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Prolonged Fructose Feeding and Aldose Reductase Inhibition: Effect on the Polyol Pathway in Kidneys of Normal Rats (42624)

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Abstract. The effects of diets with differing carbohydrate composition on the kidney polyol pathway were investigated. The diets employed were F = fructose rich, G = glucose rich, S = cornstarch rich, and were fed for 30 days to six groups of 12 normal male Sprague–Dawley rats with and without addition of the aldose reductase inhibitor tolrestat (T). Fructose feeding resulted in higher kidney sorbitol levels (F = 0.847 ± 0.152, G = 0.354 ± 0.087, S = 0.207 ± 0.041 μM/g wet wt, $P < 0.05$). This was not observed in the tolrestat-treated animals (F + T = 0.182 ± 0.024, G + T = 0.149 ± 0.021, S + T = 0.152 ± 0.020 μM/g wet wt). Aldose reductase activity was reduced with tolrestat administration (F = 0.0208 ± 0.0023, F + T = 0.0048 ± 0.0005; G = 0.0210 ± 0.0002, G + T = 0.0059 ± 0.0008; S = 0.0227 ± 0.0022, S + T = 0.0062 ± 0.0007 μU). Myoinositol levels did not differ among groups (F = 1.973 ± 0.182, G = 2.291 ± 0.307, S = 2.066 ± 0.155 μM/g wet wt), but tended to increase with aldose reductase inhibition (F + T = 2.253 ± 0.186, G + T = 2.713 ± 0.166, S + T = 2.618 ± 0.221 μM/g wet wt). Plasma glucose was higher in the fructose-fed rats (F = 10.78 ± 0.55, G = 9.09 ± 0.058, S = 9.03 ± 0.52, F + T = 9.75 ± 0.61, G + T = 8.42 ± 0.64, S + T = 8.81 ± 0.49 mM/liter). It is concluded that prolonged fructose feeding results in the accumulation of sorbitol in the kidney, caused by increased flux of glucose through the polyol pathway. This can be prevented by aldose reductase inhibition.

In recent years fructose has been proposed as a substitute for glucose or other sugars in hypocaloric diets and in diets for diabetic patients because of its ability to induce smaller increases in plasma glucose and insulin following an oral load in normal and diabetic subjects (1–4). However, a number of long-term studies performed in rats fed fructose-rich diets have shown that lesions closely resembling those of diabetic nephropathy and retinopathy developed in a high percentage of animals (5, 6). The pathogenesis of such lesions has not been unequivocally established, although the accumulation of lactate, fructose or fructose-1-phosphate has been proposed as being at its basis (7, 8). We have examined the possibility that fructose might influence the activity of the polyol pathway as increasing evidence relates the complications of diabetes to increased aldose reductase activity in target tissues such as lens (9), retina (10), peripheral nerve (11), and possibly the kidney glomerulus (12). More recently it has been found that increased sorbitol contents in the nerve are associated with decreased myoinositol (13), which in turn has been proposed to result in reduced activity in Na^+–K^+-dependent ATPase (14). Similar changes of myoinositol depletion and reduced Na^+–K^+-dependent activity have been observed in isolated glomeruli of diabetic rats (15). In the present study we have chosen to devote our attention to the kidney where the presence of both aldose reductase and sorbitol dehydrogenase has been clearly demonstrated (16). To our knowledge, the activity of the polyol pathway relative to the metabolism of fructose has not been studied extensively in this tissue, which appears to be one of the most sensitive to the possible noxious effects of prolonged fructose feeding (6). With this in mind, we have chosen to investigate (i) if sorbitol does accumulate in normal kidneys following chronic administration of fructose, (ii) if this possible accumulation of sorbitol is due to the activity of the polyol pathway, and (iii) if it can be prevented by means of a specific aldose reduc-

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tase inhibitor. In order to examine these hypotheses, we studied groups of rats fed diets with differing carbohydrate compositions (fructose rich, glucose rich, cornstarch rich), repeating the study with a new aldose reductase inhibitor (tolrestat, Ayerst) added to the food. Importantly, aldose reductase inhibitors have been shown to prevent both the accumulation of sorbitol and the decrease in myoinositol in experimental diabetes (17).

Materials and Methods. Our study consisted of two experiments. The first (referred to as "basal") was performed without tolrestat administration. Sixty male Sprague–Dawley rats (Harlan–Sprague–Dawley Breeding Laboratories, Indianapolis, IN) were used in each experiment. The rats were housed in the University Animal Care Facility, initially 6 per cage, and fed a standard natural component diet (Purina Rat Chow 5001) for 1–2 weeks. At the end of this period the animals were weighed and the 6 of lowest and 6 of highest body weight were excluded from the study. The remaining rats were then divided into four groups such that body weight would be as homogeneous as possible among groups and that to each animal there would correspond a control of equal (±2 g) body weight. The groups (each consisting of 12 animals) differed in diet treatment according to the following: group F was fed a fructose-rich diet composed of fructose 50%, cornstarch 15%, D,L-methionine 0.3%, high nitrogen content purified casein 20%, fibers 5%, AIN vitamin mix 1%, choline bitartrate 0.2%, AIN mineral mix 3.5%, and corn oil 5%; group G was fed a glucose-rich diet of the same composition except that glucose was substituted for fructose; groups S1 and S2 (controls) were fed a diet identical in composition to that fed group F except that cornstarch was substituted for fructose. All the diets were purchased from NBC Biochemical Co. (Cleveland, OH). They were used within 2 months of preparation and were refrigerated. Subsequently, all the animals were housed individually in metabolic cages, with the rats in F and S1, and G and S2 pair-fed. Food and water intake were measured daily; body weight was checked every fourth day. Food was changed daily and water every second day. The animals were maintained on a 12-h light, 12-h dark cycle. On the 30th day food was withheld and a 12-h fast was started (lasting until the moment of sacrifice on the following morning). At 0800 hr on the 31st day, the animals were sacrificed after being anesthetized with sodium pentobarbital (50 mg/kg body wt intraperitoneally). Both kidneys were removed (one for metabolite assay, the other for enzyme activity assay), immediately weighed on a Mettler digital balance, and frozen at −40°C. A sample of arterial blood was taken for plasma glucose determination. The blood was centrifuged for 30 min at 3000 rpm (4°C) and the plasma separated and stored at −20°C.

The second experiment was performed exactly as described above except that a potent new aldose reductase inhibitor with a half-life of 10 hr was added daily to the food of all the rats in a concentration to provide a dose of 25 μg/kg body weight based on the previous day's food intake. The inhibitor used was tolrestat (Ay-27773,N-([1–5 trifluoromethyl-6-methoxy-1-naftalenyl]thioxomethyl-*N*-methylglycine) which was kindly provided by Dr. D. Dvornik, Ayerst Laboratories (New York, NY).

Analytical methods. Tissues for the determination of sorbitol, myoinositol, fructose, glucose, and glycerol were homogenized in 5 vol of 1 *N* perchloric acid with a Polytron (Brinkman Instruments, Westbury, NY) for 20 sec. The homogenate then was centrifuged for 30 min at 3000 rpm (4°C), and the supernatant was aspirated and neutralized by adding 2 *N* potassium hydroxide. It was centrifuged again for 30 min at 3000 rpm, and the supernatant was aspirated and used for assay. Sorbitol, myoinositol, and fructose were measured enzymatically (18). All the reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Kidney glucose was determined with the glucose oxidase method, using a kit by Boehringer Mannheim (Indianapolis, IN). Glycerol was determined enzymatically with a modification of the method of Garland and Randle (19). Plasma glucose was measured using the same kit from Boehringer Mannheim. Aldose reductase and sorbitol dehydrogenase activity were measured in the contralateral kidney. The tissue was homogenized with a Polytron for 10 sec in 5 vol of phosphate buffer (0.2 *M*).

The homogenate then was centrifuged at 9000 rpm for 20 min at 2°C, and the supernatant was aspirated and frozen prior to assay. A 0.1-ml aliquot was assayed for aldose reductase and sorbitol dehydrogenase activities. Another 0.1-ml aliquot was analyzed for protein content according to the method of Bradford (20). Aldose reductase activity was measured spectrophotometrically, recording the change in optical density following the oxidation of NADPH to NADP, using D-L-glyceraldehyde as a substrate (21). Sorbitol dehydrogenase was measured in the reverse direction, recording the change in optical density following the oxidation of NADH to NAD, using fructose as a substrate (22). Both the enzyme activity assays were performed at 37°C. All the optical density recordings were made on a Beckman Model 25 dual-beam spectrophotometer with attached recorder. Enzymes, metabolites, and glycerol were read at 340 n*M*, glucose at 505 n*M*, and protein at 595 n*M*. Final concentrations were obtained from a simultaneously prepared five-point (triplicate assays) standard curve. The lower limits of sensitivity were sorbitol, 0.2 μg/tube; myoinosital, 0.02 μm/tube; fructose, 2 μg/ml; glucose, 10μg/ml; glycerol, 0.005 mM/tube; and protein, 2.5 μg/ml. The concentrations of sorbitol, myoinositol, fructose, glucose, and glycerol in the kidney are expressed in micromoles per gram wet weight. The concentration of glucose in the plasma is given in milligrams per deciliter. Enzyme specific activity is expressed as micromoles of coenzyme oxidized per minute per milligram of protein. Statistical analysis was performed using the analysis of variance or the Student *t* test for paired or unpaired samples as appropriate (23). All the results are expressed as means ± SEM.

Results. No significant differences were observed among groups regarding body weight gain or food or water intake (Table I). In particular, groups S1 and S2 (controls) did not differ in any respect. Accordingly, their data were pooled. Kidney weight in the basal and tolrestat-treated animals (g/kg body wt) was significantly higher (9–11%) in the fructose-fed animals ($P < 0.05$). Finally, plasma glucose was significantly greater (19%) in the fructose-fed animals ($P < 0.05$), with the same pattern in the tolrestat-treated animals ($P = 0.05$).

Metabolites. Kidney sorbitol levels (Fig. 1) were significantly higher in the animals fed a fructose-rich diet ($P < 0.05$). In the tolrestat-treated rats, the tissue sorbitol levels were lower, and no significant differences were present among groups.

Myoinositol concentration in the kidney (Fig. 1) was similar in the "basal" animals. In the tolrestat-treated animals there was a tendency (although not significant) for myoinositol levels to be higher in all groups.

Glucose concentration (Fig. 1) was significantly higher (62%) in kidneys from fructose-fed rats ($P < 0.05$). In the animals treated with tolrestat, the differences between groups were reduced ($P > 0.05$).

The amount of fructose (Fig. 1) in the kidney was significantly higher (123%) in animals fed a fructose-rich diet ($P < 0.05$). A similar pattern was noted in the tolrestat-treated animals ($P > 0.05$), although absolute levels were lower in all groups.

Kidney glycerol content was slightly higher in group F ($P > 0.05$), and it was not affected by aldose reductase inhibition (*P*

TABLE I

	Body wt[a] (g)		Kidney wt (mg/g body wt)		Food intake[a] (g/day)		Water intake (ml/day)		Plasma glucose (mg/dl)	
	Basal	Tolrestat	Basal	Tolrestat	Basal	Tolrestat	Basal	Tolrestat	Basal	Tolrestat
Fructose	318 ± 6.1	310 ± 5.6	3.44 ± 0.05*	3.35 ± 0.04*	15.5 ± 0.18	17.4 ± 0.73	21.7 ± 1.28	22.9 ± 1.30	194 ± 10*	175 ± 12*
Starch 1	317 ± 8.7	314 ± 8.2	3.09 ± 0.12	3.06 ± 0.10	15.4 ± 0.17	17.5 ± 0.57	21.4 ± 0.75	21.6 ± 0.96	163 ± 9	157 ± 9
Glucose	314 ± 9.4	305 ± 7.6	3.06 ± 0.04	3.08 ± 0.06	15.4 ± 0.27	17.8 ± 0.77	23.7 ± 0.82	21.3 ± 0.78	164 ± 11	152 ± 17
Starch 2	316 ± 8.0	311 ± 8.0	3.12 ± 0.12	3.02 ± 0.06	15.4 ± 0.22	17.6 ± 0.76	22.3 ± 0.52	19.6 ± 0.7	160 ± 8	159 ± 6

[a] Rats were pair-weighed (226 ± 2 g) at start of the feeding regimen and controls were pair-fed to the amount eaten by their experimental pairmate the previous day. $N = 12$ each group.

* Significantly different from control and glucose groups $P < 0.05$.

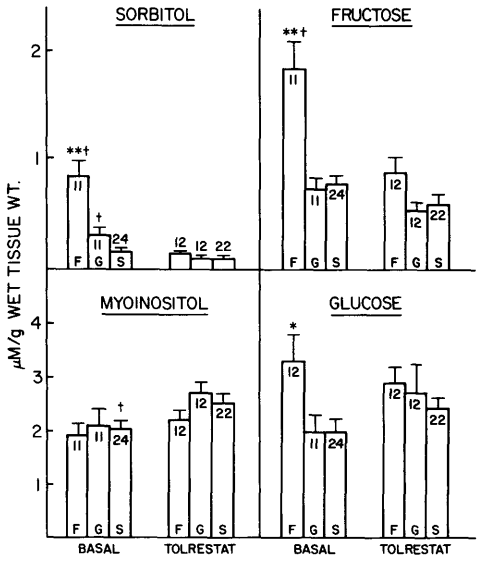

FIG. 1. Kidney sorbitol, myoinositol, fructose, and glucose levels in rats fed 30 days with diets high in fructose (F), glucose (G), or starch (S) in the absence or presence of the aldose reductase inhibitor tolrestat. *Significantly different from appropriate diet group (*$P < 0.05$, **$P < 0.01$); †Significantly different from appropriate tolrestat group, $P < 0.05$. Numbers in bars, numbers of observations; vertical bars, SEM.

> 0.05), except for a slight decrease in the fructose-fed animals.

Enzyme activity. Aldose reductase activity did not differ significantly among groups ($P > 0.05$), and it was dramatically reduced in the animals treated with tolrestat ($P < 0.05$; Fig. 2). Sorbitol dehydrogenase activity was significantly higher in the fructose-fed animals ($P < 0.05$), and was decreased in the rats treated with tolrestat. The ratio aldose reductase/sorbitol dehydrogenase was significantly lower in group F ($P < 0.05$) and in rats treated with tolrestat ($P < 0.05$), although the absolute values were decreased in all groups.

Discussion. No significant differences in body weight or food and water intake were observed among groups, nor did the administration of tolrestat appear to affect significantly these parameters. Although in previous studies (24) rats fed a fructose-rich diet showed a delayed body weight gain during the first days on the diet, this was not the case in the present study, perhaps due to the fact that the animals were accurately pair-fed, or, alternatively, it is possible that, since we measured body weight every fourth day, we might have missed differences occurring in the first 3 days of study.

We have investigated the effects of fructose feeding on the polyol pathway in the whole kidney (entire kidney homogenized and assayed) rather than in only isolated glomeruli. Due to the complex morphology of the kidney, localization of aldose reductase by immunoreactive staining has been difficult, but it is clear that this enzyme is present throughout the kidney at multiple locations within the cortex, medulla and pelvis (25).

Kidney weight was significantly higher in the animals fed a fructose-rich diet and this finding also was reproduced in the fructose-fed rats treated with tolrestat, but to a lesser magnitude. Although we did not look at its cause specifically, it might result from tissue hypertrophy or hyperplasia, or even an increased content of glycogen in the kidney, since it has been shown that at least in the liver the glycogenogenic effect of fructose is greater than that of glucose (26). Results were normalized to gram of wet tissue weight in consideration of the difference of kidney weights among the various groups.

The concentration of sorbitol in the kidney was greater in the fructose-fed group; a similar finding has been observed previously in the lens and retina (27) and sciatic nerves (28) of diabetic rats fed chronically a fructose-rich diet. This accumulation of sorbitol in the kidney can be almost completely prevented by aldose reductase inhibition (Fig. 1).

Effects of both diabetes and aldose reductase inhibitor administration have been observed previously on sorbitol and fructose concentrations in whole kidney of diabetic rats (29). The observed differences were not statistically significant, however, due to great variations in the sugar concentrations. A difficulty with the previous study (29) may well be the small number of animals employed per group ($n = 4$) as compared to the data presented herein. The inhibition of sorbitol accumulation by aldose reductase inhibition is evidence that increased kidney sorbitol levels originate from the activity of the polyol pathway following transformation of

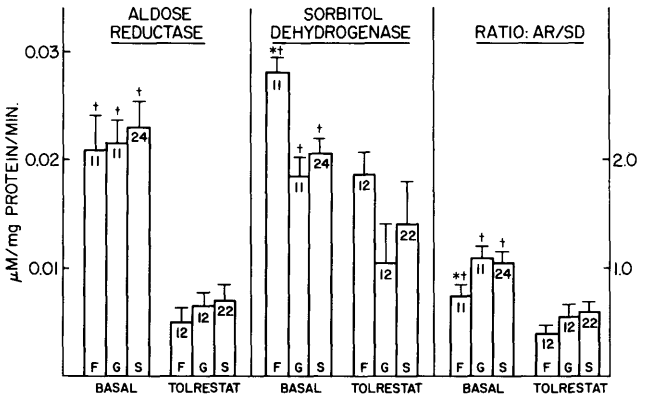

FIG. 2. Kidney aldose reductase and sorbitol dehydrogenase activity in rats fed 30 days with diets high in fructose (F), glucose (G), or starch (S) in the absence or presence of the aldose reductase inhibitor tolrestat. *Significantly different from appropriate diet group, $P < 0.05$; †significantly different from appropriate tolrestat group, $P < 0.05$. Numbers in bars, numbers of observations; vertical bars, SEM.

fructose to glucose with its subsequent reduction to sorbitol by aldose reductase. An alternative possibility which cannot be excluded by our study could be that the sorbitol is derived totally or in part from fructose following reduction by sorbitol dehydrogenase acting in the reverse direction since, at intracellular pH, sorbitol dehydrogenase is more active in the reverse direction (30), assuming enough fructose is present. However, the latter mechanism does not appear to be effective in this case since aldose reductase inhibition causes a dramatic reduction of sorbitol and fructose levels in the fructose-fed group; if the activity of sobitol dehydrogenase in the reverse direction were metabolically significant, the administration of tolrestat would not affect tissue sorbitol concentration to such an extent. Thus, the data presented herein support the suggestion that the forward direction was favored. The increased activity of sorbitol dehydrogenase observed in the fructose-fed animals (not treated with tolrestat) might result from increased substrate flow through the polyol pathway. Although detailed data regarding regulation by sorbitol of sorbitol dehydrogenase activity are not available from this study, it can be hypothesized that chronically increased tissue sorbitol levels can lead either to allosteric activation or to new synthesis of the enzyme. It has been shown (16) that in diabetic rats, together with increased flux of glucose through the polyol pathway, the activity of sorbitol dehydrogenase in the glomeruli appears to be higher than in normoglycemic controls.

Tolrestat proved to be an effective inhibitor of aldose reductase in the kidney, leading to a reduction of enzyme activity to approximately 20% that of untreated animals. This confirms previous reports of tolrestat efficacy in the rat lens (31) and nerve (32).

Myoinositol levels in the whole kidney were not affected significantly by differing diet treatments (although they tended to be slightly lower in the organs from fructose-fed animals) and tended to be higher in the rats treated with the aldose reductase inhibitor, demonstrating in the kidney the existence of an inverse relationship between sorbitol and myoinositol levels, although not so evidently as in peripheral nerves (33). It may be necessary to use isolated glomerular preparations (12) rather than whole kidney preparations to increase the sensitivity of the analysis.

Glycerol levels were slightly higher in the fructose-fed animals. This finding can be explained by an increased tissue concentration of fructose, which can be broken down (prior transformation to fructose-1-P) to glyceraldehyde and dihydroxyacetone phosphate by the action of aldolase B, the activity of which is increased following prolonged fructose feeding (34). Glyceraldehyde can be subsequently reduced to glycerol by action of al-

dehyde reductase, alcohol dehydrogenase, or aldose reductase itself (35). The administration of tolrestat led to a decrease in glycerol levels in the fructose-fed animals, although it did not cause any change in the other dietary groups.

As already noted, aldose reductase activity was much lower in the animals treated with the inhibitor. Reduced activity was observed for sorbitol dehydrogenase following tolrestat administration which probably can be accounted for by the decreased availability of its substrate, sorbitol (assuming allosteric activation or induced synthesis of the enzyme by its substrate as noted above); an alternative explanation could be that tolrestat had some direct inhibitory activity on sorbitol dehydrogenase. Actually, another aldose reductase inhibitor, alrestatin, has been shown to partially inhibit sorbitol dehydrogenase (30). The ratio aldose reductase/sorbitol dehydrogenase was diminished in the tolrestat-treated animals due to a greater reduction in aldose reductase activity than in sorbitol dehydrogenase activity.

Kidney fructose levels were higher (not surprisingly) in the fructose-fed animals, although in the tolrestat-treated rats they were significantly lower, probably due to the fact that the fraction of fructose derived from glucose through the polyol pathway was greatly reduced following aldose reductase inhibition. Such a fraction, according to our data, can amount to as much as 50–60% of the total fructose present in the tissue.

Finally, we must note that prolonged fructose feeding resulted in higher levels of glucose, both in the plasma and in the kidneys of normal rats. Similar findings (although slightly less marked) were noted in the animals treated with the aldose reductase inhibitor. The phenomenon of glucose intolerance and a mild form of diabetes may originate from a state of insulin resistance induced by chronic fructose feeding, which has previously been observed to occur in the rat and man (36, 37), a finding of some importance in view of the increasing use of fructose in human diets.

In conclusion, we can state that feeding with a fructose-rich diet for 30 days can lead to an accumulation of sorbitol in whole kidneys of normal rats, probably following an increased flux of glucose through the polyol pathway. This can be prevented by administration of tolrestat, a potent inhibitor of aldose reductase. Also, the concentration of fructose in the kidney can be reduced by aldose reductase inhibition, leading us to conclude that a significant portion of it is originated through the polyol pathway. Finally, lesions in the kidneys of rats fed a fructose-rich diet may have a common biochemical basis with those proposed for diabetes due to induced changes of aldose reductase, sorbitol, and myoinositol.

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