Early enzyme changes caused by a dietary fiber (dikanut) in the kidney of streptozotocin-induced diabetic rats

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A study was conducted on the metabolism of streptozotocin-induced diabetic rats fed a supplement of a soluble fiber (dikanut) for 4 weeks.

The activities of key enzymes of carbohydrate and lipid metabolism were determined. There was an increase in glucose utilization through heightened metabolism by dikanut. Such improved utilization of glucose may retard the tendency toward diabetic-induced nephropathy. The reduction in kidney transaminase as a result of diabetes was restored by the dikanut supplement. However, cellulose, an insoluble fiber employed in similar experimentation, failed to curtail the loss of kidney alanine transaminase. Glucose-6-phosphatase activity was also significantly increased in rats fed dikanut, but not those fed cellulose.

These observations suggest that the integrity of kidney membrane and functions are sensitive to different fiber sources. (J. Nutr. Biochem. 5:193–196, 1994.)

Keywords: dietary fiber; diabetes; enzymes; kidney

Introduction

Dietary fiber is a group of heterogenous substances that are resistant to human gastrointestinal enzymes. It has been found to be crucial for health and is effective in treating several diseases often described as "fiber-related ailments".[1] Prominent among the diseases linked to decreased fiber intake is diabetes mellitus.[2,3] Diabetes mellitus is a heterogenous broad-spectrum disease often characterized by disordered carbohydrate and lipid metabolism.[4]

In the past, a number of structural and functional effects of fibers have been attributed to increased luminal bulk. Now it is better understood that some fibers are not simply inert bulking agents, but are metabolically active luminal substances.[5] The metabolic effect of dietary fiber on carbohydrate and lipid metabolism and its probable role in diabetic renal pathology is not yet known.

In this study, we investigated the early enzyme changes that may lead to diabetic nephropathy and the effect of feeding a supplement of dikanut (the endosperm of the viscous seeds of an African perennial plant, *Irvingia gabonesis*) on reversing the changes in enzyme activities caused by diabetes. Cellulose (an insoluble fiber) was also fed to diabetic rats for comparison purposes. The status of some enzymes of carbohydrate and lipid metabolism in the kidney of streptozotocin-induced diabetic rats fed dikanut supplement was thus the focus of the study.

Methods and materials

Four groups of rats (eight rats/group; average body weight of 167 g) were employed in the study. Three of the groups (diabetic) received a single intraperitoneal (i.p.) injection of streptozotocin (65 mg/kg body weight in 0.05 M citrate buffer, pH 4.5) and the last group (normal) was injected a sham equivalent amount of buffer i.p. Diabetes was confirmed 8 days later when blood glucose (obtained by cutting the tail) was four times in excess of normal. Two of the diabetic groups were assigned to dikanut- and cellulose-supplemented diets. The last diabetic group and the group not induced with diabetes received the normal diet *(Table 1)*.

All rats were allowed free access to their respective diets and water. The feeding lasted for 28 days. Body weight change and food consumption were recorded. Twenty-four-hour urine samples were collected and preserved for each rat. Pooled weekly collection was used for glucose determination. The kidney was excised, homogenized, centrifuged, and the supernatant was used for the enzyme assays. Urinary glucose was determined by a spectrophotometric method.[7] The activities of transaminase in the kidney were obtained by measuring the amount of pyruvate released from the

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Received March 8, 1993; accepted November 30, 1993.

Table 1 Composition of diets (%)

	Normal	Supplements	
		Dikanut	Cellulose
Ground corn*	53.7	49.7	49.7
Groundnut oil*	3.0	3.0	3.0
Soybean*	38.0	38.0	38.0
Salt Mix†	4.0	4.0	4.0
Vitamin Mix‡	1.0	1.0	1.0
Dikanut*	—	4.0	—
Cellulose§	—	—	4.0
Methionine§	0.3	0.3	0.3

*Obtained from a local market in Benin, Nigeria.
†American Institute of Nutrition (AIN).[6]
‡Vitamin Mixture (Vitadol) was from Tuco Products Company, Orangeville, Ontario, Canada.
§Product of Merck, Darmstadt, Germany.

appropriate substrate.[8] The activities of phosphofructokinase, pyruvate kinase, ATP-citrate lyase, and NADP-isocitrate dehydrogenase in the kidney were determined by measuring the change in extinction due to NADP reduction or NADH oxidation.[9] Glucose-6-phosphatase activity was determined by measuring the amount of inorganic phosphate released following incubation with glucose-6-phosphate.[10]

All results were expressed as means ± SEM. Analysis of variance (ANOVA) was used to show overall treatment effect and Duncan's multiple range test was applied to indicate significant differences among the means ($P < 0.05$).[11]

Results

The effects of 4% dikanut and cellulose supplementation were determined separately on food consumption, weight changes, and glucose levels in the blood and urine *(Table 2)*. The rats fed cellulose consumed the most food but the normal and dikanut-fed rats had higher body weights. The weight of the kidney was significantly higher in the diabetic group on normal diet when compared with the other groups. Dikanut and cellulose supplementation caused a significant decrease in the level of urinary glucose and there was evidence of glycemic control with the supplementation. The dikanut-fed rats approached normal blood glucose levels but were rarely normalized. *Table 3* shows the effect of dikanut and cellulose on kidney aspartate and alanine transaminase. Aspartate transaminase was significantly low in diabetic rats when compared with normal rats. A similar trend was recorded for alanine transaminase activity, except that the level of reduction did not attain statistical significance. Treatment with dikanut and cellulose raised aspartate transaminase to the normal level, while alanine transaminase activity remained depressed with both supplements. *Table 4* shows the effect of dikanut and cellulose supplements on the activities of some enzymes of carbohydrate and lipid metabolism in the kidney. The activities of most of the enzymes were significantly reduced in diabetic rats when compared with the normal values. Dikanut supplementation significantly increased pyruvate kinase activity but failed to increase phosphofructokinase activity. Cellulose had no effect on the activities of both of these enzymes. The supplemented diets significantly increased the activities of kidney ATP-citrate lyase, NADP-isocitrate dehydrogenase, malic enzyme, glucose-6-phosphate dehydrogenase, malic enzyme, and 6-phospho-gluconate dehydrogenase. The activity of glucose-6-phosphatase was significantly increased in diabetic rats on normal diet, but dikanut and cellulose supplementation significantly reduced the elevated level of this enzyme in the diseased state toward normal.

Discussion

Animals consuming dikanut are less hyperphagic. This may represent better glycemic control and a less catabolic state, but it is also possible that the diet may be less palatable to the rats.

The regulatory reactions of glycolysis are catalyzed by phosphofructokinase and pyruvate kinase, while glucose-6-phosphatase accelerates an important reaction of gluconeogenesis. Key reactions of pentose phosphate pathway are catalyzed by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These latter reactions generate NADPH for the synthesis of steroids and fatty acids.[12] Malic enzyme similarly generates NADPH for reductive biosynthesis and ATP-citrate lyase produces acetyl CoA for the synthesis of steroids and fatty acids.[13] Murphy et al.[14] reported that diabetes mellitus exerts considerable

Table 2 Weight records and glucose levels in blood and urine

	Groups			
	Normal	Diabetic	Dikanut	Cellulose
Food intake (g/wk)	97.9 ± 3.3^{a}	119.0 ± 2.5^{b}	105.9 ± 1.6^{c}	120.0 ± 1.4^{b}
Initial body wt (g)	167 ± 2^{a}	167 ± 3^{a}	168 ± 3^{a}	168 ± 3^{a}
Final body wt (g)	186 ± 2^{a}	155 ± 3^{b}	177 ± 3^{c}	165 ± 2^{d}
Kidney weight (g)	1.3 ± 0.1^{a}	1.7 ± 0.1^{b}	1.4 ± 0.1^{a}	1.4 ± 0.1^{a}
Glucose				
Plasma (mg%)	57.1 ± 1.0^{a}	280 ± 1.1^{b}	87.8 ± 1.7^{a,d}	113.3 ± 1.3^{c}
Urine (g/dl/wk) × 10^{-1}	—	34.0 ± 1.1^{a}	7.0 ± 2.0^{b}	11.0 ± 0.3^{c}

Means ± SEM, values in horizontal columns with different letter superscripts are significantly different ($P < 0.05$). Fasting blood glucose was measured after an overnight fast. Initial blood glucose for normal rats was 64.0 ± 1.9 mg%, while the initial blood glucose for diabetic rats and those that received cellulose and dikanut supplements ranged from 310.0 ± 2.7 to 324.3 ± 1.9 mg%.

Table 3 Effect of dikanut and cellulose supplements on kidney aspartate and alanine transaminase activities

Kidney	Groups			
	Normal	Diabetic	Dikanut	Cellulose
	(mg pyruvate/mg protein/min × 10^{-4})			
Asp transaminase	10.8 ± 0.4[a]	8.4 ± 0.6[b]	10.7 ± 0.7[a]	10.4 ± 0.3[a]
Ala transaminase	17.7 ± 1.6[a]	15.0 ± 1.7[a]	9.6 ± 1.9[b]	13.4 ± 0.6[b]

Means ± SEM, values in horizontal columns with different letter superscripts are significantly different ($P < 0.05$).

Table 4 Effect of dikanut and cellulose supplements on the activities of some enzymes of carbohydrate and lipid metabolism in the rat kidney

Kidney	Groups			
	Normal	Diabetic	Dikanut	Cellulose
	(nmole/mg protein/min)			
Phosphofructokinase	6.3 ± 0.3[a]	2.8 ± 0.2[b]	3.5 ± 0.2[b]	3.5 ± 0.2[b]
Pyruvate kinase	16.1 ± 2.7[a]	19.5 ± 0.4[a]	29.5 ± 2.2[b]	22.6 ± 1.0[a]
ATP-citrate lyase	7.8 ± 0.2[a]	2.6 ± 0.1[b]	6.7 ± 0.3[c]	6.7 ± 0.3[c]
NADP Isocitrate d'nase	20.7 ± 0.3[a]	6.5 ± 0.2[b]	14.8 ± 1.0[c]	14.6 ± 0.5[c]
Malic enzyme	14.2 ± 0.2[a]	4.0 ± 0.2[b]	12.4 ± 1.7[a]	12.7 ± 0.7[a]
Gluc-6-P d'nase	5.4 ± 0.4[a]	4.1 ± 0.2[b]	5.0 ± 0.3[a,b]	2.1 ± 0.6[c]
6-Phosphogluco d'nase	16.5 ± 1.2[a]	8.1 ± 0.6[b]	18.9 ± 1.0[a,c]	15.1 ± 1.3[a,d]
Glucose-6-phosphatase	64.0 ± 5.0[a]	138.0 ± 5.0[b]	85.0 ± 5.0[c]	118.0 ± 5.0[d]

Means ± SEM, values in horizontal columns with different letter superscripts are significantly different ($P < 0.05$).

stress on metabolism in the kidney and had found glomerular membrane lesions. Lee[15] reported that high dietary fiber supplementation in the diet of the genetically diabetic mouse caused a decrease in diabetic renal pathology. Adamson and Esionye,[16] however, did not observe improvement in kidney function in diabetic rats fed dikanut and therefore suggested that kidney acid phosphatase may not be a good indicator of metabolic changes leading to diabetic nephropathy. It was, therefore, imperative for us in this study to further investigate the effects of dikanut and cellulose supplements on several of the enzymes of carbohydrate and lipid metabolism that may respond to metabolic control of diabetes due to dietary fiber ingestion.

Our data suggest an improvement of glucose metabolism occasioned by streptozotocin injection through the glycolytic route and the pentose phosphate pathway, while gluconeogenesis is promoted in the kidney. Khandelwal et al.[17] reported a significant increase in glycogen synthase activity and a three-fold increase in glucose-6-phosphate level and then attributed their findings to an increase in the accumulation of glycogen in the kidney of diabetic animals. Sochor et al.[18] also reported elevated hexokinase and glucose-6-phosphate in diabetic mice due to eventual accumulation of basement membrane materials in the glomerulus. The kidney does not require insulin for glucose uptake and phosphorylation and has been found to show glucose overutilization under hyperglycemic conditions.[19,20] Early experimental diabetes has been reported to cause glucose overutilization in the kidney.[21,22] Seyer-Hansen[23] reported a positive correlation of diabetic hypertrophy with the degree of hyperglycemia. Our findings of impaired glucose metabolism therefore may be due to a severe diabetic-induced damage to the kidney and then hypertrophy. The decrease in the activity of kidney transaminase in diabetic rats may possibly be due to leakage of enzyme from hypertrophied kidney tissue, but this is required to be corroborated by high levels of the isoform in the blood. The increase in glucose-6-phosphatase activity in diabetic rats, on the other hand, may be due to improved cellular glucose metabolism in the kidney.

Dikanut supplementation did not increase the activities of phosphofructokinase and glucose-6-phosphate dehydrogenase in this short-term study, but the slight increase in activity may indicate gradual restoration of a glycolytic and pentose phosphate regulatory mechanism. This was supported by the gross anatomical observation of the enlarged kidney in the treated rats that returned to normal. The marked increase in the activities of ATP-citrate lyase, NADP-isocitrate dehydrogenase, and malic enzyme in the dikanut-fed rats shows a heightened demand for NADPH by the kidney. Frenkel[24] reported earlier that the kidney has a relatively low rate of lipogenesis. It is therefore unlikely that this change is related to the elevation of lipogenic capacity as has been established for the liver.[25] It is rather suggested that the NADPH and acetyl CoA produced by these enzymes are used for energy purposes and for hormone synthesis toward restoration of nutrient homeostasis in the kidney.

The transaminase enzymes were studied to give an indication of the integrity of the kidney membrane and renal sufficiency. An earlier study with whole kidney acid phosphatase did not show any sensitivity to the dietary supplements with either of the supplements.[16] Distinct changes in enzyme activities that may be sensitive to the dietary supplements may have been possible if separate activities were determined for the tubular and glomerular tissues. In the present study the effect of the supplements on the transaminase produced variable results. While both dietary supplements restored

aspartate transaminase (mol. wt. = 91 k) level in the kidney to normal, the effect on alanine transaminase (mol. wt. = 53 k) resulted in a further decrease in activity. It is possible that the kidney membrane was not fully restored to prevent undesirable transmembrane movement of the smaller molecular weight enzyme from the kidney tissue. Although the concentrations of transaminase enzymes are relatively low in the kidney, the abnormal levels may indicate that full renal function may not have been attained in the short period of 4 weeks of supplementation with dikanut.

Acknowledgments

We are grateful to The Third World Academy of Sciences, Trieste, Italy for a grant awarded to I.A. in partial support of this study.

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