Low-Carbohydrate Diet and Oxidative Stress in Diabetic and Nondiabetic Rats

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ABSTRACT: Hyperglycemia of diabetes has been implicated in increased tissue oxidative stress, with consequent development of secondary complications. Thus, stabilizing glucose levels near normal levels is of utmost importance. Because diet influences glycemic control, this study investigated whether a low-carbohydrate (5.5%) diet confers beneficial effects on the oxidative status of the heart, kidney, and liver in diabetes. Male and female normal and diabetic rats were fed standard chow (63% carbohydrates) or low-carbohydrate diet for 30 days. Elevated glucose, HbA_{1c}, and alanine and aspartate aminotransferases in diabetic animals were reduced or normalized by the low-carbohydrate diet. While diabetes increased cardiac activities of glutathione peroxidase and catalase, low-carbohydrate diet normalized cardiac glutathione peroxidase activity in diabetic animals, and reduced catalase activity in females. Diabetic rats fed low-carbohydrate diet had altered activities of renal glutathione reductase and superoxide dismutase, but increased renal glutathione peroxidase activity in diabetic animals was not corrected by the test diet. In the liver, diabetes was associated with a decrease in catalase activity and glutathione levels and an increase in glutathione peroxidase and γ-glutamyltranspeptidase activities. Decreased hepatic glutathione peroxidase activity and lipid peroxidation were noted in diet-treated diabetic rats. Overall, the low-carbohydrate diet helped stabilize hyperglycemia and did not produce overtly negative effects in tissues of normal or diabetic rats.
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KEYWORDS: **Liver; Kidney; Heart; Rat; Oxidative Stress; Carbohydrate; Diabetes**

INTRODUCTION

Diabetes, a disease characterized by the chronic presence of hyperglycemia, is usually accompanied by specific long-term complications such as diabetic nephropathy, retinopathy, neuropathy, and cardiovascular complications. Persistent high glucose concentration in tissues has been convincingly linked to the overproduction of reactive oxygen species (ROS) [1] and increased cellular oxidative stress [2] that are postulated to play a central role as the initiators of the complications of chronic diabetes [3,4]. A number of possible mechanisms linking glucose to ROS include the polyol pathway [5], glucose auto-oxidation [6], advanced glycation end product formation [7], protein kinase C, and NADPH oxidase activation [8,9]. The products of these pathways modify the expression and characteristics of cellular and extracellular components, compromising their ability to function normally and leading to tissue injury. The damage caused by ROS becomes inevitable when the generation of ROS overwhelms the capacity of tissues to quench them. The sensitivity and response of tissues to changes in redox status appear to be tissue specific, resulting in distinct pathological effects in each tissue type [10,11]. In a similar manner, a wide variety of exogenous compounds that reverse the diabetes-induced alterations of antioxidant markers [12] are tissue and antioxidant-marker specific.

Diet is one of the parameters that may be manipulated to improve blood glucose control in diabetic patients. One outcome of diet studies has been the categorization of types of carbohydrates and fats into those that are beneficial and those that are detrimental to glucose control, weight loss, and desirable lipid profile. A low-carbohydrate, higher fat diet, if incorporating beneficial fats (monosaturated fatty acids of a cis-confuguration), appears to be superior to a high-carbohydrate diet in achieving good glucose control as well as better lipid profile independent of weight loss,

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so long as the desired caloric content is maintained, in diabetic and glucose intolerant subjects [13–18]. In type 1 diabetics, a diet high in monounsaturated fats benefits fasting lipoprotein profiles [16]. Furthermore, when the insulin regimen is strictly tailored to individual requirements and carbohydrates are restricted to 30 g a day, both HbA_{1c} and lipid profiles dramatically improve compared to the baseline values when subjects eat an average carbohydrate diet [19]. A comprehensive review of the efficacy and safety of low-carbohydrate diets has cited over 2600 articles for relevance and comparison [20].

Since chronic hyperglycemia is associated with increased oxidative stress, we hypothesized that a diet, which leads to a consistently lower level of blood glucose, would also correlate with a reduced generation of ROS and more normal antioxidant levels. This study used the streptozotocin-induced diabetic rat as a model of type 1 diabetes to investigate whether, in addition to the favorable effects on blood glucose resulting from a low-carbohydrate diet, diabetes-induced changes in the oxidative status of the liver, kidney, and heart would also be reversed.

MATERIALS AND METHODS

Reagents

Bovine serum albumin, Folin-Ciocalteau phenol, Tris-HCl, reduced glutathione (GSH), oxidized glutathione (GSSG), NADPH, glutathione reductase, cumene hydroperoxide, cytochrome C, xanthine, xanthine oxidase, glycylglycine, l-glutamyl-*p*-nitroanilide, *N*-ethylmaleimide, *o*-phthalaldehyde, metaphosphoric acid, and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest reagent grade. Deionized water was used for all assays.

Diets

Carbohydrates (primarily corn, wheat, molasses, oats, alfalfa, beet pulp) contributed 62%, and fats (mainly soybean oil) 12% of total calories in the control diet, Purina rat chow (no. 5012, St. Louis, MO). The low-carbohydrate diet was prepared by Dyets Inc. (Bethlehem, PA) to supply about 68% calories from fat (corn oil, flaxseed oil, sunflower oil, and coconut oil) and 5.4% calories from carbohydrate (primarily corn starch; see Table 1). The proportion of protein calories in the two diets was similar, and both were primarily soy based. Vitamins and minerals added as premixtures are included within the calculation of other nutrients. Distribution of saturated and unsaturated fats in the two diets is shown in Table 2.

TABLE 2. Approximate Breakdown of Fatty Acid Composition in Control and Test Diets

Fats (% by wt of Total Diet)	*Purina Rat Chow (Control Diet)*	*Low-Carbohydrate Diet (Test Diet)*
Linoleic acid (omega-6)	1.8	3.8
Omega-3 fatty acids	0.3	0.8
Total saturated fatty acids	0.7	29.7
Total monounsaturated fatty acids	1.0	3.2
Cholesterol (ppm)	180	200

Animals

A total of 92 Sprague-Dawley rats (Harlan, Indianapolis, IN), half males and half females weighing between 75 and 100 g were given 3 days to acclimatize to their new environment. Each gender was randomly divided into four groups ($n = 10$–14). Two groups of each sex were rendered diabetic by intraperitoneal injection of 75 mg/kg streptozotocin in citrate buffer. Glucose levels were measured in blood samples from the tail using a glucometer (Elite XL kit). Treatment was initiated only after blood glucose levels above 350 mg/dL confirmed diabetes. Within each sex, one nondiabetic group (normal control) and one diabetic group (diabetic control) were fed standard rat chow diet and acted as controls. The other nondiabetic (normal test) and diabetic group (diabetic test) of each gender were fed the low-carbohydrate diet (test diet). Rats received

TABLE 1. Nutrient Composition of Diets per Weight and Calorie

	Purina Rat Chow (5012)			*Low-Carbohydrate (Test) Diet*		
Nutrient	*% Weight (g/100 g)*	*Calorie (kcal/g)*	*% Calorie*	*% Weight (g/100 g)*	*Calorie (kcal/g)*	*% Calorie*
Protein	22.5	90	26	36.9	136	27
Carbohydrate	52.6	212	63	4.0	27	5
Fat	4.0	36	11	37.5	337	68
Total		338	100		500	100

Minerals and vitamins were premixed with vehicle, the energy content of which is included in the values of carbohydrate, fat, and protein.

the diet for 30 days before sacrifice. Food and tap water were supplied ad libitum, and a normal daylight cycle was maintained. Animal care and handling followed the National Institutes of Health guidelines [21].

Assay of Tissue Markers of Oxidative Stress

Animals were sacrificed after inhalation of 2% isoflurane, administered by appropriate vaporizer with compressed air as the carrier gas. There was no evidence of hypoxia. Blood was drawn by cardiac puncture and sent to the ASCP-accredited pathology lab at Bloomington Hospital for analysis of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Glycated hemoglobin (HbA_{1c}) was measured using the Sigma kit 441-B (St. Louis, MO). Organs were removed, weighed, immediately frozen in liquid nitrogen and stored at $-80^{\circ}C$ awaiting analysis. An aliquot of 0.25 g of each frozen tissue was homogenized with a Brinkmann Polytron homogenizer in 4.75 mL of ice-cold 0.1 M phosphate buffer at pH 7.6. The homogenate was then centrifuged for 1 h at $55{,}000\times g$, $4^{\circ}C$. The pellet was resuspended in 0.1 M Tris-HCl/$MgCl_2$ buffer, pH 9.0, and assayed for γ-glutamyl transferase (GGT) activity [22], whereas the supernatant (5% cytosol) was used to carry out the following assays.

The activities of glutathione reductase [23], glutathione peroxidase [24], catalase [25], and superoxide dismutase [26] were measured spectrophotometrically. In the liver, the amounts of reduced glutathione (GSH), oxidized glutathione (GSSG), and malondialdehyde (MDA) were measured using Bioxytech® GSH/GSSG-412™ kit and Bioxytech® MDA-586™ kit, respectively, following the manufacturer's instructions (Oxis International, Portland, OR). For the measurement of GSH and GSSG in kidney and heart, fresh 5% cytosol was prepared as above but using a 0.1 M sodium phosphate/5 mM EDTA solution to which was added an appropriate volume of a 25% HPO_3 solution as the buffer. The fluorometric method of Hissin and Hilf [27] was then followed. Protein levels of all tissue preparations were measured by the Lowry method [28]. All assays were performed in duplicate along with blanks and standards, and results calculated relative to the amount of protein present.

Statistical Analysis

Results are given as mean ± standard error of mean and analyzed by one-way ANOVA followed by Duncan's multiple range test. Significance was set at $p \leq 0.05$. Data for each tissue were compared within sexes only. Each group was compared to the nondiabetic control, and the two diabetic groups were also compared.

RESULTS

Body Weight and Organ Weights

There was no significant difference in body weights among the rats at the beginning of the study (data not shown). After the induction of diabetes, diabetic rats on the standard diet ate about 50% more food than nondiabetic controls (Table 3), but remained significantly lighter than nondiabetics throughout the study (Table 4). Diabetic animals on the low-carbohydrate diet consumed amounts equivalent in calorie content to normal rats (Table 3), and their weight was significantly lower than that of diabetic controls throughout the study (Table 4). There was no significant difference in body weight between normal controls and nondiabetic rats receiving the treatment diet in both male and female groups. Therefore, the low-carbohydrate diet did not trigger an increase in body weight in the normal rats and the gain in body weight in the diabetic animals was minimal.

Diabetes was associated with an increase in liver-to-body weight ratio (Table 4) in both males and females. The low-carbohydrate diet did not alter this ratio in the diabetics but increased it in the nondiabetic female rats. Diabetes correlated with an increase in kidney- and heart-to-body-weight ratios which was exacerbated by the low-carbohydrate diet in both male and female animals (Table 4).

Serum Markers

Treatment with low-carbohydrate diet significantly reduced both glucose and HbA_{1c} levels (Table 5) in the diabetics (~62% and 88% of diabetic control, respectively), but did not normalize them. The activities of serum enzymes ALT, AST, and ALP (Table 5) were significantly higher in diabetic rats than in normal

TABLE 3. Food Intake in 24 h Period after 30 Days on Normal or Low-Carbohydrate Diet

Treatment Groups (Male)	*n*	*kcal/24 h*	*kcal/100 g Body wt/24 h*
Normal control	12	8110 ± 66	50.7 ± 1.13
Normal + test diet	12	8150 ± 216	50.2 ± 0.28
Diabetic control	18	12600 ± 740 [a]	62.1 ± 5.63 [a]
Diabetic + test diet	16	7780 ± 232 [b]	36.1 ± 1.54 [c]

Values are means ± SEM, $p \leq 0.05$.
[a] Significantly different from normal controls.
[b] Significantly different from diabetic controls.
[c] Significantly different from both normal and diabetic controls.

TABLE 4. Body and Tissue Weights of Animals in Treatment Groups

	n	*Body Weight (g)*	*Liver Weight (g)*	*Liver Weight/ Body Weight (%)*	*Kidney Weight (g)*	*Kidney Weight/ Body Weight (%)*	*Heart Weight (g)*	*Heart Weight/ Body Weight (%)*
Males								
Normal control	10	316 ± 2	12.2 ± 0.1	3.86 ± 0.02	2.29 ± 0.02	0.72 ± 0.00	1.15 ± 0.01	0.36 ± 0.00
Normal test diet	10	317 ± 1	12.4 ± 0.3	3.91 ± 0.05	2.31 ± 0.01	0.73 ± 0.00	1.21 ± 0.01	0.38 ± 0.00
Diabetic control	12	223 ± 16[a]	11.3 ± 0.7	5.01 ± 0.22[a]	2.76 ± 0.19[a]	1.25 ± 0.02[a]	0.86 ± 0.06[a]	0.38 ± 0.01
Diabetic test diet	14	170 ± 4[c]	7.9 ± 0.2[c]	4.76 ± 0.03[a]	2.28 ± 0.05[c]	1.45 ± 0.03[c]	0.94 ± 0.05[a]	0.55 ± 0.02[c]
Females								
Normal control	10	216 ± 2	7.7 ± 0.1	3.58 ± 0.02	1.49 ± 0.02	0.69 ± 0.01	0.82 ± 0.01	0.38 ± 0.00
Normal test diet	10	209 ± 1	8.6 ± 0.1[a]	4.14 ± 0. 03[a]	1.50 ± 0.01	0.72 ± 0.01	0.82 ± 0.01	0.39 ± 0.00
Diabetic control	13	163 ± 8[a]	9.3 ± 0.3[a]	5.72 ± 0.11[a]	2.02 ± 0.09[a]	1.24 ± 0.02[a]	0.67 ± 0.02[a]	0.41 ± 0.01[a]
Diabetic test diet	13	135 ± 2[c]	7.5 ± 0.1[b]	5.62 ± 0.04[a]	2.11 ± 0.02[a]	1.59 ± 0.01[c]	0.69 ± 0.01[a]	0.51 ± 0.00[c]

Values are means ± SEM, $p \leq 0.05$.
[a] Significantly different from normal controls.
[b] Significantly different from diabetic controls.
[c] Significantly different from both normal and diabetic controls.

TABLE 5. Selected Characteristics of Animals, Including Activities of Serum Enzymes

	n	*Glucose (mg/dL)*	*HbA1c (%)*	*ALT (Units/L)*	*AST (Units/L)*	*ALP (Units/L)*
Males						
Normal control	10	77 ± 1	1.96 ± 0.04	56.4 ± 0.5	61.3 ± 0.7	221 ± 1.6
Normal test diet	10	79 ± 1	1.90 ± 0.03	62.2 ± 0.6	72.8 ± 1.3	525 ± 9.0[a]
Diabetic control	12	417 ± 11[a]	4.00 ± 0.19[a]	116 ± 6.4[a]	98.4 ± 7.3[a]	1010 ± 84[a]
Diabetic test diet	14	119 ± 14[c]	3.53 ± 0.08[c]	77.5 ± 1.1[c]	61.6 ± 1.0[b]	884 ± 12.8[c]
Females						
Normal control	10	82 ± 0	2.03 ± 0.04	52.7 ± 0.5	62.8 ± 0.7	186 ± 3.3
Normal test diet	10	77 ± 1	2.12 ± 0.07	59.7 ± 0.6	67.3 ± 0.6	514 ± 12.5[a]
Diabetic control	13	418 ± 45[a]	4.42 ± 0.12[a]	201 ± 28[a]	207 ± 34.6[a]	1030 ± 82.7[a]
Diabetic test diet	13	258 ± 18[c]	3.84 ± 0.21[c]	86.8 ± 1.9[b]	69.2 ± 1.5[b]	826 ± 9.7[c]

Values are mean ± SEM, $p \leq 0.05$.
[a] Significantly different from normal controls.
[b] Significantly different from diabetic controls.
[c] Significantly different from both normal and diabetic controls.

controls. The low-carbohydrate diet had the beneficial effect of normalizing the activity of AST and significantly reducing the activity of ALT and ALP. In contrast, in both male and female nondiabetic animals, treatment with low-carbohydrate diet elevated serum ALP activity while the activities of AST and ALT were not affected.

Hepatic Markers of Oxidative Stress

Levels of enzyme activity in the liver are presented in Figure 1. No significant changes from the normal control were observed in activities of GRx and SOD. CAT activity was decreased in both males and females by diabetes and not impacted by the test diet. The low-carbohydrate diet also decreased CAT activity in the nondiabetic females. There was a mixed effect of diabetes, low-carbohydrate diet, and gender on GPx; however, the low-carbohydrate diet decreased GPx activity in diabetic animals when compared to diabetic controls. A diabetes-associated decrease in hepatic GSH (Figure 2) was not reversed by the treatment diet, whereas both diabetes and the treatment diet were associated with elevations in GGT activity (Figure 2). GSSG was reduced in diabetic animals on the low-carbohydrate diet only. The low-carbohydrate diet beneficially reduced MDA levels (Figure 2) in male nondiabetics.

Renal Markers of Oxidative Stress

Figure 3 shows results of enzyme activity assays in the kidney. The low-carbohydrate diet reduced the activity of SOD only in the diabetics relative to both control groups. The activity of renal CAT was depressed by diabetes in the males and not affected by the low-carbohydrate diet. While diabetes lowered the activity of GRx in females, the treatment diet normalized this activity and in the males increased it above the nondiabetic control. The diabetes-induced elevation of GPx activity (>60% above nondiabetic control) was not modified by the low-carbohydrate diet. Activity of GGT (Figure 4) was reduced by diabetes in the females but not affected by the low-carbohydrate diet.

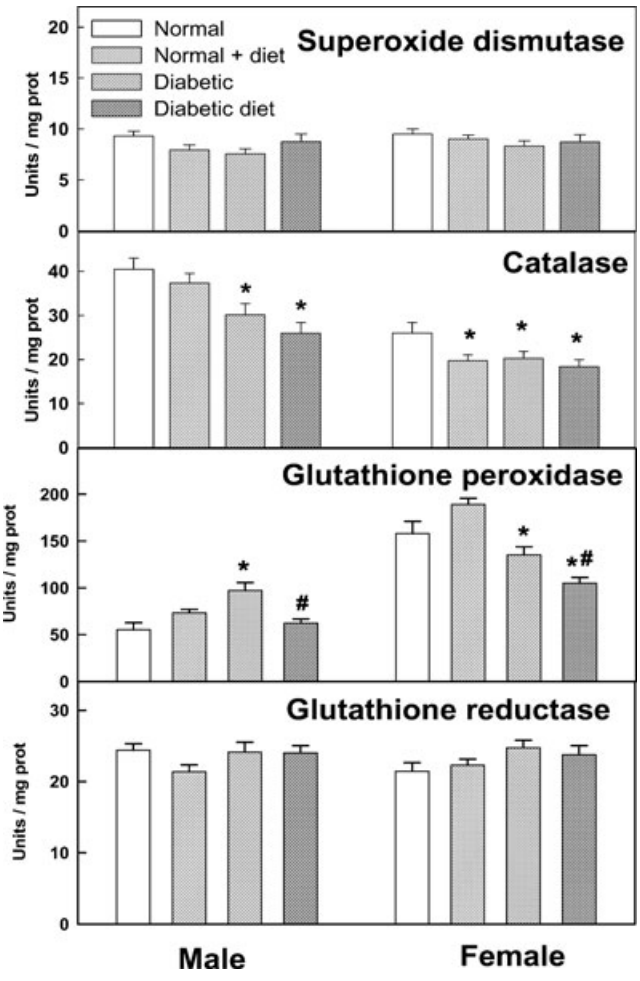

FIGURE 1. Activity of hepatic superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. One unit of superoxide dismutase activity is that which produces 50% inhibition of the reduction of cytochrome C in the presence of superoxide radical. One unit of catalase activity liberates half of the peroxide oxygen from solution in 100 s at 25°C. One unit of glutathione reductase or glutathione peroxidase activity oxidizes 1 nmol of NADPH/min at 30°C. Values are mean ± SEM and $p \leq 0.05$ when compared with the nondiabetic control (*) or diabetic control (#), $n = 10$–13.

No differences were observed in concentrations of GSH (Figure 4), while only trace amounts of GSSG were observed. The only change seen in the treated nondiabetic animals was an elevation in GPx (40% above nondiabetic control) in the males.

Cardiac Markers of Oxidative Stress

No changes were observed in activities of cardiac SOD and GRx as shown in Figure 5. Diabetes was associated with increases in cardiac CAT activity (>425% above nondiabetic control), while the low-carbohydrate diet restored it back toward normal. The activity of GPx raised by diabetes was reversed but not normalized by the low-carbohydrate diet. Diabetes decreased the ratio of GSH/GSSG (Figure 6) in both males and females, and reduced the amounts of GSSG in the males. The low-carbohydrate diet did not modify these effects in diabetics but instead decreased GSH/GSSG ratio in the nondiabetic groups.

DISCUSSION

Because carbohydrates are absorbed as glucose, they cause an immediate increase in blood glucose whose concentrations will then depend on how fast and how much glucose is absorbed from the gut and how fast it is used up or stored once in circulation [29]. A low-carbohydrate diet, having more of its contents absorbed as triglycerides, will cause only a minimal increase in glucose concentrations, and therefore would mitigate one of the major problems encountered in diabetes. The reduction in glucose levels is expected to translate to a reduction in the generation of ROS and an improvement in oxidative status.

This examination of a type 1 diabetic rat model noted a decrease, though not normalization, of glucose and HbA_{1c} levels in diabetic animals fed a low-carbohydrate diet. Other investigations of high- and low-carbohydrate diets in noninsulin-dependent [13,14,18,30,31] and insulin-dependent [16] diabetics have also observed that a low-carbohydrate high-fat diet results in a decrease in the levels of glucose and triglycerides when compared to baseline values.

As observed elsewhere [32], diabetic animals gained less weight than nondiabetic animals (54–62% of normal control). The association of diabetes with fatty liver may explain the increase in weight of liver relative to body weight, although a decrease in apoptosis has also been noted in diabetic liver [33]. In this regard, the low-carbohydrate diet was more beneficial to the males (not affecting the nondiabetic and reducing liver-to-body weight ratio in diabetics) and unfavorable to the females, where it increased this ratio in the nondiabetic animals and did not reduce it in the diabetics.

The increase in heart-to-body-weight ratio in diabetics may be due to an increase in the ventricle-to-body-weight ratios [34]. A number of studies of nondiabetic models have found that hypertrophied hearts in the absence of heart failure show increased resistance to oxidative stress when subjected to ischemic stress, and exhibit increased SOD activity and decreased lipid peroxidation [35–38]. Thus the development of cardiomegaly in conjunction with increased activities of

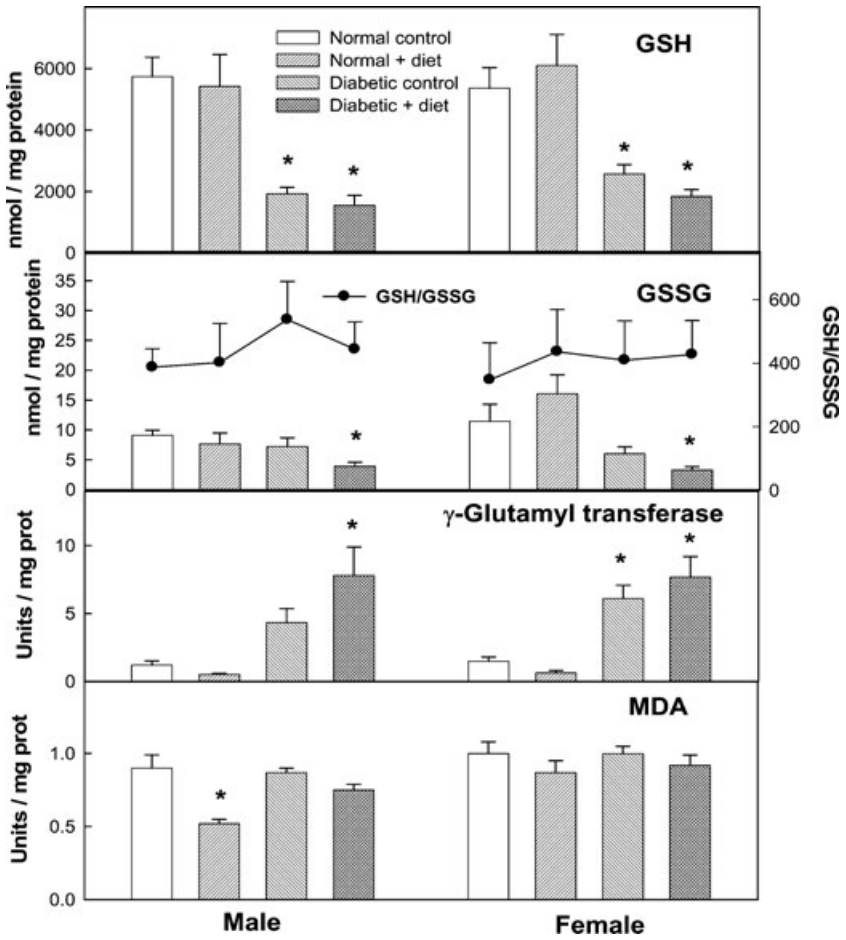

FIGURE 2. Levels of reduced glutathione (GSH) and glutathione disulfide (GSSG), γ-glutamyl transferase activity, and malondialdehyde (MDA) as a measure of lipid peroxidation in liver of male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. Connected filled circles represent the ratio of GSH to GSSG in each group. Values are mean $\pm$ SEM and $p \leq 0.05$ compared with the nondiabetic control (*) or diabetic control (#), $n = 3$–13.

some antioxidant enzymes, as seen in diabetes, appears to be an adaptation to cope with increased oxidative stress. The low-carbohydrate diet appears to intensify the cardiomegaly seen in diabetes, an effect that requires further investigation.

An increase in glomerular volume and associated renal hypertrophy is responsible for an increase in the kidney weight of hyperglycemic, STZ-induced diabetic rats [39]. Though these diabetic animals consuming a low-carbohydrate diet had significantly reduced glycemic parameters compared with the diabetic controls, a comparable reduction in kidney weight was not observed. Further research may determine exactly how tightly blood glucose must be controlled to prevent the development of abnormalities in the kidney.

The response to increased oxidative stress associated with diabetes is complex and specific to each tissue. In diabetic male rats, investigators have observed an increase in hepatic glutathione peroxidase [40,41], decrease [42,43], or no difference [32,44] when compared to nondiabetic animals. One other study noted no effect of diabetes on hepatic glutathione peroxidase activity in female rats [45]. Various confounding factors such as differences in the dose of the diabetogen (streptozotocin in all these cases), duration of diabetes, gender, or age may contribute to differing responses.

In this study, the ambiguities in hepatic glutathione peroxidase activities may resolve with longer term administration of the treatments. Since the levels of hepatic glutathione reductase activity were apparently normal, and the amounts of GSSG in the diabetics lower than control values, the decrease in hepatic GSH levels in these animals is most probably due to a decrease in de novo synthesis that could be compounded by increased flow of GSH out of the liver to supply other organs, particularly the kidney [46]. This decrease in de novo synthesis may be due to the reduced activity of γ-glutamyl cysteine ligase [47,48], the rate-limiting enzyme in the synthesis of GSH, rather than to a relative lack of amino acids, which were present in the two

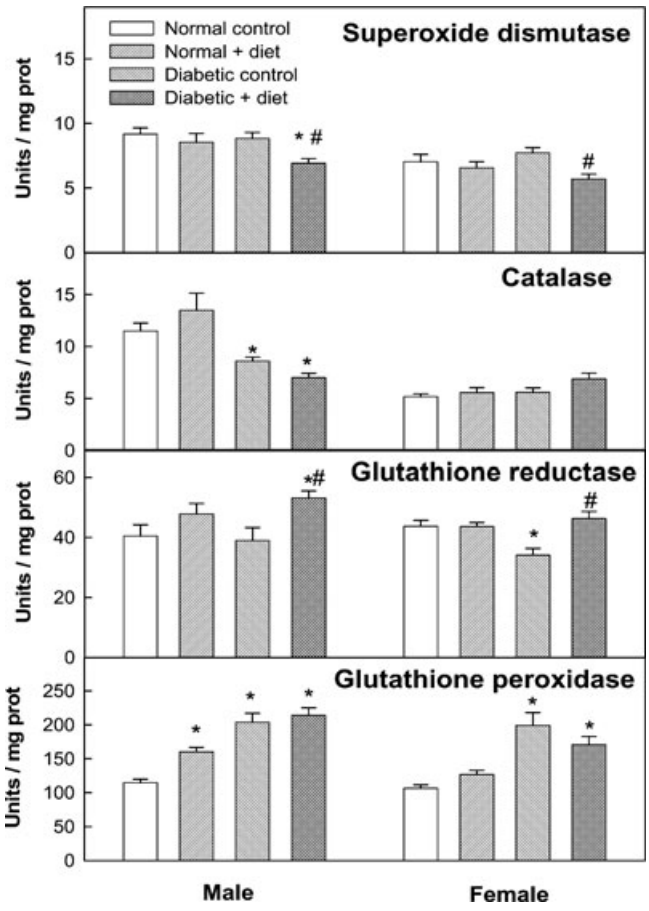

FIGURE 3. Activity of renal superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. One unit of superoxide dismutase activity is that which produces 50% inhibition of the reduction of cytochrome C in the presence of superoxide radical. One unit of catalase activity liberates half of the peroxide oxygen from solution in 100 s at 25°C. One unit of glutathione reductase or glutathione peroxidase activity oxidizes 1 nmol of NADPH/min at 30°C. Values are mean ± SEM and $p \leq 0.05$ when compared with the nondiabetic control (*) or diabetic control (#), $n = 10$–13.

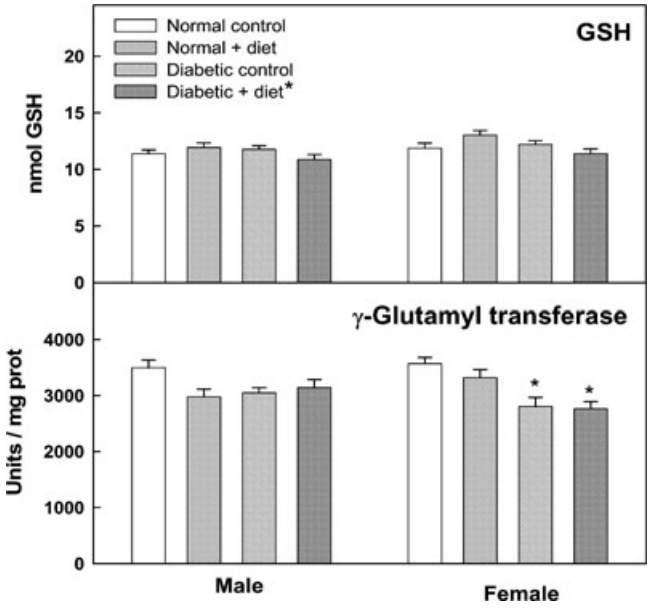

FIGURE 4. Levels of reduced glutathione (GSH) and γ-glutamyl transferase activity in kidney of male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. Values are mean ± SEM and $p \leq 0.05$ when compared with the nondiabetic control (*) or diabetic control (#), $n = 3$–13.

diets in comparable amounts. The low levels of cellular GSH, in turn, contribute to low levels of GSSG. No change in the activity of hepatic glutathione reductase was discerned in diabetics relative to nondiabetic control animals, as has been observed by others [2,32,49–51]. This would be consistent with the fact that despite a decrease in GSH, no increase in GSSG level was seen.

The diabetes-induced increase in hepatic GGT activity seen in this study, also observed in previous studies [52], was not greatly altered after 30 days of treatment with low-carbohydrate diet. GGT is closely tied with the degradation and synthesis of GSH. GSH is an important molecule in the detoxification of a number of exogenous and endogenous compounds, in the maintenance of oxidant status, and as a storage form of cysteine. GGT acts on extracellular GSH, GSSG, or GSH conjugates to release the γ-glutamyl moiety and allows for further degradation of the cysteinylglycine moiety, which in the case of GSH eventually results in the reuptake of the individual amino acids that participate in de novo synthesis of GSH or supply the cell with cysteine [53,54]. The liver is considered a major contributor to the GSH content of plasma and other organs such as the kidney. The increase in hepatic GGT in diabetes is thought to be due to a hyperglycemia-induced increase in glycation or glycosylation [52].

The fact that the activity of SOD was normal in the liver and heart indicates that the rate of superoxide anion generation may not be beyond the cells' ability to quench at this stage in the progression of diabetes. This study did not distinguish any effect of diabetes on renal SOD activity. The low-carbohydrate diet was associated with an undesirable tendency to decrease the activity of SOD, an effect that seems tied to an interaction with diabetes, as similar changes were not observed in the nondiabetic animals on the same diet.

A decrease in hepatic and renal catalase activity in diabetics relative to normal controls [2,41,45,55–57] and an increase in cardiac catalase [1,41,57–61] have been common observations. Males generally had greater hepatic and renal catalase activity than females. These varied effects on organ catalase reflect the specific function and structure of each organ. The heart, relative to the liver or kidney, appears to have a greater capacity to

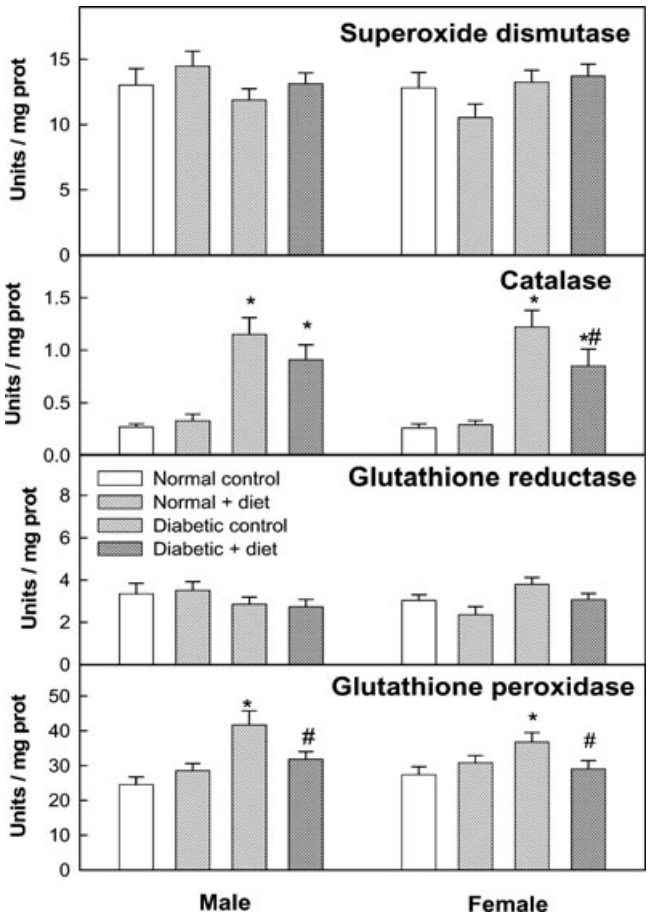

FIGURE 5. Activity of cardiac superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. One unit of superoxide dismutase activity is that which produces 50% inhibition of the reduction of cytochrome C in the presence of superoxide radical. One unit of catalase activity liberates half of the peroxide oxygen from solution in 100 s at 25°C. One unit of glutathione reductase or glutathione peroxidase activity oxidizes 1 nmol of NADPH/min at 30°C. Values are mean ± SEM and $p \leq 0.05$ when compared with the nondiabetic control (*) or diabetic control (#), $n = 10$–13.

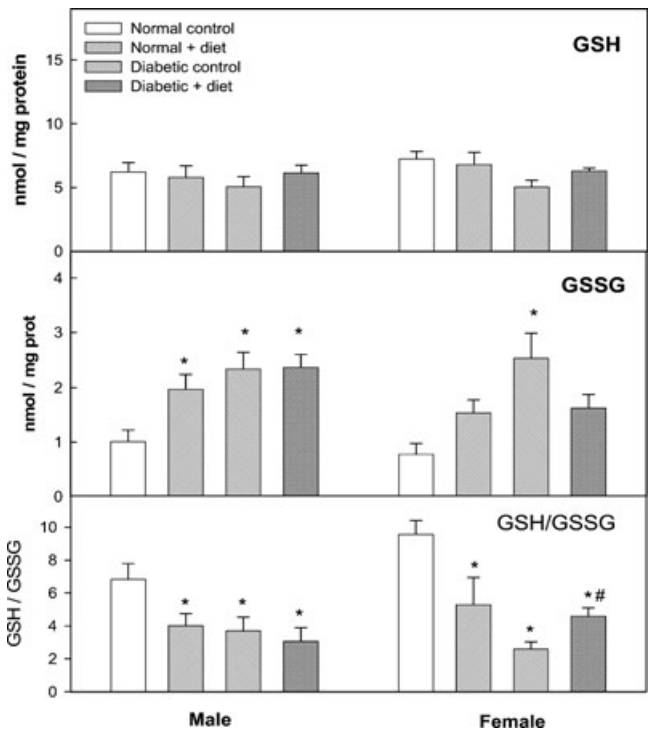

FIGURE 6. Levels of reduced glutathione (GSH) and glutathione disulfide (GSSG) and ratio of GSH to GSSG in heart of male and female normal and diabetic rats fed 30 days on standard and low-carbohydrate diets. Values are mean ± SEM and $p \leq 0.05$ when compared with the nondiabetic control (*) or diabetic control (#), $n = 3$–13.

induce an increase in the activity of catalase in response to oxidative stress. The low-carbohydrate diet tended to reverse the effects of diabetes on cardiac catalase but was neutral in the other two organs. Our conclusion is that the diet had no negative effect as far as catalase is concerned.

The increase in renal glutathione peroxidase activity in diabetes suggests an intensified peroxidative injury engendered by hyperglycemia. The low-carbohydrate diet did not modify this increased activity either directly or indirectly through the reduced, not normalized, blood glucose concentration. In contrast, the low-carbohydrate diet seems to stimulate an increase in glutathione peroxidase in the kidneys of normal male rats that could be due to an increase in peroxide production from metabolism of the predominant fuel. Overall, rats seem to possess the ability to increase activity of renal glutathione peroxidase as protection from oxidative stress.

Considering the increase in glutathione peroxidase activity and fall in both glutathione reductase (22%) and GGT (18%) activity in the kidney of diabetic female rats, one would expect a rise in the concentration of renal GSSG and concomitant fall in renal GSH values. But only trace amounts of GSSG were measured while concentration of GSH was unaffected. It is probable that changes in the activities of the above enzymes were not large enough to elicit measurable effects on GSH, but they do indicate a risk of failure to meet increased demand in case of a more pronounced oxidative attack. The low-carbohydrate diet elicited a positive effect by increasing glutathione reductase activity of diabetic animals though this was not correlated with an increase in GSH.

Conflicting results have been reported for cardiac glutathione reductase [32,44,57,62] and peroxidase [32,41,59,63–66]. An increase in glutathione peroxidase would signify an adaptation by the heart to prevent oxidative injury from H_2O_2 and lipid peroxides. The fact that activity of cardiac glutathione peroxidase was

normalized by low-carbohydrate diet in both male and female rats is indicative of improved oxidative status in these animals.

GSH and GSSG are also important in controlling the oxidant/antioxidant balance in cells. The increase in cardiac GSSG level (males) and decrease in GSH/GSSG ratio in diabetic rat hearts with no significant alterations in GSH and glutathione reductase values point to some de novo synthesis of GSH, as well as the probability that the cell is not able to reoxidize and/or extrude GSSG out of the cell at a rate commensurate with its formation. Whereas GSH is used for many other metabolic activities in the cell [54], it is also possible that the GSH/GSSG pool could be prioritized and shifted to the defense of the cell. A reduction in GSSG levels because of the normalization of glutathione peroxidase activity by the diet was not seen. The low-carbohydrate diet appears to independently alter the redox status in the heart.

In conclusion, the low-carbohydrate diet did alleviate some pathological effects engendered by diabetes (on glucose, HbA_{1c}, and glutathione peroxidase) but did not dramatically affect the oxidative status of the liver in diabetic or nondiabetic animals. The undesirable effects of the low-carbohydrate diet (seen in liver weight and GSH levels) were more pronounced in females than in males. Because blood glucose levels were not entirely normalized by the low-carbohydrate diet, it is not clear whether some of the benefits resulted directly from the diet itself or indirectly from a reduction of glucose concentrations. The test diet also led to an improved oxidant/antioxidant balance with respect to glutathione peroxidase in diabetic animals, and did not negatively affect the activities of catalase, SOD, or glutathione reductase in both diabetic and nondiabetic animals. Studies of low-carbohydrate diet in humans, primarily in type 2 diabetes, are limited, confounded by poor experimental design and of sufficiently short duration as to make predictions of long-term efficacy difficult [20]. Although this low-carbohydrate diet appears to stabilize hyperglycemia with minimal effects on antioxidant enzymes, additional information about effects over a longer treatment period would be useful.

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