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Aspirin Induced Changes in Enzymes of Colonic Energy Metabolism and ATPases of Rats Exposed to Cycas and Fed a Nigerian-Like Diet

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This study is aimed at assessing the role of the interaction between a Nigerian-Like Diet (NLD) or Western-Like Diet (WLD) and aspirin on energy metabolism and ATPases in early colon carcinogenesis. We observed that the WLD significantly ($p < 0.05$) increased intestinal length of rats compared with the NLD and interaction with aspirin slightly reversed this effect. The study also reports that the WLD increased the activities of some enzymes of energy metabolism compared with the NLD and that this increase is more than tripled with the inclusion of cycas in the diet. Aspirin administration reduced the activities of these enzymes more in the rats fed with the WLD than the NLD. We also observed that like the WLD, cycas inclusion in the diet of rats significantly raised the activity of Mg^{2+} ATPase (9% in the NLD and 34% in the WLD) and decreased Ca^{2+} ATPase (30% in the NLD to 42% in the WLD). Aspirin reversed these effects of cycas on these ATPases (37% in the NLD and 40% in the NLD for Ca^{2+} ATPase). This study demonstrates that an increase in Mg^{2+} ATPase and a decrease in Ca^{2+} ATPase is associated with early colon carcinogenesis and that while the WLD promotes these enzyme changes, the NLD have the opposite effect. The study also reveal that aspirin effect on the enzymes of energy metabolism and ATPases supports its protective role against colon carcinogenesis and that the effect of the drug is dependent on the protein content of the diet.

Key words: Colon carcinogenesis, Nigerian diet, aspirin, energy metabolism, ATPases

INTRODUCTION

In North America, Oceania and Western Europe, colon cancer is common (Greelee *et al.*, 2001) and it is believed that the etiology of the disease is of both genetic and dietary origin. Colon cancer which originates from environmental causes (Sporadic colon cancer) represents about 95% of all cases of the disease and is mostly associated with dietary risk factors (Boutron-Ruault, 2002). A variety of studies support the earlier finding of Burkitt (1984) that the regular intake of carbohydrate, particularly of resistant starch and fibre, reduces the incidence of colon cancer (Cassidy and Cummings, 1994), while populations on a high fat and high protein diet such as those in Western diets are at a high risk of developing colon cancer (Hursting *et al.*, 1990). However, epidemiologic evidence supporting the fiber hypothesis has been rather controversial. A study of colon cancer rates in South Africa concluded that the low prevalence of colon cancer in black Africans cannot be explained by dietary protective factors, such as fiber, calcium, vitamins A, C and folic acid, but may be influenced by the absence of aggressive factors, such as excess animal protein and fat and by differences in colonic bacterial fermentation (O'Keefe *et al.*, 1999). On the other hand, a recent paper reported that the risk of colorectal adenoma (the precursor of colorectal cancer) decreased by 41% for every additional 5% unit of fiber intake/day (Mathew *et al.*, 2004). Red meat fat increased the risk by 20% and white meat fat decreased the risk by 67% for every additional 5% unit of respective intake/day. Experimental studies on the role of whole diet consumed by populations in colon carcinogenesis also agrees with some of these population studies though most have been carried out using semi-purified or purified diets.

Several recent reviews (Taketo, 1998a; Taketo, 1998b; Janne and Mayer, 2000), have also provided evidence that Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) have promise as anticancer drugs as they reduced incidence of and mortality from colon cancer. NSAIDs have been shown experimentally to stimulate apoptosis and to inhibit angiogenesis, two mechanisms that help to suppress malignant transformation and tumor growth. Numerous experimental and epidemiologic (nonrandomized) studies (Thun *et al.*, 1993; Smalley *et al.*, 1999; Giovannucci *et al.*, 1994; Qiao *et al.*, 1996; Reddy *et al.*, 1993) have found that long-term users of aspirin or other NSAIDs have a lower risk of colorectal adenomatous polyps and colorectal cancer than nonusers, although some other studies show that NSAID have a deleterious effect (Lee *et al.*, 1997; Rederick *et al.*, 1993).

Little attention has been given to the interaction of drugs with certain foods particularly when such drugs have to be administered over a long period. Studies showing the beneficial effects of aspirin in colon carcinogenesis recommend long term intake of the drug thus making drug nutrient interaction important. Factors that can increase the potential for interactions include long-term drug administration, poor dietary intake, preexisting disease states (especially gastrointestinal disease). Nutritional status and diet can affect the action of drugs by altering absorption, distribution, metabolism and excretion of drugs and thus influence drug response (Hathcock, 1985). In this context the aim of the present study was to further document the role of aspirin and diet interactions on aspects which relates to early carcinogenesis, with special attention placed on energy metabolism and ATPases.

MATERIALS AND METHODS

Experimental design: Seventy two male albino rats (Wistar Strain) with an average weight of 100 ± 2 g were housed individually in stainless steel cages with wire mesh floor to prevent coprophagia. The rats were grouped into three diet classes with twenty-four animals each such that the weight difference between the groups was less than 0.2 g. One group was fed with a normal wholly compounded diet (ND) and served as the diet control class; another class of animals was fed a wholly compounded Nigerian-Like Diet (NLD), which was low in protein and high in carbohydrate and fibre. The third class of rats was fed with a Western-Like Diet (WLD) which was high in protein and fat. Both the NLD and the WLD served as test diets (Table 1). The animals of each diet class were further distributed into three subgroups of eight rats each such that the mean weight difference between all the classes was less than 0.2 g. In each class one group received the diet alone, another group received the diet and cycas and the third received the diet containing cycas and were administered with 60 mg aspirin kg^{-1} body weight. All these animal treatment were carried out in accordance with the principles of laboratory animal care of the NIN guide for Laboratory Animal Welfare as contained in the NIN guide for grants and contracts, vol. 14, No. 3, 1985.

The animals were given the food and water ad libitum. They were acclimatized with their respective diet for one week before the commencement of the study, which was for sixteen weeks. During this period, food intake and dry faecal output were measured daily, while weight gain was recorded weekly. At the end of the study period, the

Table 1: Composition of the study diets

Dietary components	Diet type					
	ND (%)	ND+C (%)	NLD (%)	NLD+C (%)	WLD (%)	WLD+C (%)
Garri ¹	51.7	51.7	59.0	59.0	30.0	30.0
Soya bean (Defatted) ¹	20.0	20.0	15.0	15.0	24.0	24.0
Palm oil ¹	5.0	5.0	3.0	3.0	25.0	25.0
Cellulose ²	5.0	5.0	15.0	11.0	6.0	6.0
Sucrose ²	13.0	8.0	2.7	1.7	9.7	4.7
Salt mix ³	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix ⁴	2.0	2.0	2.0	2.0	2.0	2.0
Methionine ²	0.3	0.3	0.3	0.3	0.3	0.3
Cycas	--	5.0	--	5.0	--	5.0

1: From a local market in Benin City; 2: Product from Merck, Darmstadt, Germany; 3: Product from Tuco Products Company, Ontario, Canada; 4: AIN-93-VX vitamin mix recommended for use with the AIN-93G and AIN-93M diet formulations; Garri is a cassava (*Manihot esculentum*) based starch

animals were fasted for 18 h and sacrificed after they were anaesthetized by intraperitoneal injection of 5 mL kg⁻¹ body weight of 25% urethane saline solution.

Blood collection and treatment: While under anesthesia blood was collected from each rat via heart puncture and transferred into fluoride tubes, where they were allowed to coagulate. The serum from each blood sample was recovered by centrifugation at 3,000 g. Serum glucose, total cholesterol, total lipid and total phospholipids levels were estimated using appropriate kits from Randox laboratories (England).

Tissue treatment: The colon (first 10 cm of the proximal end of the large intestine) was recovered from each rat, flushed several times with ice-cold normal saline solution until free of debris. The intestine was inverted and the mucosa was removed by scraping with a glass slide. The colonic tissue was homogenized, centrifuged at 4,000 g for 30 min and both the supernatant and residue were frozen until needed for assay which generally was within 24 h.

Enzyme and protein assay: Hexokinase, phosphofructokinase, lactate dehydrogenase and glucose 6-phosphate dehydrogenase, activities were estimated by measuring the oxidation or reduction of NAD(P)(H) in straight or coupled reactions (Storey and Bailey, 1978; Bergmeyer, 1974).

ATPase action was estimated by the method of (Bonting, 1970) as modified by Takeo *et al.* (1980) and the inorganic phosphate released by the action of the enzyme was determined (Fiske and Subarrow, 1925). Total protein was estimated by the method of Lowry *et al.* (1951).

Statistical analysis: The results are expressed as Mean±SEM. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was used to test for significant differences between the means (Sokal and Rohlf, 1969).

RESULTS

Statistical evaluation of the data did not reveal any significant ($p>0.05$) difference in the weight gain of rats fed the ND and NLD, but in those fed with the WLD, there was a significant ($p<0.05$) increase in the weight gained. While the WLD fed to rats significantly decreased food intake and dry faecal output, the NLD significantly increased food intake and dry faecal output compared with the control ND fed rats. The inclusion of cycas to the various diets or the administration of aspirin did not significantly alter weight gain, food intake and dry faecal output data of the rats. Feeding rats with the WLD significantly ($p<0.05$) increased their intestinal length compared with the NLD and control ND. The administration of aspirin only slightly reduced intestinal length of the animals. This study indicates that weight, food intake, faecal output and intestinal length of rats are responsive to the type diet fed to the animals (Table 2).

Exposure of rats to cycas significantly increased ($p<0.05$) fasting serum glucose in the all the diet classes. In contrast, aspirin reduced the level of glucose in the serum to levels comparable to those of the diet controls. The ND and the NLD fed rats had statistically similar levels ($p>0.05$) of glucose however these were significantly lower ($p<0.05$) than that observed in the WLD fed rats. The serum total cholesterol, total phospholipid and total lipid levels were significantly raised ($p<0.05$) by the WLD compared with the ND and NLD. Aspirin restored the levels of these lipids in the serum to levels comparable to those observed in the rats fed with the diet controls. Feeding of cycas significantly ($p<0.05$) increased serum total cholesterol in all the three diet classes. This study shows that WLD generally increased the serum glucose and lipids in rats (Table 3).

Feeding rats with the WLD significantly raised the activities of hexokinase, phosphofructokinase, lactate dehydrogenase and glucose 6-phosphate dehydrogenase compared with the ND and NLD (Table 4). When the activities of these enzymes in the NLD fed rats were

Table 2: Weight gain, food intake, dry faecal output and intestinal length of rats exposed to cycas and aspirin

Parameters	Groups								
	ND	ND-C	ND-C-A	NLD	NLD-C	NLD-C-A	WLD	WLD-C	WLD-C-A
Weight gain	1.5±0.2 ^{ac}	1.4±0.1 ^a	1.3±0.1 ^a	1.3±0.1 ^a	1.2±0.2 ^a	1.2±0.1 ^a	1.9±0.2 ^b	1.8±0.2 ^b	1.7±0.2 ^b
Food intake	13.5±1.2 ^a	13.7±1.2 ^a	13.3±1.3 ^a	16.7±1.6 ^b	16.2±1.4 ^b	16.7±1.4 ^b	10.7±0.8 ^c	10.8±0.9 ^c	10.9±1.0 ^c
Dry faecal output	1.4±0.2 ^a	1.4±0.2 ^a	1.3±0.2 ^a	1.8±0.1 ^b	1.9±0.2 ^b	1.8±0.2 ^b	0.8±0.1 ^c	0.9±0.2 ^c	0.9±0.2 ^c
Intestinal length	103.5±6.0 ^a	114.5±7.1 ^{abc}	111.8±7.9 ^a	98.7±5.4 ^a	100.7±6.8 ^a	98.6±6.1 ^a	119.6±9.2 ^{bc}	124.8±9.1 ^c	122.7±9.5 ^c

Values are Means±SEM n = 8. Means of the same row followed by different letter(s) differ significantly (p<0.05); Weight gain, food intake and dry faecal output values are in g day⁻¹ rat⁻¹. Intestinal length is in cm. ND = Normal diet, ND-C = Normal diet + Cycas, ND-C-A = Normal diet + Cycas + Aspirin, NLD = Nigerian-like diet, NLD-C = Nigerian-like diet + Cycas, NLD-C-A = Nigerian-like diet + Cycas + Aspirin, WLD = Western-like diet, WLD-C = Western-like diet + Cycas, WLD-C-A = Western-like diet + Cycas + Aspirin

Table 3: Serum glucose and lipid profile of rats exposed to cycas and aspirin

Parameters	Groups								
	ND	ND-C	ND-C-A	NLD	NLD-C	NLD-C-A	WLD	WLD-C	WLD-C-A
Fasting serum sugar	82.5±2.2 ^a	91.4±2.3 ^b	80.3±3.1 ^a	80.2±2.7 ^a	90.5±2.6 ^b	81.2±2.1 ^a	88.9±2.7 ^b	100.1±3.1 ^c	91.7±2.5 ^b
Serum total cholesterol	113.5±4.5 ^{ac}	126.7±5.6 ^{bd}	110.3±5.3 ^a	105.4±4.6 ^a	119.2±5.7 ^{bc}	104.6±5.8 ^a	130.2±6.4 ^{cd}	158.8±7.3 ^e	141.9±6.4 ^d
Serum total lipid	221.4±6.2 ^a	239.4±7.1 ^{bd}	229.3±7.3 ^{ab}	201.8±6.1 ^c	234.9±6.2 ^b	210.8±7.2 ^c	250.4±8.1 ^{cd}	295.9±9.2 ^e	265.9±8.5 ^d
Serum total phospholipid	90.5±4.0 ^{ab}	96.5±4.1 ^a	91.8±4.9 ^{ab}	88.7±3.4 ^b	93.7±3.8 ^b	87.6±3.1 ^b	104.6±3.2 ^{cd}	112.8±3.1 ^c	101.7±3.5 ^d

Values are Means±SEM n = 8. Means of the same row followed by different letter(s) differ significantly (p<0.05); Serum sugar, total cholesterol and total lipid levels are in mg dL⁻¹ serum. Total phospholipid values are in µmole inorganic phosphate; ND = Normal diet, ND-C = Normal diet + Cycas, ND-C-A = Normal diet + Cycas + Aspirin, NLD = Nigerian-like diet, NLD-C = Nigerian-like diet + Cycas, NLD-C-A = Nigerian-like diet + Cycas + Aspirin, WLD = Western-like diet, WLD-C = Western-like diet + Cycas, WLD-C-A = Western-like diet + Cycas + Aspirin

Table 4: Enzymes of colonic energy metabolism of rats exposed to cycas and aspirin

Parameters	Groups								
	ND	ND-C	ND-C-A	NLD	NLD-C	NLD-C-A	WLD	WLD-C	WLD-C-A
Hexokinase	3.1±0.3 ^a	7.4±0.5 ^b	5.8±0.5 ^c	3.2±0.4 ^a	5.5±0.4 ^c	4.7±0.6 ^c	5.1±0.4 ^c	15.3±1.1 ^d	10.1±0.9 ^c
Change (%)	---	+138.7	+87.1	---	+71.9	+46.9	---	+200.0	+49.5
Phosphofructokinase	4.5±0.5 ^a	20.7±1.6 ^b	12.0±1.3 ^c	4.0±0.6 ^a	15.8±1.2 ^d	12.1±1.4 ^c	8.7±0.9 ^d	49.8±2.9 ^e	22.6±3.1 ^b
Change (%)	---	+360.0	+166.7	---	+295.0	+202.5	---	+460.9	+159.8
Lactate dehydrogenase	6.4±0.8 ^a	12.4±1.1 ^b	9.3±1.0 ^c	5.8±1.0 ^a	10.0±1.0 ^c	8.2±0.9 ^c	7.8±1.1 ^a	38.9±2.2 ^d	25.5±3.0 ^c
Change (%)	---	+93.8	+45.3	---	+72.4	+41.4	---	+398.7	+226.9
Glucose 6-phosphate dehydrogenase	12.2±0.9 ^a	33.8±3.6 ^b	20.0±2.0 ^c	15.1±1.2 ^a	25.2±1.5 ^d	18.9±1.4 ^c	24.6±2.9 ^d	77.8±4.1 ^e	46.0±4.0 ^c
Change (%)	---	+177.0	+63.9	---	+66.9	+25.2	---	+216.3	+87.0

Values are Means±SEM n = 8. Means of the same row followed by different letter(s) differ significantly (p<0.05); The unit of enzyme activity is µmole of NAD(P) utilized min⁻¹ mg⁻¹ protein. The hexokinase and phosphofructokinase values are multiplied by 10. ND = Normal diet, ND-C = Normal diet + Cycas, ND-C-A = Normal diet + Cycas + Aspirin, NLD = Nigerian-like diet, NLD-C = Nigerian-like diet + Cycas, NLD-C-A = Nigerian-like diet + Cycas + Aspirin, WLD = Western-like diet, WLD-C = Western-like diet + Cycas, WLD-C-A = Western-like diet + Cycas + Aspirin. % Change is in relation to the diet control

compared with those of the control ND, no statistically significant (p>0.05) change was observed. In the all the diet classes, cycas inclusion in the diet resulted in significant (p<0.05) increases in the enzymes of energy metabolism assayed compared with their diet controls. The least increases in these enzymes were recorded in the NLD fed rats and the most in the WLD fed ones. The increases also varied from enzyme to enzyme. Hexokinase increased with about 71% in the NLD fed rats, but in the WLD class it was about 380% from the diet control. The increases observed in phosphofructokinase activity were much more than those observed in the other enzymes. It reached about 360, 295 and 460% in the ND, NLD and WLD fed rats, respectively. The administration of aspirin significantly (p<0.05) reversed the activities of the enzymes of energy metabolism though the degree of reversal varied. The degree of reversal was much more in the WLD fed animals than the ND before the NLD fed

ones thus indicating the importance of protein and/or fat content in the diet in aspirin effect. The observed results demonstrate that some enzymes of energy metabolism are altered by diets and aspirin in ways which can affect colonic cell metabolism.

The effect of the inclusion of cycas in the rat diets on the activity of Na⁺/K⁺ ATPase did not produce any clear cut effect. In the ND, it significantly (p<0.05) reduced Na⁺/K⁺ ATPase but increased the enzyme action in the NLD and WLD fed animals, though the increases failed to reach a statistically significant level (p>0.05). Administration of aspirin did not significantly alter the Na⁺/K⁺ ATPase activity in all the diet classes. Mg²⁺ ATPase and Ca²⁺ ATPase activities produced a clearer pattern as the inclusion of cycas significantly (p<0.05) raised Mg²⁺ ATPase in all the diet groups, it significantly reduced Ca²⁺ ATPase. The percentage increase in Mg²⁺ ATPase varied from 30 to 9 and 34% in the ND, NLD and

Table 5: Colonic ATPases of rats exposed to cycas and aspirin

Parameters	Groups								
	ND	ND-C	ND-C-A	NLD	NLD-C	NLD-C-A	WLD	WLD-C	WLD-C-A
Na ⁺ /K ⁺ ATPase	10.3±0.9 ^a	6.1±1.6 ^b	8.0±1.9 ^{ab}	14.2±2.0 ^c	16.0±2.2 ^c	13.7±2.1 ^c	6.1±1.5 ^b	7.3±1.8 ^b	6.4±1.7 ^b
Change (%)	---	-40.8	-22.3	---	+12.7	-3.5	---	+19.7	+4.9
Mg ²⁺ ATPase	9.0±0.7 ^a	11.7±1.6 ^{bd}	10.0±1.6 ^{ab}	16.2±2.1 ^{ce}	17.8±2.1 ^{ce}	15.9±2.6 ^c	14.7±2.1 ^{cd}	19.8±2.6 ^e	14.6±1.9 ^{cd}
Change (%)	---	+30.0	+11.1	---	+9.9	+1.9	---	+34.7	-0.07
Ca ²⁺ ATPase	10.4±1.3 ^{ad}	7.4±1.3 ^b	8.7±1.2 ^{ab}	18.8±2.5 ^e	13.1±2.8 ^{bd}	15.2±2.6 ^{ce}	7.3±1.0 ^b	4.2±0.8 ^f	5.5±1.9 ^{ef}
Change (%)	---	-28.8	-16.3	---	-30.3	-19.1	---	-42.5	-24.7

Values are Means±SEM n = 8. Means of the same row followed by different letter(s) differ significantly (p<0.05); ATPase activity is in μmole inorganic phosphate released h⁻¹ mg⁻¹ protein. ND = Normal diet, ND-C = Normal diet + Cycas, ND-C-A = Normal diet + Cycas + Aspirin, NLD = Nigerian-like diet, NLD-C = Nigerian-like diet + Cycas, NLD-C-A = Nigerian-like diet + Cycas + Aspirin, WLD = Western-like diet, WLD-C = Western-like diet + Cycas, WLD-C-A = Western-like diet + Cycas + Aspirin. % Change is in relation to the diet control

WLD fed rats, respectively. The decrease in Ca²⁺ ATPase varied from 28% in the ND to 30% in the NLD and 42% in the WLD fed rats. Aspirin administration caused both Mg²⁺ ATPase and Ca²⁺ ATPase activities to reverse to levels obtained in the diet controls, though the reversal were not total (Table 5). In Ca²⁺ ATPases the reversal varied from about 43 to 37 to 40% in the ND, NLD and WLD fed rats, respectively. This study reveals that ATPases are responsive to diet type and that changes in Mg²⁺ and Ca²⁺ ATPases occur with cycas treatment alone and with cycas and aspirin treatment of rats.

DISCUSSION

Most of the experimental studies on the role of diet/dietary components on colon carcinogenesis investigate the progression stage of the disease and few concentrate on the early events that lead to the development of tumor. Cycasin which is modified by the colonic microflora to Dimethylhydrazine (DMH) (a potent carcinogen) is present in cycas. As the leaves of cycad plant contain less cycasin than the nuts and with the duration of the feeding protocol, the present study will therefore relate to early events in the initiation stage of colon carcinogenesis. Also nutrient type/intake can affect drug action in the long term, thus this study assessed the interaction between aspirin and NLD or WLD on some energy metabolizing enzymes and ATPases in early colonic carcinogenesis.

The effect of the NLD, which is high in carbohydrate and fibre, on weight gain and dry faecal output observed in this study (Table 2) is consistent with those earlier observed in our laboratory (Eriyamremu *et al.*, 1995; Eriyamremu and Adamson, 1994). Potential mechanisms by which dietary fibre can protect against the development of colorectal cancer include increases in stool bulk, dilution or binding of potential carcinogens and decrease in transit time (Burkitt, 1984). Since the NLD increased faecal bulk, it will not only dilute out potential carcinogens/co-carcinogens, it will also increase the transit time of the stool thus may protect the rats from

colorectal carcinogenesis. In comparison to the NLD, the WLD which had opposite effect on dry faecal output (Table 2) will predispose rats to colorectal carcinogenesis. The effect of stool bulk on transit time becomes all the more important as this study also reveal that the WLD increased the intestinal length of the rats. There will not only be more contact between the colonic contents and the colonic mucosa in these rats but also more pressure will be exerted on the colonic wall, a process that have been shown to improve proliferation (Walsh *et al.*, 2004). Aspirin decreased intestinal length which will not only reduce transit time but will also reduce the pressure exerted by the diet on the colon and may be an important adaptive feature by which this NSAID decreases tumourigenesis. From the calculated metabolizable energy, the WLD contains more energy than the NLD and so animals consuming the WLD do not need to consume large quantities of the diet to meet their energy demand and this would have accounted for the low food intake observed in the animals on this diet (Table 2).

The observed effect of the feeding of the NLD to rats on fasting serum glucose level compared with the WLD fed rats is not surprising (Table 3). Though the NLD is rich in starch and is expected to increase blood glucose level, but it can also pass into the colon largely undigested when one considers that it decreased intestinal length (Table 2) and thus may decrease digestion and absorption. Earlier studies have shown that diets rich in fibre and resistant starch are fermented in the colon into Short Chain Fatty Acids (SCFA) (Cummings and MacFarlane, 1991) which are rapidly absorbed (Wolever *et al.*, 1989) and they affect different metabolic processes. Propionate inhibits gluconeogenesis and cholesterol synthesis and stimulates glycolysis. An earlier study had shown that the NLD produced more propionate and butyrate than the WLD (Eriyamremu and Adamson, 1995). Reduced production of propionate would in part account for the high glucose level observed in the WLD fed rats. The effect of propionate on cholesterol and triglyceride synthesis may also help explain the observed effects of the diets on serum cholesterol and triglyceride

levels. Earlier studies have shown that there is an inverse relationship between serum cholesterol and cancer (Sherwin *et al.*, 1987; Henriksson *et al.*, 1989), therefore the observed effect of cycas on serum cholesterol in all the diet classes (Table 3) is not surprising.

The WLD increased the activities of enzymes of glycolysis and of a key enzyme in the pentose phosphate pathway (Table 4) which would be needed to provide energy as well as ribose sugars to support the proliferation. These observations imply an improved utilization of glucose. Earlier studies have shown that an increase in glucose uptake is associated with cell transformation from a normal to a neoplastic state (Shapot, 1980) and that changes occur in some enzymes of energy metabolism in the progression stages of cancer to support the proliferation process (Ruggieri *et al.*, 1987). The improved chance of transformation in the colon of rats fed the WLD from a normal to a neoplastic one is heightened because the WLD contains high level of fat and will thus increase enterohepatic circulation of bile salts which increase the chance of loss of these bile salts to the colon where they increase cell proliferation and promote carcinogenesis (Bird *et al.*, 1985). On the other hand, the NLD which contains low level of fat will decrease losses of bile salts to the colon and decrease colonic cell proliferation and would account for the low levels of the enzymes of energy metabolism assayed in these rats compared with those fed with the WLD. As cycas is modified in the colon into carcinogens, the high activity of the enzymes of energy metabolism observed in the cycas fed animals would be the result of excessive proliferation in these animals. Recently, glycolytic bioenergetic changes have been suggested as a useful marker for colon and other carcinomas (Cuezva *et al.*, 2002; Isidoro *et al.*, 2004), therefore implying that the WLD may indeed support carcinogenesis as it increased the activities of glycolytic enzymes. Glucose supplies only a part of the energy utilized by the colon and butyrate nourishes the colon and may be used preferentially to glucose in colonic energy metabolism (Fleming and Floch, 1986), bypassing the glycolytic sequence. As the NLD had earlier been shown to produce more butyrate than the WLD, it may also in part contribute to the low levels of enzymes of glycolysis observed in the NLD fed rats compared with those observed in the WLD fed animals. Also, butyrate has been shown to induce apoptosis (Hague *et al.*, 1993) and may in part also contribute to the low percentage increase in enzymes of glucose metabolism in the NLD and cycas fed rats compared with the WLD and cycas fed ones (Table 4).

The mechanisms underlying the chemopreventive effects of aspirin are less well understood and are a matter

of ongoing debate. One potential mechanism of aspirin action involves inhibition of cyclo-oxygenase activity, which limits tumorigenesis by reducing the production of mutagens that result from arachidonic acid metabolism (Marnett, 1994). Protection might occur through several other pathways, including cell cycle arrest and induction of apoptosis (Hanif *et al.*, 1996; Stark *et al.*, 2001). These may explain the reduction of the enzymes of energy metabolism to levels comparable to those observed in the diet controls (Table 4). Data on the influence of aspirin on enzymes of energy metabolism are lacking and data comparison is difficult and also the data provided in this study are far from been conclusive, but suffice to say that aspirin do exert indirect effects which may have arisen from its effect on cell proliferation.

This study reveals that the WLD increases Mg^{2+} ATPase and decreases Ca^{2+} ATPases activities, while the NLD had the opposite effect and that cycas further extends these effects of the WLD (Table 5). An earlier study had shown that ATPases activities change with diet types (Adamson and Mbajiorgu, 1985). ATPases are membrane bound enzymes that transport ions from one compartment to another and from extracellular fluid into the cell. As the source of the ATPases assayed in the present study is whole tissue homogenate, the study does not provide the activity in different organelles and therefore limits its findings. Dietary fibre can bind cations and make them unavailable for absorption and could trigger a compensatory rise in ATPases to maximize absorption (Adamson and Mbajiorgu, 1985). This would account for the improved activities of ATPases in the NLD fed rats compared with the ND and the WLD fed ones (Table 5).

The increase in Mg^{2+} ATPase observed in the cycas fed rats (Table 5) may be related to improved energy production in the colon of these rats as Mg^{2+} ATPase is also closely associated with mitochondria and ATP formation. It has been observed that in carcinogenesis, there is de-regulation of cell-type-specific programmes that control mitochondria biogenesis and function in different mammalian tissues (Scarpulla, 2002). So if indeed the increase in Mg^{2+} ATPase also reflects changes in the mitochondria, then in the cycas fed animals there would be increase in energy production to meet the increased proliferation. This reflects the relevance of both the glycolytic sequence and mitochondria oxidative phosphorylation in colon carcinogenesis. As aspirin lowers Mg^{2+} ATPase it may improve the regulation of mitochondria function and lower ATP formation. Also if the aspirin decrease cell proliferation, there would be a corresponding drop in energy requirement and a decrease in Mg^{2+} ATPase activity.

Ca²⁺ ATPase activity was reduced in the cycas fed rats in all the diet groups compared with the diet controls (Table 5). This infers that a reduction in this ATPase action is an early event in improved colon proliferation and carcinogenesis. Ca²⁺ ions are known to mediate diverse array of physiological processes including gene expression and regulation (Murphy *et al.*, 1988; Hardingham *et al.*, 1997). Its subcellular concentrations is essential in these processes, but in this study however, the subcellular levels of Ca²⁺ ion or the ATPase responsible for its transport were not measured. Even with these drawbacks, the study provides information on the effect of cycas, aspirin and diet on the cellular level of activity of the ATPase. The influence of diet becomes more evident when the percentage reduction in the enzyme activity is considered. Whilst the ND caused about 28% reduction, the NLD caused about 30.3% reduction and the WLD about 42% reduction in the activity of Ca²⁺ ATPase. Thus if the reduction of the enzyme action is related to colonic cell proliferation, then the WLD may promote colonic tissue transformation to a cancerous state. It is also pertinent to note that the high percentage reduction in Ca²⁺ ATPase activity observed in the NLD fed animals compared with the controls may in part be as a result of improved binding of ions by fibre which may then affect the ATPase action (Adamson and Mbajjorgu, 1985). It was observed that the improvement of Ca²⁺ ATPase by aspirin administration (Table 5) was also diet related. Reduced Ca²⁺ ATPase action improved from 42 to 24% in the WLD and from 30 to 19% in the NLD fed rats representing about 43 and 36% improvement in the WLD and NLD, respectively. This emphasizes the importance protein on aspirin effect on ATPases action in colon carcinogenesis.

The effect of aspirin on the degrees of restoration of the activities of enzymes of energy metabolism (Table 4) and ATPases (Table 5) also seems to vary with protein content of the diets. The percentage restoration increase from the NLD to the ND to the WLD thus suggesting that aspirin/protein interaction is important for its action. High protein intake, particularly of animal sources has in itself been associated with colon carcinogenesis (O'Keefe *et al.*, 1999; Mathew *et al.*, 2004). Despite these findings, a fine balance as regards the amount and type of protein consumed is thus important. Generally, fish as a source of protein have been shown to be of more benefits in colon carcinogenesis than diary sources. In the Southern part of Nigeria, the major source of protein is fish while diary products constitute the major source of protein for the population of Northern Nigeria. Thus on balance a typical meal in Southern Nigeria with improved fish protein would be more beneficial than the Northern Nigerian diet in

relation to aspirin and colon carcinogenesis. Nutrient/aspirin interaction in colon carcinogenesis needs to be further investigated particularly as a lack of histopathological investigations in this study limits its claims.

In summary, the NLD and WLD affect colon carcinogenesis by inducing changes in the intestinal length and affecting the activities of enzymes of energy metabolism and Mg²⁺ and Ca²⁺ ATPases. The study suggests that an increase in Mg²⁺ ATPase and a decrease in Ca²⁺ ATPase are associated with early carcinogenesis and that these events are reversed by aspirin in a manner dependent on the protein content of the diet.

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