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Full Length Research Paper

Alterations in glucose-6-phosphate dehydrogenase and mitochondria oxygen consumption in rats fed with cycads, Nigerian- like and western -like folic acid supplemented diets

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Alterations in mitochondria oxygen consumption and glucose-6-phosphate dehydrogenase was studied in 96 Wister albino rats exposed to cycads and fed on Nigerian like and western like diets supplemented with folic acid. The animals were divided into three diet classes of 32 animals each. First group was fed with a wholly compounded Nigerian like diet (NLD) which was low in protein and high in carbohydrate and fiber. Another group was fed with a western like diet (WLD) which was high in protein and fat while the third group of animals was fed with a normal diet (ND) which served as the control class. The animals of each class were further distributed into four subgroups of eight rats each. In each subclass, first group received the diet alone; second group received the diet and cycads, third group received the diet and folic acid while the fourth group received the diet, cycads and folic acid. Exposing the colon of rats to NLD decreased glucose–6-phosphate dehydrogenase activity and increased mitochondria oxygen consumption. WLD increased glucose -6-phosphate dehydrogenase activity and decreased mitochondria oxygen consumption while folic acid decreased glucose-6-phosphate dehydrogenase activity but increased mitochondria oxygen consumption at the initial stage of colon carcinogenesis. These results suggest that NLD and folic acid may protect rats against colon cancer and the WLD may enhance colon carcinogenesis. Therefore, a nutritional shift from NLD to WLD is likely to cause a higher incidence of colon cancer.

Key words: Cycads, glucose-6-phosphate dehydrogenase, mitochondria oxygen consumption, Nigerian-like diet (NLD), Western-like diet (WLD).

INTRODUCTION

Cycads are gymnosperms that are widely distributed throughout the world. They contain different glycoside including macrozamin and neocycasin but the most common is cycasin. The active components of all the glycosides is methylazoxyglycoside (MAM) which is metabolized to dimethylhydrazine (DMH) a toxic intermediate by the P450 enzymes present in the liver and in the olfactory epithelium in the nose. To release MAM, cycasin must be cleaved. This cleavage is made by the enzyme betaglucosidase which is found in microbes in the gut. For this reason, cycasin must be taken orally in order to be toxic. Cycasin is found in all cycad genera (De luca, 1990) and is found to be carcinogenic in five animal species which are rat,

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hamster, guinea-pig and fish inducing tumor in various organs (De luca, 1990). Cancer is considered to be a group of diseases of multiple causes which occurs when cells become abnormal and divide without control or order (ACS, 1990). Epidemiological studies show that colon cancer is prevalent in Europe and North America (ACS, 1990). High fat and low fiber intakes are major features of western diet whereas high fiber and low fat diets prevail in Africa, Asia and other areas of the world where the risk of colon cancer is low (Burkitt, 1971). In which, they consume a lot of refined food as against a low incidence recorded in areas where unrefined foods are consumed (Burkitt, 1978).

Studies have shown that the Japanese and Chinese have low incidence of cancer (King and Lock, 1980), but when they migrate to the U.S. the incidence of colon cancer in these population rise to the same level as that of U.S. whites (Burkitt, 1984). Colon cancer is termed a nutritionally based cancer and can either be genetic or sporadic. Lack of the adenomatous polyposis coli (APC) gene has also been linked with the disposition of colon cancer. Sporadic cancers are more and account for 80% of all cancer cases and diet has mostly been linked as a causative/contributory agent of colon cancer (Boutron–Ruault, 2002). Colorectal cancer is a leading cause of death in Western world and has remained so for the past 50 years. Therefore, the search for strategies to prevent the development and progression of colorectal cancer has markedly intensified. Chemoprevention is one of such strategies. Accumulating evidence suggests that folic acid, a water soluble vitamin, could be an effective chemopreventive agent for colorectal cancer. Low folic acid and low fiber intake have been implicated in the aetiology of colon cancer.

The findings of experimental and epidemiological survey reveal a relationship between high intake of fiber and folic acid to lower risk of colon cancer (Bingham, 2003). However, there exist controversies as regards the role of folic acid and diet type in colon carcinogenesis. Whilst some studies report a protective effect (Choi and Mason, 2000), others show that it aggravates the problem (Cole and Baron, 2007). Studies that relate diet to colon carcinogenesis have mostly focused on the role of diet components on the promotion/progression stages of the disease. Only few studies have been reported on the role of diet on the initiation stage of colon carcinogenesis. The aim of this study was to assess the role of wholly compounded Nigerian and Western like diets supplemented with folic acid on Glucose-6-phosphate dehydrogenase and mitochondria oxygen consumption at the early stages of colon carcinogenesis.

MATERIALS AND METHODS

Animals

96 Wister albino rats of average body weight of 30 to 35 g purchased from the animal house, College of Medicine, Ambrose Alli University, Ekpoma, were used for this experiment. They were housed in groups. The rats were weighed and grouped into three diet classes with 32 animals each; first group was fed with a wholly compounded ND which served as the control class. Second class of animals was fed wholly on compounded NLD which was low in protein and high in carbohydrate and fibre. The third class of rats was fed with a WLD which was high in protein and fat. Both the NLD and WLD served as test diets. The animals of each diet class were further distributed into four subgroups of eight rats each. In each class, first group received the diet alone, second group received the diet and cycads, third group received the diet and folic acid while the fourth group received the diet, cycads and folic acid. The animals were given food and water *ad libitum* and were fed with folic acid and cycads such that they consumed 0.5 mg/kg body weight. The animals were acclimatized with their respective diets for one week before the commencement of the study which lasted for twelve weeks (Table 1).

Feed preparation

The cycads leaves were obtained from Santua garden, opposite University of Benin, Ugbowo, Benin City, Edo State, Nigeria where it was also identified. They were washed and dried in the oven at 50°C for 2 h and then blended into powder. The soya beans which was obtained from Uselu market, Benin City, Edo State, was cooked for about 5 h, dried in the oven at 60°C for 2 h and blended into powder. The sugar, palm oil and white garri were obtained from Uselu market, Benin City, Edo State, the multivitamin and folic acid were obtained from Edoma pharmacy, Siluko road, Benin City while the sawdust was obtained from Ogbodu sawmill Siluko road, Benin City, Edo State. These other food components which were already in powder or liquid form were mixed in their various proportions. The ND was patterned after previously fed diets by Schuette and Richard (1986) in their study of the effects of diets high in fats and/or fiber on colonic absorption of DMH in rats. The diet rich in carbohydrate and fiber was patterned after that of Anderson and Gustafson (1987) in their study of the hypolipidaemic effect of a high carbohydrate and high fat diet. While the WLD was patterned after that of Eriyamremu and Adamson (1994), in their study of early changes in energy metabolism in rats exposed to an acute level of deoxycholate and fed NLD.

Isolation of the colon

At the end of the study period, the animals were fasted over night, sacrificed under ether anaesthesia and the colon excised. The colon was flushed several times with ice cold normal saline solution until free of debris. The intestine was inverted and the mucosa was removed by scrapping with a glass slide. The tissue and mucosa were kept separately in plane bottles and stored in the freezer for analyses.

Isolation of crude mitochondria

A combined method of Douce et al. (1987) and that of Johnson and Lardy (1985) with slight modification was used for the isolation of the mitochondria. Two grammes of the mucosa and tissue from the colon of each rat were placed separately in the extraction medium. Mitochondria extraction medium was prepared by weighing 85.6 g of sucrose, 1.9 g of ethylenediaminetetraacetate (EDTA), 0.4 g of ethylene glycol tetra acetic acid (EGTA), 0.2 g of dithioerythritol, 0.2 g of bovine serum albumen (BSA) and 1.6 g of Hepes tris. They were all put together in a conical flask. Little amount of distilled water was added to them which was then made up to 100 mls and homogenized. The homogenate was centrifuged at 3000 rpm for 3

Table 1. Diet pattern for each group.

Group	Diet type
Normal diet	
1	Normal diet (ND)
2	Normal diet and cycads (NDC)
3	Normal diet and folic acid (NDF)
4	Normal diet, cycads and folic acid (NDCF)
Nigerian-like diet	
1	Nigerian-like diet (NLD)
2	Nigerian-like diet and cycads (NLDC)
3	Nigerian-like diet and folic acid (NLDF)
4	Nigerian-like diet, cycads and folic acid (NLDCF)
Western-like diet	
1	Western-like diet (WLD)
2	Western-like diet and cycads (WLDC)
3	Western-like diet and folic acid (WLDF)
4	Western-like diet, cycads and folic acid (WLDCF)

min. The supernatant was discarded and the pellets resuspended in the extraction medium. This method was repeated. The pellet suspended in the extraction medium was centrifuged at 8000 rpm for 10 min to pellet nuclear and unbroken cells. The supernatant was collected and centrifuged at 15000 rpm for 10 min. Three layers were observed in Eppendorff microcentrifuge tube. The first layer contained the extraction medium, the second layer contained the mitochondria and third layer contained residual cell debris. The pellets (mitochondria) was washed in the medium, resuspended in 50 mM tris hydrochloric acid (HCl) and stored in the freezer until analysis.

Preparation of sample homogenate supernatant for glucose-6-phosphate dehydrogenase assay

Tissue and mucosa were homogenized separately in 5% Trichloroacetic acid (TCA) for 10 s and centrifuged at 10,000 rpm for 15 min at 4°C or on ice. The supernatant was used for the analysis.

Determination of mitochondria oxygen consumption

Reagents and chemicals used

Protease inhibitor mixture (cocktail), 2% triton x-100, 10 uM ferricyanide, few grains of dithionite and 10 Mm tris buffer pH 7.4. A volume of 0.1ml of sample in 1ml of 10 mM tris buffer pH7.4, was lysed with 2% triton x-100 in the presence of a protease inhibitor mixture. The absorbance of oxidized (by 10 uM ferricyanide) and reduced (by 0.2 g of dithionite) samples were recorded at a wavelength of 550 nm (North et al., 1996; Schagger, 1995).

Determination of glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) was estimated using Randox glucose-6-phosphate dehydrogenase kit manual.

Statistical analysis

The results were expressed as mean±SEM. Duncan's multiple range tests was used to test the significant differences between the means. Analysis of variance was used to test the differences between all the groups (Sokal and Rohlf, 1969).

RESULTS

The study presents data on alterations in glucose-6-phosphate dehydrogenase and mitochondria oxygen consumption in rats fed with cycads, Nigerian- like and Western -like folic acid supplemented diets in early carcinogenesis. From the results obtained NLD fed rats recorded high crude mitochondria oxygen consumption while WLD fed rats recorded low levels of mitochondria oxygen consumption. WLD fed rats recorded high glucose-6-phosphate dehydrogenase as against low levels observed with NLD fed rats.

DISCUSSION

Figure 1 shows mitochondria oxygen consumption in the colon of rats fed with the diet and folic acid. The WLD fed rats had the lowest value for oxygen consumption. This value was significantly ($P<0.05$) lower than the observed value with the NLD and ND fed rats. Inclusion of folic acid to the ND, NLD and WLD significantly ($P<0.05$) increase the mitochondria oxygen consumption of the rats whereas separate inclusion of cycads only significantly reduced ($P<0.05$) the mitochondria oxygen consumption of rats fed with the NLD compared with the one fed with

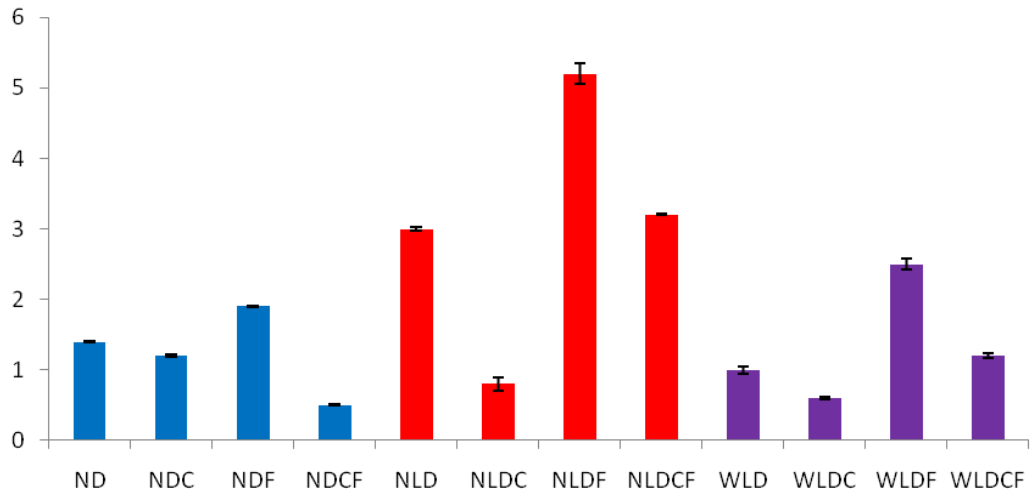

Figure 1. Presents colonic mucosa mitochondria oxygen consumption of rats fed with cycads, Nigerian- like and Western- like folic acid supplemented diets.

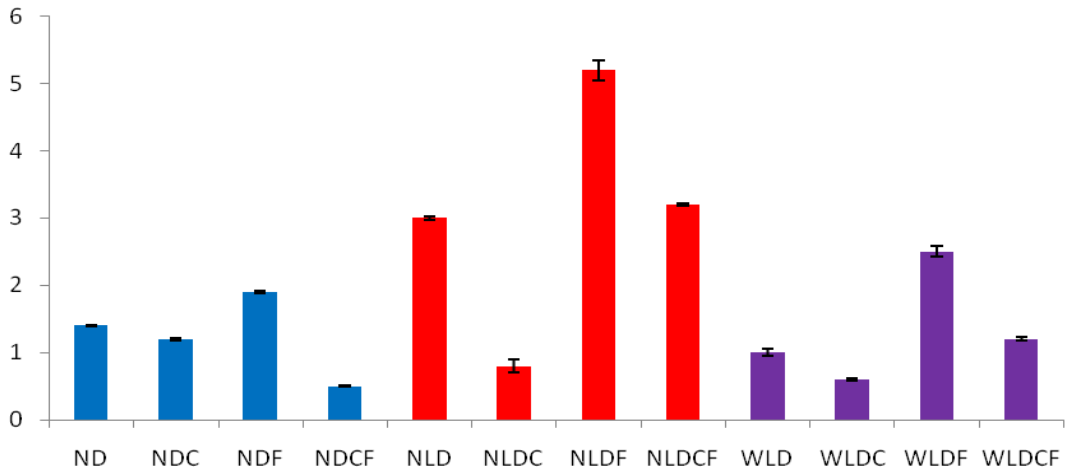

Figure 2. Presents colonic tissue mitochondria oxygen consumption of rats fed with cycads, Nigerian- like and Western- like folic acid supplemented diets.

the ND or WLD. Co-administration of cycads and folic acid to the NLD and WLD that is, (NLDCF and WLDCF) significantly (P<0.05) increases the mitochondria oxygen consumption to values that where observed in the absence of either cycads or folic acid. In figure 2, the trend observed in the colonic mucosa was repeated in the tissue considering that the mitochondria oxygen consumption of rats maintained on an NLD had a significantly (P<0.05) higher consumption of oxygen compared with ND and WLD. Similarly, inclusion of folic acid to ND, NLD and WLD significantly (P<0.05) increase the mitochondria oxygen consumption of the rats where as separate inclusion of cycads only significantly reduce (P<0.05) the mitochondria oxygen consumption of rats fed with NLD compared with the ND or WLD.

It was also observed that co-administration of cycads and folic acid to the NLD or WLD significantly (P<0.05) increase the mitochondria oxygen consumption to values that where observed in the absence of either cycads or folic acid. The primary characteristic of a cancer cell is that it is starved of oxygen and nutrient and so it reverts to non oxygen requiring (anaerobic) form of metabolism (Abrahamse et al., 1999). If there is damage caused by oxidative stress to the mitochondria membrane, it becomes permeable to ions and this in turn affects the oxygen consumption by the crude mitochondria. It would appear that NLD produces more energy and therefore may support proliferative activity. However, energy derivation for the purpose of proliferation is mostly via anaerobic glycolysis (Keith, 1979). So the low oxygen

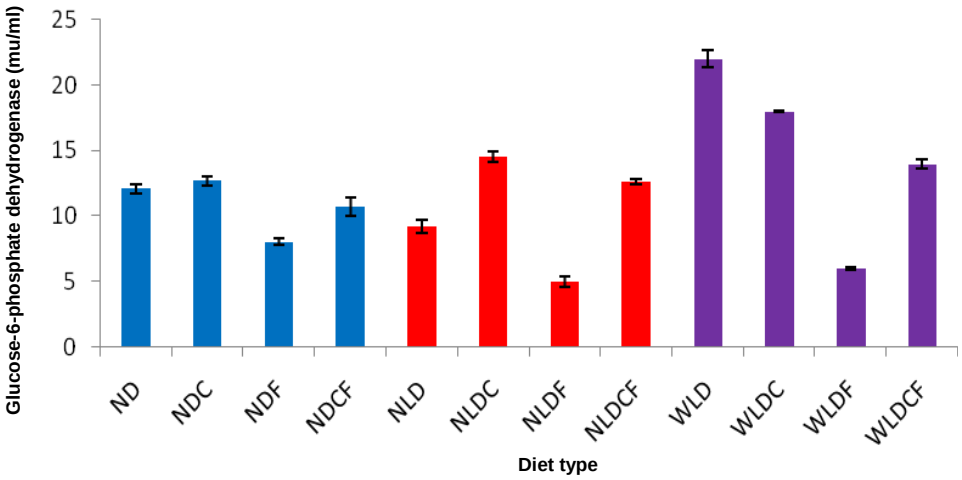

Figure 3. Colonic mucosa glucose-6-phosphate dehydrogenase of rats fed with cycads, Nigerian- like and Western- like folic acid supplemented diets.

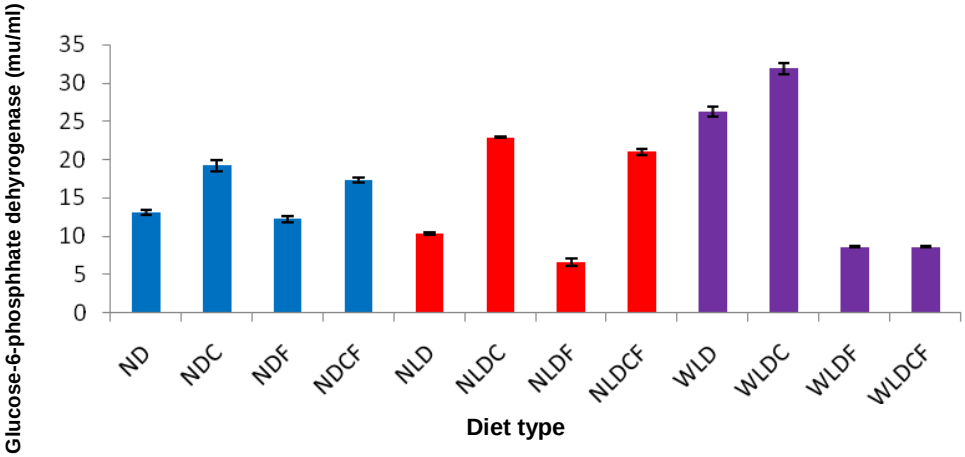

Figure 4. Colonic tissue glucose-6-phosphate dehydrogenase of rats fed with cycads, Nigerian-like and Western- like folic acid supplemented diets.

consumption which may be indicative of increased energy derivation through anaerobic glycolysis may support the agreement that there is high proliferation in the rats fed with WLD. The addition of cycads decreased crude mitochondria oxygen consumption indicating increased energy derivation through anaerobic glcolysis.

Cycads can be transformed into a potent carcinogen in gut (de luca, 1990). Thus, there is high proliferative activity in this group of animals. Addition of folic acid increased mitochondria oxygen consumption indicating low energy derivation through anaerobic glycolysis and thus low proliferative activity. Figure 3 shows colonic mucosa glucose-6-phosphate dehydrogenase level in experimental animals. The activity of the enzyme was significantly raised ($P<0.05$) in the rats fed with WLD compared to those fed with NLD and ND. Addition of cycads to the NLD significantly ($P<0.05$) increase the activity of the enzyme but the opposite trend was recorded when rats were fed with WLD. Inclusion of both cycads and folic acid to the three diet formulation did not significantly ($P>0.05$) alter the activity of the enzyme compared with the ND. This study reveals that the activity of glucose-6-phosphate dehydrogenase is responsive to diet type and cycads affect the activity of the enzyme. Figure 4 shows colonic tissue glucose-6-phosphate dehydrogenase level in experimental animals. The activity of the enzyme was significantly higher ($P<0.05$) in the rats fed WLD compared with those fed NLD or ND. Addition of cycads to the ND, NLD and WLD significantly ($P<0.05$) increased the activity of the enzyme. However, inclusion of only folic acid to the WLD significantly ($P<0.05$) reduced the activity of the enzyme compared

with its inclusion in the ND and NLD. In addition, supplementation of a WLD that contains cycads with folic acid significantly ($P<0.05$) reduced the activity of the enzyme compared with the ND.

Glucose-6-phosphate dehydrogenase is a key enzyme in the pentose phosphate pathway of glucose whose major metabolic role is to provide NADPH and ribose sugar (Vasudevan and Sreekumaris, 2007). Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) is necessary for the maintenance of glutathione. Free radicals are consistently produced in all cells. These will destroy deoxyribonucleic acid (DNA), proteins, fatty acids and all biomolecules and in turn, cells are destroyed. The free radicals are inactivated by enzyme systems containing super oxide dismutase, glutathione peroxidase and glutathione reductase. Reduced glutathione is regenerated with the help of NADPH. When cells are required to proliferate or differentiate, the requirement for D- ribose may be greater than what could be supplied by the synthetic pathway (Freeman et al., 2008). Ribose is a critical building block for nucleotide and its plays important role in energy metabolism, transcription, translation and second messenger systems (Freeman et al., 2008). Results obtained from this study shows that high levels of glucose-6-phosphate dehydrogenase was observed in the rats fed western like diets in both the tissue and mucosa.

This could mean an increased synthesis of ribose sugar and may imply an increased proliferative rate. Increased glucose-6-phosphate dehydrogenase level was observed in the colon of rats fed with NDC, NDCF, NLDC, NLDCF, WLDC and WLDCF. This could mean increased synthesis of ribose sugar and may imply an increased proliferative rate. Addition of folic acid decreased glucose-6-phosphate dehydrogenase level. This could mean a decrease synthesis of ribose sugar and hence a decreased proliferative rate and implies that folic acid may prevent colon cancer. Folic acid may protect rats from cycads induced changes in early carcinogenesis by decreasing glucose-6-phosphate dehydrogenase and improving mitochondria oxygen consumption. This effect of folate is better potentiated with the feeding of the NLD and suggests that folic acid plays a protective role in colon carcinogenesis.

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