High-fructose feeding of streptozotocin-diabetic rats is associated with increased cataract formation and increased oxidative stress in the kidney

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We examined the effects of high-fructose (FR) feeding on the development of diabetic complications in the lens and the kidney of streptozotocin (STZ)-diabetic rats. Male Wistar Furth rats were treated with one of two doses of STZ (HIGH STZ, 55 mg/kg body weight; MOD STZ, 35 mg/kg body weight) or vehicle alone (SHAM) and were then assigned to a control (CNTL) or 400 g FR/kg diet for 12 weeks. At the end of the study, body weight, plasma glucose and insulin concentrations differed among STZ groups (HIGH *v.* MOD *v.* SHAM, $P < 0{\cdot}001$) but did not differ due to diet. Plasma FR concentrations were significantly higher in FR-fed *v.* CNTL-fed groups ($P < 0{\cdot}0001$) and in HIGH-STZ groups *v.* MOD-STZ and SHAM groups ($P < 0{\cdot}0004$ and $P < 0{\cdot}0001$ respectively). Focal length variability of the lens, a quantitative measure of cataract formation, was increased in the HIGH STZ, FR group compared with the HIGH STZ, CNTL group ($P < 0{\cdot}01$). The concentration of H_2O_2 in kidney microsomes was significantly higher in HIGH STZ, FR rats *v.* HIGH STZ, CNTL rats ($P < 0{\cdot}01$). Microalbuminuria was not observed in any of the groups examined, and there was no evidence of extensive histological damage in the kidney from any rats. Under conditions of severe hyperglycaemia, high FR intake promotes the development of cataracts in the lens of the eye, and results in increased concentrations of substances indicative of oxidative stress in the kidney. Although FR has been suggested as a carbohydrate source for diabetics, a high FR diet coupled with hyperglycaemia produces effects that may promote some of the complications associated with diabetes.

Fructose: Cataracts: Oxidative damage: Diabetes

Diabetes mellitus affects approximately 5 % of the Western population. With the disease comes an increased risk of long-term complications including nephropathy and kidney failure, eye and vision problems (cataracts, retinopathy and eventual blindness), neuropathy and cardiovascular disease (Group, 1993). While glycaemic control is one of the most important factors influencing the development of diabetic complications, the interactive role of other factors, such as intake of specific nutrients, in influencing the rate or the extent of complications has yet to be clearly delineated.

Since it elicits lower blood glucose and insulin secretory responses than other simple carbohydrates, fructose (FR) has been suggested as an important dietary source of carbohydrate for diabetic patients (Bantle *et al.* 1986; Gerrits & Tsalikian, 1993). Despite its potential benefits to glycaemic control when consumed in moderate amounts (Bantle *et al.* 1986), FR supplementation has not been wholeheartedly endorsed for several reasons. First, evidence from trials of FR feeding in rats and human subjects has suggested that increasing dietary FR consumption can lead to significant increases in triacylglycerol and cholesterol concentrations (Swanson *et al.* 1992; Hollenbeck, 1993; Bell *et al.* 1996). Second, results of *in vitro* experiments demonstrate that intracellular FR, produced as part of the polyol pathway of glucose metabolism, may increase diabetic complications in the eye (Kador, 1989) and in the kidney (Beyer-Mears *et al.* 1984; Kikkawa *et al.* 1987). This alternative pathway of glucose metabolism is activated under conditions of uncontrolled hyperglycaemia and involves conversion of glucose to sorbitol via aldose

Abbreviations: CNTL, control; FR, fructose; HIGH STZ, high streptozotin-treated group; LP, lipid peroxide; MOD STZ, moderate streptozotocin-treated group; SHAM, sham-treated group; STZ, streptozotocin.

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reductase, and from sorbitol to FR via sorbitol dehydrogenase. The multiple mechanisms that may contribute to the positive relationship between intracellular sorbitol and fructose accumulation and the development of cataracts and glomerular damage include osmotic changes induced by an accumulation of sorbitol and FR which are accompanied by changes in electrolytes (Kador, 1989), decreased availability of NADPH (Lee *et al.* 1985), and disturbances of many intracellular homeostatic mechanisms (Cheng *et al.* 1989; Kador, 1989). Third, intracellular FR and its metabolites, such as fructose-3-phosphate, promote glycation, fructosylation and cross-linking of proteins (McPherson *et al.* 1988; Dills, 1993; Lal *et al.* 1995). Whether FR introduced through the diet acts to promote the development of diabetic complications, similar to FR which is produced intracellularly, is not clear.

The purpose of this project was to examine the effects of high-FR feeding on the development of diabetic complications in the kidney and the eye of streptozotocin (STZ)-diabetic rats. We tested rats that were either moderately or severely diabetic in order to evaluate the interactive effects between hyperglycaemia and FR feeding, since this could have clinical relevance in a diabetic population.

Materials and methods

Animals and streptozotocin treatment

Male Wistar Furth rats weighing approximately 250 g were lightly anaesthetized under halothane, and were made diabetic using a moderate dose (MOD STZ, 35 mg/kg body weight), or high dose of STZ (Sigma, St Louis, MO, USA; HIGH STZ, 55 mg/kg body weight) given by intravenous injection into the tail vein; rats in the control group (SHAM) received a sham injection of vehicle alone. Diabetes was allowed to develop for 1 week, at which time blood glucose levels were assessed using a glucose meter (Glucometer Elite; Miles Canada, Etobicoke, Ontario, Canada). Rats were then assigned to either the high FR or the control (CNTL) diet. The composition of the diets is shown in Table 1. Rats were housed in hanging, stainless-steel cages, 2–3 animals per cage with a reverse 12 h light–dark cycle. This study was approved by the Animal Care Committee at the University of Waterloo, Ontario, Canada in accordance with the Guidelines of the Canadian Council on Animal Care.

Table 1. Composition of experimental diets (g/kg diet)

Ingredient	Control diet	High-fructose diet
Casein	200	200
DL-Methionine	3	3
Cornstarch	325	250
Dextrose	325	–
Fructose	–	400
Cellulose	50	50
Corn oil	50	50
Mineral mixture*	35	35
Vitamin mixture†	10	10
Choline bitartrate	2	2

* Mineral mixture (g/kg): calcium phosphate, dibasic 500·0; sodium chloride 74·0; potassium citrate, monohydrate 220·0; potassium sulfate 52·0; magnesium oxide 24·0; manganous carbonate 3·5; ferric citrate 6·0; zinc carbonate 1·6; cupric carbonate 0·3; potassium iodate 0·01; sodium selenite 0·01; chromium potassium sulfate 0·55; sucrose (finely powdered) 118·03.

† Vitamin mixture (g/kg): thiamin HCl 0·6; riboflavin 0·6; pyridoxine HCl 0·7; niacin 3·0; calcium pantothenate 1·6; folic acid 0·2; biotin 0·02; vitamin B_{12} (1 g/kg) 1·0; vitamin A palmitate 0·8; vitamin D_3 0·25; vitamin E acetate 10·0; menadione sodium bisulfite 0·08; sucrose (finely powdered) 981·15.

Diets

FR and CNTL diets were both AIN 76-A-based (Bieri *et al.* 1977), with 200 g casein, 50 g corn oil and 700 g carbohydrate/kg and are outlined in Table 1. The carbohydrate source in the CNTL diet was a mixture of dextrose and cornstarch (no FR) while the FR diet contained a mixture of FR (400 g/kg diet) and cornstarch. Rats were allowed free access to their assigned diets for 12 weeks. Tap water was available at all times except where noted.

Regular monitoring

Rats were weighed weekly throughout the study. Food disappearance was measured for each cage, three times per week, and was estimated for individual rats based on the number of rats per cage. Whole blood was drawn from the tail vein prior to the induction of diabetes and after 12 weeks of the experimental diets. Blood was drawn in the mornings, approximately 3 h into the dark cycle. Blood samples were transferred into microcentrifuge tubes containing sodium fluoride and heparin and were centrifuged (Eppendorf Microcentrifuge, Model no. 5415C; VWR Scientific, Mississauga, Canada). The plasma was removed and stored at −20°C until determination of glucose (Glucose Trinder method; Sigma) and insulin (radioimmunoassay; Linco, St Charles, MO, USA). Urine samples were collected from individual rats in metabolic cages after 11 weeks of dietary treatment for determination of albuminuria; no water was available during this time. Urine samples were analysed within 24 h of collection using a spectophotometric assay (Sigma). Kumar (1995) defined microalbuminuria as a urine albumin concentration excretion rate of between 20 and 200 μg/min.

Measures at autopsy

After 12 weeks of dietary treatment, rats were given an overdose of sodium pentobarbital (Euthanyl, (Maple Leaf Foods, Cambridge, Ontario, Canada) 65 mg/kg body weight), and a blood sample was taken, by cardiac puncture, into heparinized vacutainers that were then centrifuged and the plasma removed and frozen at −80°C until used for determination of FR concentrations. Plasma FR was determined according to the method of Beutler (1983) in samples that had been deproteinized and deglucosed according to the method of Aebi (1984). Animals were killed approximately 3 h into the dark cycle.

Kidneys, pancreas, and eyes were removed quickly from the carcass. One kidney was divided in half along the longitudinal axis and from this, a small section was fixed in phosphate-buffered formalin (10 %) for later histological examination for changes that would be indicative of diabetic nephropathy. The remaining portion of this half

kidney and the pancreas were placed in ice-cold 0·1 M-Tris-HCl buffer in 9 g saline/l (pH 8·3) for determination of lipid peroxides (LP) and H_2O_2 from microsomal samples (see details later).

Refractive changes in the lens

The eyes from each animal were carefully removed and placed in M199 (Gibco, Life Sciences Technology, Gaithersberg, MD, USA) including Hepes, $NaHCO_3$, and penicillin–streptomycin (1×10^5 units/l) at 37°C for quantification of the optical quality of the lens. Lenses were excised under a dissection microscope by cutting the globe below the cornea with small iris scissors. Surgical sponges were used to clean the vitreous from the posterior of the lens. The lenses were transferred immediately into a two-part culture chamber containing M199 (Gibco; 37°C, pH 7·4), 1×10^5 units/l each of penicillin and streptomycin, and 8 % fetal bovine serum. The lower chamber consisted of a silicone rubber base, a flat round glass bottom, a small chamber filled with culture medium, and a bevelled plastic washer (inner diameter 3·6 or 4·0 mm) upon which the lenses rested. The upper portion, a square piece of borosilicate tubing, was inserted into the lower chamber, filled with culture medium to a volume of 30 ml, and topped by a sterile plastic 35×10 mm Petri plate. The culture chamber is fully described in Sivak *et al.* (1986).

The scanning lens monitor was used to provide an indication of the optical integrity of the lens as in previous work (Sivak, 1995). A detailed report of the optical quality of the lens has been published elsewhere (Herbert *et al.* 1999). The scanning lens monitor is a laser-based optical lens scanner developed at the University of Waterloo. The laser is a low power (3·0 mW) He–Ne laser positioned by an X–Y table using stepping motors. Two cameras, 90° from each other, capture the laser-beam image and transfer it to a computer where the beam's initial position and the slope of the refracted beam are recorded. The scanning lens monitor finds the optical centre of the test lens, that is, the point where the slope of the laser beam approaches infinity. The scanning lens monitor then scans eighteen readings across the rat lens at intervals of 0·2 mm for a total range of 3·6 mm, in both X and Y directions. Lenses were scanned immediately after dissection. When available, lenses of right and left eyes were pooled for a maximum of seventy-two focal length recordings per animal. Lenses showing damage from dissection were not included, therefore data were obtained for the remaining lens only. For every animal at least one lens was scanned.

Focal length results were analysed for the eighteen scanning positions in terms of the mean focal length and the focal length variability (spherical aberration or sharpness of focus). Lens damage does not often result in obvious change in mean focal length, presumably because the effect of a change in lens curvature (for example, due to swelling) might be neutralized by an opposite effect created by a change in the lens refractive index distribution. However, lens damage upsets the balance of factors responsible for controlling spherical aberration and for maintaining a well-defined focal point in the lens as a whole. Data collected from all lenses in each experiment can be expressed graphically, either as mean focal lengths (for all eighteen beam positions), or as focal length variability (standard error of the mean focal lengths). Thus it is possible to differentiate between a change in focal length and a change in sharpness of focus (Sivak, 1995).

Indices of oxidative damage: lipid peroxides and hydrogen peroxide concentration

Microsome samples from kidney and pancreas were measured for LP and inorganic peroxides. For the procedure, tissues (approximately 0·1–0·3 g) were homogenized in 0·1 M-Tris-HCl buffered saline (pH 8·3) using a Teflon homogenizer. The homogenate was centrifuged (2500 ***g***, 10 min), the pellet was discarded, and the supernatant (microsomes) was centrifuged again (23 000 ***g***, 30 min, 4°C). This second supernatant was divided into portions and stored at −20°C for analysis of protein. LP were determined by the thiobarbituric acid test (Uchiyama & Mihara, 1978). This assay measures lipid breakdown products, such as malonaldehyde, and it is used as evidence of tissue damage. Each microsome sample (200 μl) was added to a solution containing orthophosphoric acid (1·5 ml, 1 %), thiobarbituric acid (0·5 ml, 0·7 %) and distilled water (50 μl). Samples were vortexed, boiled (45 min), cooled to room temperature, and chloroform (1·5 ml) and glacial acetic acid (0·5 ml) were added. Samples were centrifuged (3500 ***g***, 10 min) and absorbance of the supernatant was measured. The difference between absorption at 535 nm and at 520 nm was used to reduce interference (Sawada & Carlson, 1985), and the results were expressed in terms of the change in relative absorbance per μg protein. 1,1,3,3-Tetraethoxypropane (malenaldehyde) was used as the standard. Microsomal samples from kidney and pancreas were also measured for inorganic peroxides as an indicator of H_2O_2 formation. This procedure measures the oxidation of *o*-dianisidine after treatment of samples with peroxidase to liberate molecular oxygen (Meiattini 1985; Shi *et al.* 1994). The absorption was measured at 600 nm, and H_2O_2 served as the standard.

Histological changes in the kidney

Formalin-fixed kidney tissue was dehydrated and embedded in paraffin for sectioning (3–5 μm thick). Sections were stained with haematoxylin and eosin and examined by a pathologist for determination of changes to the glomerulus and the convoluted tubules. A subset of the kidney samples were Ag-stained for confirmation of the changes observed by haematoxylin and eosin staining. The pathologist was 'blinded' to the treatment groups of the samples.

Statistical analysis

Differences between experimental groups for most outcome variables were evaluated using two-way ANOVA (Statview; Abacus Concepts Inc., Berkeley, CA, USA). Bonferonni's test was used in *post-hoc* analyses to assess differences between groups. For some of the outcome

variables (H_2O_2, focal length variability), variances among groups were not homogeneous. Based on residual plots there was no consistent pattern of non-constant variance, therefore *t* tests for non-homogeneous samples were conducted to determine differences between groups. For all analyses, a *P* value of $<0{\cdot}05$ was considered to be significant. All values reported are the mean with standard error of the mean unless otherwise noted.

Results

Body weight and plasma glucose, insulin and FR concentrations of HIGH STZ, MOD STZ and SHAM rats at the end of the study are shown in Table 2. Prior to STZ treatment and the introduction of the experimental diets, there was no difference in body weight, glucose or insulin concentrations among any of the groups (data not shown; plasma FR was not measured). After 12 weeks of experimental diets there was a significant main effect of STZ treatment on body weight ($P < 0{\cdot}0001$). Rats in the HIGH STZ group weighed significantly less than rats in the MOD STZ and SHAM groups ($P < 0{\cdot}0001$), and rats in the MOD STZ group weighed significantly less than those in the SHAM group ($P < 0{\cdot}0001$). There was no significant difference in body weight between dietary groups within any of the levels of STZ treatment.

Plasma glucose concentrations were significantly elevated in MOD STZ and HIGH STZ-treated rats compared with SHAM injected rats at the end of the experiment (main effect of STZ, $P < 0{\cdot}0001$). Glucose levels also differed significantly between HIGH STZ and MOD STZ groups ($P < 0{\cdot}0001$). Circulating plasma glucose did not differ significantly between the two dietary treatments within either of the STZ groups or within the SHAM group. Plasma insulin concentrations were significantly reduced in both groups of STZ-treated rats (main effect of STZ, $P < 0{\cdot}001$) relative to rats in the SHAM group and were significantly lower in rats from the HIGH STZ *v.* the MOD STZ groups ($P < 0{\cdot}03$). Dietary treatment had no effect on circulating insulin concentrations within the different STZ or SHAM treatment groups.

Dietary FR resulted in higher levels of plasma FR in the SHAM and both of the STZ-treated groups, compared with rats fed the CNTL diet ($P < 0{\cdot}001$). There was a significant main effect of diabetes on plasma FR ($P < 0{\cdot}0001$) such that the mean plasma FR concentration of rats in the HIGH STZ group was significantly higher than rats in the MOD STZ ($P < 0{\cdot}0004$) and SHAM groups ($P < 0{\cdot}0001$), but did not differ significantly between the MOD STZ and SHAM groups.

Damage to the lens was assessed by comparing the focal length and the focal length variability (Table 3) of lenses from rats in the different experimental groups using an

Table 3. Focal length and focal length variability of lenses from high streptozotocin, moderate streptozotocin, and sham-treated rats fed a control or fructose-based diet for 12 weeks*

(Mean values with standard errors of the means for ten to twelve rats per group)

Experimental group	Focal length (mm)		Focal length variability† (mm)	
	Mean	SEM	Mean	SEM
HIGH STZ				
CNTL	4·33‡	0·53	0·48‡[a]	0·04
FR	6·11§	0·85	0·95§[b]	0·11
MOD STZ				
CNTL	5·96	0·13	0·30[c]	0·06
FR	4·80	0·47	0·38[c]	0·07
SHAM				
CNTL	6·11	0·04	0·01[c]	0·00
FR	6·03	0·08	0·11[c]	0·01

HIGH STZ, high streptozotocin-treated group; CNTL, control diet; FR, fructose-based diet; MOD STZ, moderate streptozotocin-treated group; SHAM, sham-treated group.

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}01$).

* Moderate streptozotocin dose was 35 mg/kg body weight intravenously; high streptozotocin dose was 55 mg/kg body weight intravenously. For details of diets see Table 1. For details of measurement procedures see pp. 576–577.

† There was a significant diet×STZ interaction ($P < 0{\cdot}05$).

‡ 2/10 lenses were not scanned in this group due to thickness of cataract.

§ 4/9 lenses were not scanned in this group due to thickness of cataract.

Table 2. Body weights, fed-state glucose, insulin and fructose concentrations of high streptozotocin, moderate streptozotocin and sham-treated rats fed a control or fructose-based diet for 12 weeks*

(Mean values with standard errors of the means for ten to twelve rats per group)

Experimental group	Body weight (g)		Plasma glucose (mM)		Plasma insulin (ng/ml)		Plasma fructose† (μmol/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HIGH STZ								
CNTL	211[a]	8	35·7[a]	1·1	0·30[a]	0·04	490	65
FR	220[a]	8	39·9[a]	1·2	0·25[a]	0·04	1035	197
MOD STZ								
CNTL	314[b]	10	25·6[b]	1·7	0·70[b]	0·13	324	43
FR	307[b]	10	27·6[b]	2·0	0·94[b]	0·18	580	43
SHAM								
CNTL	380[c]	6	7·2[c]	0·5	2·67[c]	0·53	144	36
FR	374[c]	8	7·9[c]	0·4	3·46[c]	0·40	406	61

HIGH STZ, high streptozotocin-treated group; CNTL, control diet; FR, fructose-based diet; MOD STZ, moderate streptozotocin-treated group; SHAM, Sham-treated group.

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}001$).

* Moderate streptozotocin dose was 35 mg/kg body weight intravenously; high streptozotocin dose was 55 mg/kg body weight intravenously. For details of diets see Table 1.

† Statistically significant main effect of dietary fructose ($P < 0{\cdot}001$) and main effect of diabetes ($P < 0{\cdot}0001$). For details see p. 578.

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Table 4. Kidney and pancreas lipid peroxide and hydrogen peroxide concentrations from high streptozotocin, moderate streptozotocin and sham-treated rats fed a control or fructose-based diet for 12 weeks*

(Mean values with standard errors of the means for ten to twelve rats per group)

Experimental group	Lipid peroxide (change in relative absorbance (535–520 nm)/μg protein×10^{-6})				Hydrogen peroxide (μmol/μg protein)			
	Kidney		Pancreas		Kidney†		Pancreas	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HIGH STZ								
CNTL	4·65[a]	0·50	3·36[c]	0·44	0·010[c]	0·001	0·040[a]	0·006
FR	6·25[a]	0·44	3·54[c]	0·38	0·015[a]	0·001	0·036[a]	0·003
MOD STZ								
CNTL	4·96[a]	0·58	4·36[a]	0·57	0·006[b]	0·001	0·029[a]	0·004
FR	5·01[a]	0·61	4·74[a]	0·58	0·007[b]	0·001	0·030[a]	0·004
SHAM								
CNTL	3·35[b]	0·54	4·92[a]	0·49	0·006[b]	0·001	0·033[a]	0·004
FR	2·54[b]	0·29	4·45[a]	0·65	0·006[b]	0·001	0·030[a]	0·003

HIGH STZ, high streptozotocin-treated group; CNTL, control diet; FR, fructose based diet; MOD STZ, moderate streptozotocin-treated group, SHAM, sham-treated group.

[a,b,c]Mean values within a column with unlike superscript letters were significantly different (a *v.* c, $P < 0·05$; b *v.* c, $P < 0·01$; a *v.* b, $P < 0·001$).

* Moderate streptozotocin dose was 35 mg/kg body weight intravenously; high streptozotocin dose was 55 mg/kg body weight intravenously. For details of diets see pp. 576. For details of measurement procedures see pp. 576–577.

† There was a significant diet×STZ interaction ($P < 0·03$).

automated scanning laser system. There is a number of factors involved in controlling focal length, so that changes in the curvature of the lens may be neutralized (partially or fully) by opposite effects in lens refractive index, and focal length may not be altered even if lens damage is present. In contrast, if damage to the lens upsets the balance of any of the factors involved in controlling the spherical shape of the lens then a sharply defined focal point is no longer possible and focal length variability is increased. In this study, after 12 weeks, cataract development in the HIGH STZ groups had progressed to the point that lenses from two of ten rats in the CNTL-fed group, and from four of nine rats in the FR-fed group could not be scanned. These rats were removed from further analyses of the lens. Average focal length did not differ significantly among the various experimental groups. However, focal length variability was significantly greater in rats from the HIGH STZ groups relative to the other groups and focal length variability was greatest in HIGH STZ, FR-fed rats (difference significant *v.* HIGH STZ, CNTL-fed rats, $P < 0·01$).

There was a statistically significant interaction between diet and STZ treatment on H_2O_2 concentration in the kidney (Table 4; $P < 0·03$). Rats in the HIGH STZ group fed the FR diet had levels of H_2O_2 which were approximately 50 % higher than those observed in rats in the HIGH STZ group fed the CNTL diet ($P < 0·001$). The extent of diabetes also had a significant impact on the H_2O_2 concentrations observed in kidney microsomes. Kidney microsomes from rats in the HIGH STZ group had higher concentrations of inorganic peroxide compared with microsomes from rats in the MOD STZ ($P < 0·001$) and SHAM groups ($P < 0·0001$).

LP in kidney microsomes were significantly elevated in MOD STZ and HIGH STZ groups compared with SHAM groups (Table 4; $P < 0·0001$) but did not differ between dietary groups. In pancreatic microsomes, the concentration

Table 5. Kidney weights (absolute and relative) and urinary protein excretion rates of high streptozotocin, moderate streptozotocin and sham-treated rats fed a control or fructose-based diet for 12 weeks*

(Mean values and standard errors of the means for ten to twelve rats per group)

Experimental group	Kidney wt (g)		Relative kidney wt (g/kg body weight)		Urinary protein (mg/24 h)	
	Mean	SEM	Mean	SEM	Mean	SEM
HIGH STZ						
CNTL	1·75[a]	0·07	8·3[a]	0·3	7·5[a]	1·3
FR	1·91[a]	0·08	8·7[a]	0·3	12·6[a]	3·1
MOD STZ						
CNTL	1·72[a]	0·12	5·6[b]	0·5	15·1[b]	2·9
FR	1·93[a]	0·18	6·4[b]	0·5	17·8[b]	1·5
SHAM						
CNTL	1·08[b]	0·03	2·9[c]	0·1	15·2[b]	1·8
FR	1·21[b]	0·03	3·2[c]	0·1	15·5[b]	1·9

HIGH STZ, high streptozotocin-treated group; CNTL, control diet; FR, fructose-based diet; MOD STZ, moderate streptozotocin-treated group; SHAM, sham-treated group.

[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P < 0·001$).

* Moderate streptozotocin dose was 35 mg/kg body weight intravenously; high streptozotocin dose was 55 mg/kg body weight intravenously. For details of diets see pp. 576. For details of measurement procedures see pp. 576–577.

of LP was significantly lower in HIGH STZ *v.* MOD STZ and SHAM groups ($P < 0{\cdot}03$), although there was no difference between dietary groups. H_2O_2 concentrations in microsomes from the pancreas did not differ significantly among experimental groups.

Kidneys from HIGH STZ and MOD STZ rats were significantly heavier than those from SHAM-injected rats (Table 5; $P < 0{\cdot}001$). Kidney weight relative to body weight was significantly higher in the HIGH STZ group compared with the MOD STZ and SHAM groups ($P < 0{\cdot}0004$ and $P < 0{\cdot}0001$ respectively), and was higher in rats from the MOD STZ group compared with the SHAM group ($P < 0{\cdot}0001$). There was no significant difference between dietary groups.

Rats in the HIGH STZ groups showed signs of hyperfiltration since urine output was significantly elevated in the HIGH STZ group (data not shown) but the protein concentration in urine was significantly lower in rats in the HIGH STZ groups compared with rats in the MOD STZ and SHAM groups (Table 5; $P < 0{\cdot}03$). Protein concentration in the urine did not differ between CNTL and FR-fed groups. In histological examination of kidney sections, a moderate degree of vacuolization was noted from the apical surface of the epithelium lining of the convoluted tubules in MOD STZ and HIGH STZ rats, but this did not differ between dietary groups. The degree of damage to the convoluted tubules was not considered to be extensive or irreversible. Regional accumulation of protein was noted in tubules from some, but not all, rats in the MOD STZ, FR and HIGH STZ, FR groups but was not observed in any kidney sections from rats fed the CNTL diet. There were no changes to glomerular structure and no nuclear changes observed in any of the sections of either haematoxylin-and-eosin-stained slides, or Ag-stained slides.

Discussion

Under conditions of severe hyperglycaemia, high dietary FR intake led to increased plasma FR concentrations, promoted the development of cataracts in the lens of the eye and increased the concentrations of H_2O_2 and LP in the kidney over a 12-week period. This suggests that high levels of dietary FR and severe hyperglycaemia have interactive effects which may contribute to the development of complications associated with diabetes. Dietary FR did not improve glycaemia, circulating insulin concentrations or growth in any of the diabetic rats. In human subjects and rats, high FR intake increases plasma lipid levels (Gerrits & Tsalikian, 1993; Hollenbeck, 1993; Bell *et al.* 1996). Thus, although FR supplementation has been advocated for patients with diabetes mellitus, the lack of positive effects on glycaemic control coupled with adverse side-effects suggests that high FR intake is not recommended for diabetic populations whose glucose concentrations are uncontrolled.

In this present study, long-term intake of the high FR diet resulted in significantly increased plasma FR concentrations compared with rats fed the CNTL diet. The plasma FR concentrations observed in the SHAM, FR group in this study are similar to those observed by Boot-Handford & Heath (1981) after 6 months of sucrose feeding, and by Topping & Mayes (1971) after an acute intragastric fructose load. Under normal conditions, approximately 55–71 % of FR absorbed from the intestine is extracted by the liver and quickly phosphorylated by fructokinase, thus peripheral FR concentrations are very low (Topping & Mayes, 1971; Mayes, 1993). Feeding rats a high FR diet has been shown to increase the mRNA, transport activity (Miyamoto *et al.* 1993), and expression (Darakhshan *et al.* 1998) of the fructose transporter, GLUT5, in the intestine, and to increase hepatic fructokinase activity (Shafrir & Orevi, 1984). Darakhshan *et al.* (1998) have also reported increased GLUT5 expression in the kidney of high FR-fed rats, but no change in GLUT5 in skeletal muscle or adipose tissue. These tissue-specific adaptations to high FR intake may promote increased absorption and reabsorption of FR without changing FR uptake by peripheral tissues and thus may result in increased circulating FR concentrations. The increased plasma FR concentrations observed in groups fed the high FR *v.* CNTL diet may also suggest that the fructokinase changes in the liver induced by high FR intake do not completely balance the increased intestinal absorption, and that there is some overspill of dietary FR into the plasma.

Severe hyperglycaemia was also shown to independently increase plasma FR concentrations (Table 2). Experimental diabetes has been shown to increase intestinal absorption of sugars and to increase intestinal expression of GLUT5 by 80–90 % (Castello *et al.* 1995), while at the same time dampening the diet-induced adaptation of hepatic fructokinase by approximately 50 % and reducing GLUT5 expression in adipocytes by approximately 75 %. The changes reported by other researchers are in directions that might contribute to higher peripheral FR concentrations among HIGH STZ- compared with MOD STZ- and SHAM-treated rats.

Intake of the high FR diet was associated with increased focal length variability among rats in the HIGH STZ group (*v.* HIGH STZ, CNTL), despite similar plasma glucose levels. This indicates more severe cataract development in FR-fed rats with marked hyperglycaemia. It has been suggested that increased cataract formation in diabetic patients involves activation of the polyol pathway in the lens (Cheng *et al.* 1989; Gaynes & Watkins, 1989; Lal *et al.* 1995; Lindsay *et al.* 1998). This alternative pathway of glucose metabolism is known to exist in cells of the lens (Cheng *et al.* 1989; Gaynes & Watkins, 1989; Lal *et al.* 1995, 1997), and the retina (Hotta *et al.* 1997). Once active, the polyol pathway produces high levels of intracellular sorbitol, fructose, and their metabolites (e.g. fructose-3-phosphate), and this is associated with increased glycation of lens crystalline (Lal *et al.* 1995), hyperosmotic effects, and decreased levels of several metabolites which ultimately may lead to opacification of the lens (Kador, 1989; Bron *et al.* 1993). If high plasma FR levels further contribute to the FR load in the lens of hyperglycaemic rats, then this may promote cataract formation. In this present study, the concentration of FR in the lens was not determined since lenses were placed in short-term tissue culture for quantification of cataract formation.

In the present study, severe diabetes led to increases in the concentrations of H_2O_2 and LP in kidney microsomes,

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but the levels of these substances were highest in the HIGH STZ, FR group (Table 4). Although these outcome measures are somewhat crude, our results are consistent with increased oxidative stress among the most severely diabetic rats, and the FR diet acting to promote this stress. Increased oxidative damage in the tissues of diabetics is well documented (Wolfe & Dean, 1987; Baynes, 1991) and may result from a combination of factors. For example, with diabetes, reactive oxygen species are increased and have been shown to be associated with the amount of protein glycation (Hunt *et al.* 1988). Diabetes also reduces the activity of the antioxidant enzymes catalase and superoxide dismutase in rat kidney (Wohaieb & Godin, 1987). Together, these effects may combine to increase oxidative stress under diabetic conditions and therefore could contribute to the increased inorganic peroxide concentrations observed in the kidney of HIGH STZ groups relative to MOD STZ and SHAM groups (see Table 4).

Mechanisms through which dietary FR acts to further increase oxidative stress in severely hyperglycaemic rats are speculative. Using *in vitro* methods, Kaneto *et al.* (1996) and Takagi *et al.* (1995) noted that high concentrations of reducing sugars (50–100 mM), particularly fructose, triggers oxidative modification of proteins more effectively than similar concentrations of glucose. These modifications seem to result in an increased formation of hydroxyl radicals (Takagi *et al.* 1995). Yan & Harding (1997) have shown *in vitro* that fructose is able to inactivate, and causes a loss of antigenicity, of catalase and SOD. If such changes occur *in vivo*, then they may contribute to increased oxidative damage in the HIGH STZ, FR group. Thus it would appear that the combination of hyperglycaemia and high dietary FR may have interactive effects which could contribute to compromised antioxidant enzyme action.

Although high dietary FR coupled with severe hyperglycaemia for 12 weeks was associated with evidence of increased oxidative damage in kidney microsomes there was no obvious functional or histological damage to the kidney. Kidney hypertrophy was similar in both groups of HIGH STZ-treated rats suggesting that this organ was responding appropriately to the severe hyperglycaemia and that diet did not affect this response. Nyengaard *et al.* (1997) observed microalbuminuria in only one out of eight STZ-diabetic rats after 16 weeks of diabetes, and increases in glomerular and mesangial volume after 7 months of diabetes. Diabetic rats showed glomerulosclerosis ranging from mild to severe after 6 months of diabetes (Boot-Handford & Heath, 1981). Thus, overt histological and functional damage in the kidney of diabetic rats may require more than 12 weeks to develop, although markers of damage may be present earlier. Interestingly, the functional damage in the lens was marked at the 12 week time-point in HIGH STZ rats. This suggests that there are tissue specific differences in susceptibility to damage from the combination of severe hyperglycaemia and dietary FR intake.

In summary, this study has shown that under conditions of severe hyperglycaemia, high FR intake promotes the development of cataracts in the lens of the eye, and increases the concentrations of hydrogen peroxide and LP, suggestive of increased oxidative stress in the kidney. The mechanisms contributing to the interactive effects of high dietary FR intake and hyperglycaemia on development of diabetic complications are likely to be multifactorial, and this study suggests that those related to oxidative stress and polyol pathway kinetics are important for further investigation. From a clinical perspective, this study suggests that high FR intake may have adverse manifestations in addition to the effects on lipidaemia, particularly in uncontrolled diabetics. Titration of the interactions between levels of FR intake and the degree of hyperglycaemia, along with more extensive documentation of the timing of onset of adverse effects may help to guide clinical recommendations in the future.

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