In a particular study, Ntui et al. (2006) reported that about half of the diabetic patients that participated in the study consumed mainly plantain as the main starch with beans, in various cooked forms. The need for diversification necessitated the present study on traditional diets.

In the present study, there was a significant drop in fasting blood glucose (FBG), after fifteen-day feeding of rats with the traditional diets. Pastors et al. (2002) reported from randomized controlled trials, observational studies and meta-analyses that nutrition intervention improves metabolic outcomes, such as blood glucose in individuals with diabetes. Several other studies have also, consistently shown that the soluble fibre and other similar leguminous fibres are more effective in improving blood glucose, glycated haemoglobin and serum lipid concentrations (Briony, 1994). A mixture of carbohydrate and fibre generates a smaller rise in glycaemia than the same type and amount of carbohydrate consumed alone. The diets studied fits so well into the description. A typical Nigerian meal from the Southeast is heavy with starchy items, light on meat and generous on fat (Umoh, 1998). Semi – solid starchy mass, made by pounding boiled yam and garri (made with cassava) is served with soups, and most Nigerian soup meals have as their constituents meat, fish, palm oil, green leafy vegetables, crayfish, seasonings and water. Soup is a mixture of ingredients obtained from both plants and animals.

In this study, the untreated diabetic rats showed combined hyperlipidaemia and hypercholesterolemia, similar to the earlier reports of Abdul Rahman et al. (1995) and Idogun et al. (2007) among diabetic patients. Also, Essien et al. (1992) had reported that the mean total cholesterol level for diabetes mellitus was slightly higher than those of controls. Alterations in serum lipid profiles are known in diabetics, and are likely to increase the risk of coronary heart disease (Massing et al., 2001). Consequently, Garg et al (1988) suggested partial replacement of complex carbohydrates with monounsaturated fatty acids in the diet of patients with type 2 diabetes, which according to them, does not increase the level of LDL cholesterol, and may improve glycemic control and the levels of plasma triglycerides and HDL cholesterol. Also Chandalia et al (2000) reported that high-fibre diet resulted in a lower fasting plasma total cholesterol concentration by 6.7%, a lower triglyceride concentration by 10.2% and a lower VLDL cholesterol concentration by 12.5%, the fasting plasma LDL cholesterol concentration was 6.3% lowered and no change in HDL cholesterol concentration. This probably may be the case with our diets in this study, where observably total and LDL cholesterol were significantly reduced upon feeding with the traditional diets, but without any change in HDL cholesterol. Research has shown that low fat diets are usually associated with moderate weight loss, which can be maintained as long as the diet is continued (Lichtenstein et al., 1994). Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease (Huang et al., 1988). Therefore, lowering of serum lipid levels whether diet or drug induced, would no doubt decrease the risk of vascular disease and related complications. This obviously would account for the observed hypolipidemic effects of these diets.

Changes in serum biochemistry following a therapeutic intervention, usually reflects the effect of such intervention protocol on liver and kidney; two vital organs pre-occupied with

the function of xenobiotic metabolism and excretion, respectively (Lieberman and Marks, 2009). The test diets in the present investigation showed null effect on these measured indices, suggesting that they were safe within the study period. Moreover, their effect was not different from the reference diet. These diets form part of the traditional food system of the South Eastern Nigerians, hence may not really constitute any treat to safety. They can therefore offer the diabetic patient the needed social satisfaction, diet diversification and variety. Srinivasan (2005) had advocated in his review on antidiabetic plants, the preference of edible vegetables, since according to him, the biological system is well adapted to them, hence may constitute little or no toxicity problem.

5. CONCLUSION

In conclusion, from a biochemical stand point, the results of this study suggest that the traditional Nigerian diets studied, can potentially substitute the classical standard diets, recommended in diabetes management, thereby offering the patients the needed dietary diversification.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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