Pakistan Journal of Nutrition 6 (4): 399-403, 2007
ISSN 1680-5194

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

H.A. Oboh, C.O. Omofoma, F.E. Olumese and B. Eiya
Department of Medical Biochemistry, School of Basic Medical Science,
College of Medicine, University of Benin, Benin City, Nigeria

Abstract: The effects of High Carbohydrate Low Fat (HCLF) diet on biochemical indices were evaluated in rabbits. The diet consisting of 85% carbohydrate and 5% fat as % of total energy in a wholly compounded form was administered to rabbits. Twelve rabbits were randomly divided into two groups of six rabbits per group. The groups were Group I fed the control diet and the second group II was fed with the low protein high carbohydrate diet. At the end of eight weeks, the animals in each group were sacrificed and analysis done on blood samples of the rabbits. Results showed that the total protein, albumin and globulin were significantly ($p<0.05$) lowered for the rabbits fed a HCLF diet. Aspartate transaminase EC2.6.1.1 (AST) and Alanine transaminase EC.2.6.1.2 (ALT) levels increased significantly ($p<0.05$) while Alkaline phosphatase EC.3.3.3.1 (ALP) decreased significantly ($p<0.05$). The bilirubin level for rabbits on the experimental diet was within the normal levels. Serum electrolyte concentrations show a significant ($p<0.05$) increase in sodium and chloride ions when compared with control. Potassium reduced significantly and bicarbonate ions had no significant change ($p<0.05$). Creatinine values showed a significant ($p<0.05$) decreased levels while urea levels increased significantly ($p<0.05$) Cholesterol levels were significantly ($p<0.05$) reduced. The lipoprotein fractions showed significantly ($p<0.05$) elevated HDL and triacylglycerol levels. LDL-Cholesterol were significantly ($p<0.05$) reduced. The HCLF Nigerian diet promotes hypertriglyceridemia, decreased serum proteins and increased AST, ALT and urea levels suggestive of hepatocellular damage. The reduced ALP levels is suggestive of arrested bone growth. The implication of consuming this diet is discussed.

Key words: High-carbohydrate low fat diet (HCLF), serum lipid, serum enzymes, electrolytes

Introduction

The typical Nigerian diet consists of low protein and high carbohydrate and fiber levels. Meat, fish, milk and eggs are expensive and out of the reach of most families. New research has shown the effect of a low protein high carbohydrate diet on some biochemical indices (Jeppesen *et al*., 1997; Darlene *et al*., 1997; Parks and Hellerstein, 2000; Nicholas *et al*., 2004). Lipid profile of humans consuming HCLF diet shows increases in fasting triacylglycerol (Connor and Connor, 1997; Katan *et al*., 1997). HDL-cholesterol, LDL-cholesterol and total cholesterol are also reduced. In humans, hypertriglyceridemia can be induced endogenously by a high fat diet (Austin *et al*., 1998) or it can be carbohydrate induced (Knittle and Ahrens, 1964; Reaven *et al*., 1965). A high triacylglycerol (TAG) flux rate was observed in subjects fed HCLF diet (Abbot, 1990). The TAG increase observed in humans on a HCLF diet may have been from de novo lipogenesis (Parks and Hellerstein, 2000).

Most previous studies examining carbohydrate induced hypertriglyceridemia have used a maximum of 10 days feeding protocols (Nestel *et al*., 1970), or diets with simple sugars (Huff and Nestel, 1982) and liquid diet devoid of fat (Schonfeld, 1970). Such diet may be metabolized differently from whole foods containing high fiber, low-fat diets as consumed normally in Nigeria. This present study was designed to examine the changes in some biochemical indices on rabbits fed a wholly compounded HCLF Nigerian-like diet.

Materials and Methods

Animals and management: 3 months old New Zealand white rabbits (Initial mean weight 1.75Kg) 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 12hr-light and 12hr dark cycle and the temperature was uniform. The animals were acclimatized on growers mash (Bendel feed and flour mills BFFM Limited, Ewu, Nigeria) for two weeks. Prior to the study, food and water was given *ad libitum*.

The rabbits were divided into two groups of six rabbits per group, according to body weight similar after the adaptation period. One group was fed the control diet and the second group was fed the High carbohydrate low fat diet for a total period of eight weeks.

The composition of both diets is shown in Table 1. Fresh feed was provided on daily basis while stale remnants were discarded after weighing. On the average each rabbit received about 150g/feed/day. Clean drinking water was provided *ad lib.* 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 18hr and baseline blood samples were drawn from the rabbit ear veins using 21-gauge syringes. At the end of the feeding period, the rabbits were anesthetized with pentobarbital (60mg/Kg body weight). Insertion was made into the heart region for collection with the use of a needle and syringe. The blood samples were collected into labelled bijou bottles containing heparin as anticoagulant and centrifuged immediately (3,000×g for 10 minutes), to obtain the serum. The serum samples were stored in the biofreezer at-10°C until analyzed. Duplicate serum samples for each animal group were analyzed for Total proteins (Brown, 1976), albumin, globulins, (Baertl, 1974) electrolytes (Na^+,K^+,Hco_3 ,Cl^-), $^-$(Kinsley and Schaffert, 1953), lipid, (Anderson *et al*., 1971), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) (Moss *et al*., 1971) urea and creatinine (Carr, 1959) were measured using commercial kits (Boehringer Mannheim).

Statistical analysis: Data were expressed as mean±Standard error of the mean (SEM) for each group of rabbits. Comparison between the control and experimental set of data was analyzed by the student's t-test and P values<0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical package.

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 analysis showed that there was a significant decrease in weight gain, feed and water intake and feed efficiency in the experimental diet when compared with the control diet.

The values observed for total protein, albumin and globulin are given in Table 3. The values were observed for total proteins (1.28mg/dl), albumin (0.26mg/dl) and globulin (1.75mg/dl). The rabbits on the experimental diet had significantly (p<0.05) lowered values compared to the control diet. The values obtained for AST, ALT, ALP and bilirubin are shown in Table 4. The AST and ALT levels significantly (p<0.05) increased while ALP levels significantly (p<0.05) decreased. The bilirubin level for rabbits on the experimental diet (0.37mg/dl) is within the normal levels and no significant difference was observed between the control and experimental diet.

Table 5 shows the level of electrolytes, creatinine and urea in rabbits. The rabbits fed the HCLF diet had a significant increase (p<0.05) in sodium and chloride ions content when compared with the control (p<0.05). Potassium ions decreased significantly (p<0.05) and bicarbonate ions had no significant (p<0.05) change.

Table 1: Composition of Experimental Diets

	Diet type	
Dietary Component	Control	HC/LFP
Garri	60.0g	85.4g
Fish	15.4g	8.0g
*Mineral and vitamin mix (*OPIMIX PREMIX)	1.0g	1.0g
Palm oil	18.0g	5.0g
Methionine	0.60g	0.60g

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

*OPIMIX PREMIX

Vitamin A	8,000,000IU	Copper	5gm
Vitamin D	1,600,000IU	Iron	20gm
Vitamin E	5,000IU	Iodine	1.2gm
Vitamin K	2,000mg	Selenium	200mg
ThiamineB1	1500mg	Cobalt	200mg
Riboflavin B2	4,000mg	Cholin chloride	200gm
Pyridoxine-B6	1500mg	Anti oxidant	125gm
Niacin	15,000mg	Manganese	80gm
Vitamin B12	10mg	Zinc	50gm
Pathothenic acid	5,000mg	Biotin	20mg
Folic acid	5,000mg		

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

Group	Control diet	Experimental Diet
Weight gain (g/rabbit)	650±8.0[a]	120±6.0[b]
Feed intake (g/rabbit/day)	53.8	50.5
Water intake(ml/rabbit/day)	20.50±2.0[a]	16.80±2.50[b]
Feed efficiency (g/body weight/g feed)	12.08	2.37
Dry fecal output (g/rabbit/day)	5.24±1.14[a]	3.25±1.02[b]

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

Table 3: Mean concentrations of total protein, albumin and globulin of rabbits on the control and experimental diet

Parameters	Control Diet	Experimental Diet
Total Protein	1.28±0.41[a]	0.40±0.17[b]
Albumin	0.26±0.15[a]	0.07±0.02[b]
Globulin	1.75±0.52[a]	1.23±0.23[b]

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 on the control and experimental diet

Parameters	Control Diet	Experimental Diet
AST(IU/dl)	2.90±0.07[a]	8.35±1.65[b]
ALT(IU/dl)	7.65±0.47[a]	10.33±0.62[b]
ALP(IU/dl)	32.5±16.48[a]	5.0±0.21[b]
Bilirubin (mg/dl)	0.28±0.09[a]	0.37±0.05[b]

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

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

Parameters	Control Diet	Experimental Diet
Potassium	0.47±0.11[a]	0.28±0.27[b]
Sodium	17.0±2.55[a]	27.00±4.14[b]
Bicarbonate	3.67±0.58[a]	6.33±0.62[a]
Chloride	12.00±0.58[a]	15.5±2.30[b]
Creatinine	0.46±0.06[a]	0.2±0.07[b]
Urea	8.33±3.69[a]	17.68±3.38[b]

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 on rabbits fed the control and experimental diet

Parameters	Control Diet	Experimental Diet
Total Cholesterol	50.18±7.75[a]	2.80±17.5[b]
HDL-Cholesterol	6.5±0.96[a]	9.34±5.23[b]
LDL-Cholesterol	32.18±7.22[a]	13.50±5.23[b]
Triacylgl;ycerol	10.5±7.59[a]	28.50±4.41[b]

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

The Creatinine values showed a significant ($p<0.05$) decrease while urea levels increased significantly ($p<0.05$) when compared with control diet.

Table 6 shows the values of serum lipids of rabbits fed the control and experimental diet. Serum cholesterol levels were significantly ($p<0.05$) reduced. The lipoprotein fractions were also significantly altered. HDL and TAG levels were significantly elevated ($p<0.05$) when compared with the control. LDL-cholesterol concentrations were also significantly reduced in the experimental groups when compared with the control diet.

Discussion

The rabbits fed the Nigerian like HC/LPF diet showed a severe reduction of body weight. A reduction in weight gain has been reported in a similar study with low protein intake (Hays *et al*., 2004; Barons, 1987).

The total protein, albumin and globulin levels in rabbits fed the experimental diet were significantly lowered. This showed that the rabbits were protein malnourished. Similar low albumin and globulin values have been observed in earlier studies when rabbits were fed protein deficient diet (Mayne, 2001). Low concentration of plasma protein and albumin is a clear indication of a low protein diet.

Rabbits fed the Experimental diet had significantly increased AST and ALT levels. The low protein diet led to adaptative changes in the liver enzymes. These enzymes have been reported to be increased in plasma during hepatocellular injury due to the increased permeability of the cell membrane serum enzymes (Okolie and Osagie, 1999). The increases in plasma AST and ALT may be linked to degenerative changes in the liver and kidney following the administration of a protein deficient diet (Mcintyre and Rosalki, 1994). The decrease concentration of ALP in the rabbits fed the experimental diet show that the levels of fat used in this study did not adversely interfere with the calcification and other metabolic activities as mediated by ALP.

The bilirubin levels had no significant change in comparison to the control. This indicates that the experimental diet did not affect the maintenance of the normal excretory function of the liver.

Creatinine and Urea have been widely used to measure excretory function especially in conditions where there is prerenal alteration of urea metabolism as in low protein diet, (Balis, 1976).

Creatinine levels for rabbits fed the experimental diet decreased significantly. This is an indicator of normal kidney function. The levels of electrolytes in rabbits fed the experimental diet had significantly higher sodium and chloride ions. Low protein diet is associated with edema due to increase in total body water. Sodium ion and chloride ion is distributed principally in the extracellular fluid and so associated with its retention (Etukudo *et al*., 1999).

Increase in sodium retention has been shown to increase the risks of an individual current blood pressure (Kotchen and Kotchen, 1997).

The 86% carbohydrate diet utilized in this study was supplied by Garri (a processed form of cassava). This cassava based carbohydrate source has a high glycemic index (Ebuehi *et al*., 2005). This will stimulate insulin production and result in a higher insulin sensitivity index. Mcneel and Mersmann (2005), reported similar results in rats fed a high carbohydrate diet. Insulin also causes the retention of sodium ions (Volpe, 1993). This will cause the retention of fluid which, causes high blood pressure and congestive heart failure. A raised insulin level may trigger the sympathetic nervous system which will cause arterial spasm or constriction of the arteries which may consequently raise blood pressure (Kaufman *et al*., 1991).

Significantly reduced potassium ion levels in serum were observed in this study. An inverse relationship between increased intracellular sodium ion and potassium ion deficiency results in hypokalaemia (Krishner *et al.*, 1987). Potassium levels are usually maintained in a very narrow range. Any deviation from the normal levels can have devastating effects on the heart and Central Nervous System (CNS). Moderate potassium depletion could lead to muscle fatigue (Kritensen *et al.*, 2006).

The total serum cholesterol and LDL cholesterol were found to be significantly lowered in the rabbits fed the experimental diet while, HDL and TAG increased significantly. Mensink and Katan (1987) and Ullmann *et al*. (1991) have shown similar changes in healthy men and women fed a strictly controlled diet rich in carbohydrate. Katibi *et al.* (2004), also reported similar results in men and women with type II diabetes mellitus.

This carbohydrate induced elevation of TAG could be explained as a reduced clearance of TAG rich lipoprotein and increased production.

There is also a possibility that reduced lipoprotein lipase activity on a low fat diet may limit the conversion of VLDL to LDL.

Mclaughlin *et al*. (2000) have also shown that a high carbohydrate diet markedly increase fatty acid synthesis and denovo lipogenesis. A high carbohydrate diet will cause fat to be deposited, while slowing down the burning of existing fat. This is linked to a greater potential to store fat (Hudgins, 2000).

The typical Nigerian diet consists of high carbohydrate and low protein. This is consumed from early childhood into adulthood. This predisposes the individual to the development of obesity, hypertension, liver dysfunction and diabetes at middle age. These diseases are increasing at an alarming rate in the Nigerian populace. Longevity might be positively influenced by a reduction in the percentage of carbohydrate and increase in protein and fiber content of the diet. This will prevent diseases and enhance good health.

References

Abbot, W.G., 1990. Effect of a high carbohydrate low fat saturated fat diet on apolipoprotein B and triglyceride metabolism in Pima indians. J. Clin. Invest., 86: 642-650.

Anderson, J.T., F. Grande and A. Keys, 1971. Effect on man serum lipid of two proteins with different amino acid composition Am. J. Clin. Nutr., pp: 24524-530.

Austin, M.A., J.E. Holkanson and K.L. Edwards, 1998. Hypertriglyceridemia as a cardiovascular risk factor. Am. J. Cardiol., 81: 7B-12B.

Baertl, J.M., R.P. Placko and G.G. Graham, 1974. Serum proteins and plasma free amino acids in severe malnutrition Am. J. Clin. Nutr., 27: 733-742.

Balis, M.E., 1976. Uric acid metabolism in Man. Adv. Clin. Chem., 18: 213-246.

Barons, D.N., 1987. A short Textbook of chemical pathology Ed., London, Hodder and stoughon, Richard Clay.

Brown, B.A., 1976. Heamatology, London, Butterworth, 3.

Carr, J.J., 1959. Determination of Creatinine with alkaline 3,5-dinitrobenzoate. Anal. Chem., 25: 1859.

Connor, W.E. and S.L. Connor, 1997. Should low-fat, high-carbohydrate diets be recommended for everyone? N. Engl. J. Med., 337: 562-563.

Darlene, M. Dreon, Harriett A. Fernstrom, Paul T. Williams and Ronald M. Krauss, 1997. LDL Subclass Patterns and Lipoprotein Response to a Low-Fat, High-Carbohydrate Diet in Women Arteriosclerosis, Thrombosis and Vascular Biol., 17: 707-714.

Ebuehi, O.A.T., O.O. Babalola and Z.A. Ahmed, 2005. Nutritive and anti-nutritive composition of Cassava (Manihot esculenta) tuber and leaf. Eu. J. Sci. Res., 9: 41-50.

Etukudo, M.H., E.O. Agedana, O.O. Akinyinka and B.O. Osifo, 1999. Plasma electrolytes, total cholesterol, liver enzymes and selected antioxidant status in Protein Energy Malnutrition. Af. J. Med. Sci., 28: 81-85.

Hays, N.P., R.D. Starling, X. Liu, D.H. Sullivan, T.A. Trappe, J.D. Fluckey and W.J. Evans, 2004. Effects of an *Ad Libitum* low fat, high carbohydrate diet on body weight, body composition and fat distribution in older men and women. Arch. Intern Med., 164: 210-217.

Hudgins, L.C., M.K. Hellerstein, C.E. Seidman, R.A. Neese, J.D. Remaroli and J. Hirsch, 2000. Relationship between carbohydrate induced hypertriglyceridemia and fatty aid synthesis in lean and obese subjects. J. Lipid Res., 41: 595-604.

Huff, M.W. and P.J. Nestel, 1982. Metabolism of apolipoproteins CII, CIII1, CIII2 and VLDL-B in human subjects consuming high carbohydrate diets. Metab., 31: 493-498.

Jeppesen, J., P. Schaaf, C.Z. Jones, M.Y. Zhon, Y.D. Chen and G.M. Reaven, 1997. Effects of low fat, high carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am. J. Clin. Nutr., 65: 1027-1033.

Katan, M.B., S.M. Grundy and W.C. Willett, 1997. Beyond low-fat diets. N. Engl. J. Med., 337: 563-566.

Katibi, I.A., A.A. Akande and A.K. Salami, 2004. Lipid abnormalities among type II diabetes mellitus patients: our experience in Ilorin, Nigeria Med. Edu. Rresource Af., 14: 254-255.

Kaufman, L.N., M.M. Peterson and S.M. Smith, 1991. Hypertension and sympathetic hyperactivity induced in rats by high fat and glucose diets. Am. J. Physiol. Endocrinol. Metab., 260: E95-E100.

Kinsley, G.S. and R.R. Schaffert, 1953. Direct micro determination of sodium, potassium and calcium Anal. Chem., 25: 1738.

Knittle, J.L. and E.K.J. Ahrens, 1964. Carbohydrate metabolism in two forms of hyperglyceridemia. J. Clin. Invest., 43: 485-495.

Kotchen, T.A. and J.M. Kotchen, 1997. Dietary sodium and blood pressure: interactions with other nutrients Am. J. Clin. Nutr., 65: 708.

Krishner, G.G., P. Chusid and R.D.L. Hoeldtre, 1987. Mild potassium depletion promoted renal sodium retention. J. Lab. Clin. Med., 109: 724-730.

Kritensen, M., T. Hansen and C. Juel, 2006. Membrane proteins involved in potassium shifts during muscle activity and fatigue. Am. J. Physiol. Regulatory Integrative Comp. Physiol., 290: 766-772.

Mayne, P.D., 2001. Clinical chemistry in Diagnosis and treatment 6th Edition ELST Arnold, London, pp: 280-348.

Mclaughlin, T., C. Abbasi, H. Lamendota and G. Yeni-Komshian, Reaven, 2000. Carbohydrate induced hypertriglyceridemia: An insight into the link between plasma insulin and triglyceride concentration J. Clin. Endocrinol. Metab., 85: 3085-3088.

Mcintyre, N. and S. Rosalki, 1994. Tests of the functions of the liver. Scientific foundations of Biochemistry in Clinical Practice 2nd Edition. Williams D and Marks, V. (Eds). Butterworth-Heinemann Ltd. Oxford, London, pp: 383-398.

Mcneel, R.L. and H.J. Mersmann, 2005. Low and high carbohydrate diets: Body composition differences in rats. Obesity Res., 13: 1651-1600.

Mensink, R.P. and M.B. Katan, 1987. Effect of monosaturated fatty acid versus complex carbohydrate on HDL in healthy men and women. Lancet, 17: 122-125.

Moss, D.W., D.N. Baron, P.G. Walker and J.H. Wilkinson, 1971. Standardization of clinical enzyme assays. J. Clin. Pathol., 24: 740-743.

Nestel, P.J., K.F. Carroll and N. Havenstein, 1970. Plasma triglyceride response to carbohydrates, fats and calorie intake. Metab., 19: 1-18.

Nicholas, P., N.P. Hays, Raymond D. Starling, Xiaolan Liu, M.D., Dennis H. Sullivan, Todd A. Trappe, James D. Fluckey and William J. Evans, 2004. Effects of an *ad libitum* low-fat, high-carbohydrate diet on body weight, body composition and fat distribution in Older men and women. Arch. Intern Med., 164: 210-217.

NRC, 1985. National Research Council Guide for the care and use of laboratory animals. Publication no. 85-23 (rev). National institutes of Health, Bethesda, MD.

Okolie, N.P. and A.U. Osagie, 1999. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure Food and Chem. Toxicol., 37: 745-750.

Parks, E.J. and M.K. Hellerstein, 2000. Carbohydrate induced hypertriglyceridemia: historical perspective and review of biological mechanism I. Am. J. Clin. Nutr., 71: 412-433.

Reaven, G.M., D.B. Hill, R.C. Gross and J.W. Farquhar, 1965. Kinetics of triglyceride turnover of very low density lipoproteins in human plasma. J. Clin. Invest., 44: 1826-1833.

Schonfeld, G., 1970. Changes in the composition of very low density lipoprotein during carbohydrate induction in man. J. Lab. Clin. Med., 75: 206-211.

Ullmann, D., W.E. Connor, L.F. Hatcher, S.L. Connor and D.P. Flavell, 1991. Will a high-carbohydrate, low-fat diet lower plasma lipids and lipoproteins without producing hypertriglyceridemia. Arterioscler Thromb., 11: 1059-1067.

Volpe, M., C. Tritto, N. Deluca, S. Rubatta, M.A. Rao, F. Larrenza, A. Mirante, I. Enea, V. Rendina and A.F. Mele, 1993. Abnormalities of sodium handling and of cardiovascular adaptations during high salt diet in patients with heart failure Circulation, 88: 1620-1627.