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Effects of Low Carbohydrate High Fat Nigerian-Like Diet on Biochemical Indices in Rabbits

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Abstract: The effects of a low carbohydrate high fat Nigerian like diet on lipoprotein levels, serum electrolytes, liver and renal functions were investigated. The diet consist of 28% carbohydrate and 35% fat as percentage of total energy in a wholly compounded form. Twelve rabbits were randomly divided into two groups. The groups were: Group I that was fed the control diet and the second group II were fed with the low carbohydrate high fat diet. At the end of eight weeks, biochemical analyses were done on blood samples of the animals. There were significant ($p \leq 0.05$) decrease in weight for the experimental animals when compared with the control group. Total protein, albumin and globulin did not change significantly ($p \leq 0.05$), for the rabbits fed on the low carbohydrate high fat diet. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) levels increased significantly ($p \leq 0.05$) while Alkaline Phosphatase (ALP) levels decreased significantly ($p \leq 0.05$). The bilirubin level for rabbits on the experimental diet did not change significantly ($p \leq 0.05$). Serum electrolyte concentrations showed no significant change in sodium, potassium, chloride and bicarbonate ions when compared with control. Creatinine and urea values showed no significant ($p \leq 0.05$) changes. Total Cholesterol levels were significantly ($p \leq 0.05$) reduced in the experimental diet. The lipoprotein fractions showed significant increases ($p \leq 0.05$) in High Density Lipoprotein (HDL) and Triacylglycerol (TAG) levels when compared with controls. Low Density Lipoprotein (LDL)-Cholesterol did not change significantly ($p \leq 0.05$). The results showed that the Low Carbohydrate High Fat (LCHF) Nigerian-like diet promotes hypertriglyceridemia and weight loss. This could also increase the risk of ischemic heart disease.

Key words: Low carbohydrate high fat diet, serum lipids, electrolytes, liver function, renal function

INTRODUCTION

Food is important to the physical well-being, energy needs, growth and repair of damaged tissues and the regulation of body function in humans. A balanced diet containing adequate amount of nutrient should include a wide variety of foods from different food groups. The food guide pyramid outlines the group of foods for which recommendations have been established. The diet type consumed by individuals is a major factor that can determine the propensity to develop cardiovascular disease or are likely to be diagnosed with adult-onset diabetes (Krauss *et al*., 2006).

Reducing calories is a proven way to make humans lose weight (Rocette *et al*., 2006). In Nigeria; this has become the practice among those who are overweight. It facilitates weight loss by promoting the metabolism of adipose tissue. The campaign for low calorie diet was due to a massive increase in the proportion of people who are overweight or obese. The benefits of a low carbohydrate diet includes weight loss, improved values in conventional tests for hyperlipidemia (Volek and Westman, 2002), fewer free radicals (Westman *et al*., 2003), a boost in antioxidant enzymes (Westman *et al*., 2002), strengthening of the immune systems, reduction in blood glucose and insulin levels, anticancer and antiaging effect (Bravata *et al*., 2003). The Atkins Diet consists of a low carbohydrate, high fat and protein diet. It has been recommended to be safe and effective, producing weight loss despite ad-libitum consumption of fatty meat, butter, other high-fat dairy and protein products (Parker *et al*., 2002). The efficacy of the Atkins Diet is raising medical and nutrition questions; the adverse effect of a high protein diet is negative on renal and hepatic function, calcium balance and insulin sensitivity (Astrup *et al*., 2004).

The guidelines for fat consumption have undergone a dramatic shift and are currently being evaluated. Restricted intakes of animal fats are recommended, because of their content of saturated fats and cholesterol (Ravnskov, 2000) Although, previous research has not determined the amount of dietary saturated fat that actually benefits a person's health, a high fat or ketogenic diet could lead to Obesity, high blood pressure, heart disease, diabetes, immunosuppression, atherosclerosis and chronic fatigue (Yancy *et al*., 2004).

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Several reports show that various groups existed that consumed relatively high amounts of fat yet were free of heart disease (Bang *et al*., 1980). The associated risk of a high fat diet was the amount of trans fat and not saturated fat consumed (Ghafoorunissa, 2008). Trans fatty acids are formed when vegetable oil is hydrogenated or heated to high temperature in deep oil frying. Trans fatty acids raise serum low density lipoprotein and lower high density lipoprotein in humans (Mozaffarian and Clarke, 2009). Therefore, the effects of trans fatty acids on risk profile for cardiovascular disease is unfavorable. Labels of food products should state the trans fatty acid content as part of nutritional information.

Palm oil is widely consumed in Nigeria for use in cooking soup, stew and some traditional foods. It is obtained from the mesocarp (pulp) of the fruit Palm-*Elaeis guineensis* by squeezing the pulp of boiled and pounded palm fruit. The pulp is discarded and the liquid obtained is boiled to give thickened red liquid, called Red Palm oil (RPO). The oil is consumed fresh. It is rich in beta-carotene which can be used to prevent vitamin A deficiency. The presence of tocols (tocopherols and tocotrienols) makes palm oil act as an antioxidant. (Schroeder *et al*., 2006).

The typical Nigerian-like diet is consumed by combining a carbohydrate based meal (Cassava flour, rice, cocoyam, potatoes, yam or plantain) with soup or stew cooked in palm oil and very small amount of protein. Proteins (meat, fish, milk and eggs) are expensive and out of the reach of a very large number of the populace. Fruits and vegetables which have high quantity of dietary fiber and numerous nutrients (vitamins, minerals and antioxidants) are in abundance in Nigeria. They promote good health and prevent diseases. However, Nigerians do not consume enough quantity.

Several studies have reported the effects of either a low carbohydrate or high fat/protein diet fed to humans (Volek *et al*., 2004). This was done by the utilization of refined processed food products. Such diets may be metabolized differently. Nigerians do not consume their food in this form. This study was designed to examine the effects of combining a low carbohydrate, high fat wholly compounded Nigerian-like diet on biochemical indices in rabbits. The experimental animals have similar lipid metabolism with humans (Zhang *et al*., 2008).

MATERIALS AND METHODS

Animals and management: New Zealand white rabbits (Initial mean weight 1.75 kg), three months old, were used in the present study.

The rabbits were housed in individual stainless steel animal cages with wire mesh floors to prevent coprography. Light was a 12 h-light and 12 h-dark cycle and the room temperature was uniform. The animals were acclimatized on growers' mash for two weeks. Prior to the study, food and water were given *ad libitum*. The rabbits were divided into two groups of six rabbits per group, each group having animals of similar weight after the adaptation period. One group was fed the control diet and the second group was fed the low carbohydrate high fat diet for a total period of eight weeks.

Diet: The composition of both diets is shown in Table 1. Red Palm Oil (RPO) was obtained from the Nigerian Institute for oil Palm Research (NIFOR), Benin, Benin City, Nigeria. Garri and fish were obtained from an open air market in Benin City, Nigeria. The fish was oven dried (Gallenkamp, UK) for 16hr to a constant weight, skinned and deboned. The dried fish was cooled in a dessicator and milled to powder in warring blender. The different components of the diet were stored in air-tight containers at -10^{o}C until used as feed for the rabbits. Fresh feed was provided on daily basis while stale remnants were discarded after weighing. On the average each rabbit received about 150 g/feed/day. Clean drinking water was provided. During this period, feed intake, water intake and dry fecal output were measured daily. Weight gain was recorded weekly.

Animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the use of Laboratory Animals (NRC, 1985).

Blood samples, collection and analysis: The animals were fasted for 18 h and baseline blood samples were drawn from the rabbit ear marginal veins using 21-gauge syringes. After eight weeks of feeding, the rabbits were anesthetized with pentobarbital (60 mg/Kg body weight). Insertion was made into the heart region for collection of blood samples with the use of a needle and syringe. The blood samples were collected into labeled bijou bottles containing heparin as anticoagulant and centrifuged immediately (3,000 x g for 10 min), to obtain the serum. The serum samples were stored in the biofreezer until analyzed. Duplicate serum samples for each animal group were analyzed for total proteins, albumin, globulins, Baertl *et al*. (1974), electrolytes (Na^+, K^+, $HC0_3^-$, Cl^-) Kinsley and Schaffert (1953), lipoprotein profile, LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C), Triacylglycerol (TAG) and Total Cholesterol (TC). Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) Anderson *et al*. (1971) urea and creatinine Carr (1959) were measured using commercial kits (Boehringer, Mannheim).

Statistical analysis: Data were expressed as Standard Error of the Mean (SEM) for each group of rabbits. Significant differences between the control and experimental set of data were analyzed by the student's t-test. P $\leq$ values 0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical (2000, GraphPad 3.06 software, Inc. San Diego, CA).

RESULTS

Table 1 shows the composition of control and experimental diet fed to the rabbits. The weight gain, feed and water intake, feed efficiency and dry fecal output of the rabbits in the control and experimental groups are presented in Table 2. Statistical analyses showed that there were significant ($p \leq 0.05$) decreases in weight gain, feed and water intake and feed efficiency in the experimental diet when compared with the control diet.

Table 1: Composition of experimental diets

	Diet type	
Dietary components	Control	LC/HF
Garri	65.0 g	28.2 g
Fish	28.4 g	35.2 g
Mineral and vitamin mix (*Opimix premix)	1.0 g	1.0 g
Palm oil	5.0 g	35.0 g
Methionine	0.6 g	0.6 g

Note: Garri is a cassava based meal, rich in carbohydrate and commonly consumed in Nigeria. It contributed to the fiber in the Nigerian- like diet.

*Opimix premix:

Vitamin A = 8,000,000IU	Copper = 5 gm
Vitamin D = 1,600,000IU	Iron = 20 gm
Vitamin E = 5,000IU	Iodine = 1.2 gm
Vitamin K 2,000 mg	Selenium = 200 mg
Thiamine (B_1) = 1500 mg	Cobalt = 200 mg
Riboflavin (B_2) = 4,000 mg	Cholin chloride = 200 gm
Pyridoxine (B_6) = 1500 mg	Anti oxidant = 125 gm
Niacin = 15,000 mg	Manganese = 80 gm
Vitamin (B_{12}) = 10 mg	Zinc = 50 gm
Pathothenic acid = 5,000 mg	Biotin = 20 mg
Folic acid = 5,000 mg	

Table 2: Weight gain, feed intake, water intake, feed efficiency and dry fecal output of rabbits in the control and experimental groups

Groups	Control diet	Experimental diet
Weight gain (g/rabbit)	650±8.0[a]	150±6.0[a]
Feed intake (g/rabbit/day)	53.8	42.5
Water intake(ml/rabbit/day)	20.50±2.0[a]	16.80±2.50[a]
Feed efficiency (g/body weight/g feed)	12.08	3.52
Dry fecal output (g/rabbit/day)	5.24±1.14[a]	3.25±1.02[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

The values observed for total protein (0.73 mg/dl), albumin (0.05 mg/dl) and globulin (1.75 mg/dl) in the experimental diet are given in Table 3. There were no significant differences ($p \leq 0.05$) between the control and experimental diet. The values obtained for AST, ALT, ALP and bilirubin are shown in Table 4. The AST and ALT increased significantly ($p \leq 0.05$) and ALP levels decreased significantly ($p \leq 0.05$). The bilirubin levels for rabbits in the control and experimental diet showed no significant difference ($p \leq 0.05$).

Table 3: Mean concentrations of total proteins, albumin and globulins of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Total protein (mg/dl)	1.28±0.41[a]	0.73±0.32[a]
Albumin (mg/dl)	0.26±0.15[a]	0.05±0.27[a]
Globulin (mg/dl)	1.75±0.52[a]	1.75±0.32[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

Table 4: Mean Concentrations of serum enzymes and bilirubin of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
AST (IU/L)	2.90±0.07[a]	9.75±3.43[b]
ALT (IU/L)	7.65±0.47[a]	11.50±2.60[b]
ALP (IU/L)	32.5±16.48[a]	4.75±1.25[b]
Bilirubin (mg/dl)	0.28±0.09[a]	0.24±0.02[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

Table 5 shows the serum levels of electrolytes, creatinine and urea in rabbits. The rabbits fed with the LCHF diet had no significant difference at ($p \leq 0.05$) in the creatinine, urea, sodium, potassium, bicarbonate and chloride ions content when compared with the control samples.

The values of serum lipids of rabbits fed the control and experimental diet are presented in Table 6. Serum cholesterol levels were significantly ($p \leq 0.05$) reduced. The lipoprotein fractions were also significantly altered. HDL and TAG levels were significantly elevated ($p \leq 0.05$) when compared with the control. LDL- cholesterol did not have a significant change ($p \leq 0.05$) in the experimental groups when compared with the control diet.

DISCUSSION

The 28% carbohydrate and 35% fat diet utilized in this study was supplied by *garri* (a processed form of cassava) and palm oil, respectively. The rabbits fed the Nigerian like LCHF diet showed significant weight loss when compared to the control animals. Significant body weight reduction was observed in humans on the LCHF. (Meckling *et al*., 2002). There were no significant changes in total protein, albumin and globulin levels in rabbits fed the experimental diet. This showed that the percentage of protein in the diet was adequate for the synthesis of plasma proteins.

There were significant increases in the serum AST and ALT levels of the experimental animals. Increases in serum levels of these enzymes are usually indicative of possible liver damage but the levels observed in this present study is lower than the normal physiological range of 20-90 IU/L. This can be correlated to the fact

Table 5: Mean concentrations of electrolytes, creatinine and urea of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Potassium (mM/L)	0.47±0.11[a]	0.28±0.47[a]
Sodium (mM/L)	17.0±2.55[a]	17.25±4.70[a]
Bicarbonate (mM/L)	3.67±0.58[a]	2.6±0.41[a]
Chloride (mM/L)	12.00±0.58[a]	12.73±2.59[a]
Creatinine (mg/dl)	0.46±0.06[a]	0.25±0.05[a]
Urea (mg/dl)	8.33±3.69[a]	8.5±10.97[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (p<0.05)

Table 6: Mean Concentrations of plasma lipids of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Total Cholesterol (mg/dl)	50.18±7.75[a]	35.00±17.9[b]
HDL-Cholesterol (mg/dl)	6.5±0.96[a]	10.50±2.06[b]
LDL-Cholesterol (mg/dl)	32.18±7.22[a]	30.76±1.49[a]
Triacylglycerol (mg/dl)	15.5±7.59[a]	52.5±8.27[b]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (p<0.05)

that the bilirubin levels had no significant change in comparison to the control. This indicates that the experimental did not affect the maintenance of the normal excretory function of the liver or caused damage of liver cells. Moderate consumption of palm oil supports normal enzyme levels (Jones, 1975). The decreased concentration of ALP in the rabbits fed the experimental diet could indicate that the levels of fat used in this study did not adversely affect metabolic activities as mediated by ALP.

Creatinine levels for rabbits fed the experimental diet did not change significantly and this is an indication of normal kidney function. The levels of electrolytes in rabbits fed the experimental diet had no significant changes.

The total serum cholesterol decreased significantly. LDL Cholesterol did not show significant changes, while HDL and TAG increased significantly. Similar results have been observed in HDL and total cholesterol levels, when palm oil was used as the source of fat in the diet (Karaji-Bani *et al.*, 2006). In this present study, the total cholesterol and HDL Cholesterol levels tend to improve with weight loss, but hypertriglyceridemia was manifested.

The improvement of total cholesterol and HDL levels accompanied by weight loss could be due to the low carbohydrate content of the diet and increased fat intake of 35%. This may have caused the body cells to use all the dietary fats and then breakdown fats (lipolysis) in skeletal and adipose tissues for energy, leading to hypertriglyceridemia and weight loss as observed in the study.

The HDL increase may be due to down regulation of HDL-Cholesterol receptors which bind HDL-Cholesterol and facilitate reverse cholesterol transport to the liver, this may be regulated by the dietary fat (Tan *et al.*, 1991). The hypercholesterolemic risk of consuming high levels of palm oil has been investigated extensively in experimental animals and in human subjects in various countries with different types of diets. All these studies have established that palm oil does not behave like a saturated fat in its effects on blood cholesterol or blood clotting, as might be anticipated from its fatty acid composition (Chong, 1991). The vitamin E tocotrienols present in palm oil are known to reduce circulating cholesterol concentrations in humans, this effect is attributed to a dose-dependent inhibition by tocotrienols of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (Haave *et al.*, 1990). Thus inhibiting the in vivo synthesis of cholesterol in the liver and thereby lowering serum cholesterol, particularly of the Low-Density Lipoprotein (LDL) fraction. Although, in this study, the LDL fraction did not change in the experimental animals. Palm oil, like many other vegetable oils, is rich in the Mono-Unsaturated Fatty Acid (MUFA) oleic acid (40%), which has recently been shown to have a hypocholesterolaemic influence (Kamisah *et al.*, 2005). This is believed to be the cardio-protective fraction of palm oil.

The potential of any dietary saturated fat for causing hypercholesterolemia is particularly related to the level of its consumption. When dietary saturated fats are consumed at high levels, they can become important risk factor in the development of hypercholesterolemia and cardiovascular disease. At low to moderate levels of palm oil consumption, such as prevailing among the bulk of the Nigerian population that kind of risk may not apply. There may be a risk in populations with a high fat intake. The typical Nigerian diet contains significant amounts of invisible fats rich in PUFA, high dietary fiber which has hypocholesterolemic factors.

Conclusion: Low Carbohydrate High Fat (LCHF) Nigerian like diet leads to improved atherogenic dyslipidemia in the presence of weight loss but with the appearance of hypertriglyceridemia and increased HDL levels. The use of human volunteers on this LCHF Nigerian like diet could give more accurate responses on formulating dietary management for people suffering from metabolic diseases.

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