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Research Communications

Effects of dietary carbohydrate intake on antioxidant enzyme activity and copper status in the copper-deficient streptozotocin (STZ) diabetic rat

B.M. McDermott,* J.J. Strain, and P.R. Flatt

Human Nutrition and Diabetes Research Groups, University of Ulster, Coleraine, Northern Ireland, UK

The aim of this study was to investigate how dietary lactose, compared with sucrose, in association with copper deficiency influences the antioxidant and copper status in the diabetic rat. Two groups of male rats (n = 12) were fed copper-deficient diets containing either 300 g/kg of sucrose or 300 g/kg of lactose in a pair-feeding regime for 35 days. Six rats from each group were injected with streptozotocin to induce diabetes. After a further 16 days the animals were killed and the liver, heart, and kidney removed for the measurement of copper levels and the activities of antioxidant and related enzymes. Diabetes resulted in higher hepatic and renal copper levels compared with controls. The copper content of the heart and kidney in diabetic rats consuming sucrose was also significantly higher than in those consuming lactose. Catalase activity in the liver, heart, and kidney was significantly increased in diabetic rats compared with controls. Hepatic glutathione S-transferase and glucose-6-phosphate dehydrogenase and cardiac copper zinc superoxide dismutase activities were also higher in diabetes. Sucrose, compared with lactose feeding, resulted in higher cytochrome c oxidase and glutathione peroxidase activities in the kidney while glucose-6-phosphate dehydrogenase activity was lower. The combination of lactose feeding and diabetes resulted in significantly higher activities of cardiac managanese superoxide dismutase and catalase and renal manganese superoxide dismutase and glucose-6-phosphate dehydrogenase. These results suggest that sucrose consumption compared with lactose appears to be associated with increased organ copper content and in general decreased antioxidant enzyme activities in copper-deficient diabetic rats.
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Keywords: sucrose; lactose; copper deficiency; diabetes; antioxidant enzymes; rat

Introduction

Copper deficiency is associated with a number of known risk factors for coronary heart disease such as glucose intolerance[1,2] hypercholesterolemia,[3] hypertension,[4] and hyperuricemia.[5] In addition, copper deficiency can also result in increased lipid peroxidation[6–8] and impaired antioxidant enzyme activity,[9] phenomena which have also been reported in diabetes.[10] Lower copper status, altered copper metabolism, and accompanying changes in activities of copper-dependent enzymes in diabetes have been reviewed.[10] The severity of copper deficiency at least in rats is influenced by the type of carbohydrate consumed with the fructose moiety of sucrose having the most detrimental effect.[11]

Lactose-based diets are poor sources of copper,[12] and consumption of dietary lactose increases the severity of atherosclerosis[13] and results in hypercholesterolemia in animals[14–17] and man.[18] The objective of this study was to investigate how dietary lactose, compared with sucrose, in

* Present address: Occupational Health Services, 2nd Floor, West Wing, Royal Hospitals Trust, Grosvenor Road, Belfast BT12 6BA, Northern Ireland, UK.
Address reprint requests to Prof. J.J. Strain at the Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK.
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association with copper deficiency influences antioxidant and copper status in the diabetic state.

Methods and materials

Two groups (n = 12) of male weanling Sprague–Dawley rats were randomly divided and individually housed. The rats were fed modifications of the AIN recommended diet for rats and mice[19] containing either 300 g/kg of sucrose with 1.02 mg/kg of copper or 300 g/kg of lactose with 0.82 mg/kg of copper as determined by atomic absorption spectrophotometry (AAS).[20] Sucrose-fed rats were pair fed against those rats consuming the lactose-based diet, and deionized water was provided ad libitum.

After 35 days, six rats from each group were injected intraperitoneally with a 50 mg/kg body weight dose of streptozotocin (STZ) dissolved in 0.5 M citrate buffer, pH 4.5, to induce diabetes. Control rats were injected with the vehicle alone. The diabetic rats were pair-fed against control rats in the same dietary group. After a further 16 days, the rats were anesthetised using diethyl ether and blood was removed by cardiac puncture. The animals were killed and the liver, heart, and kidneys were removed immediately and placed in ice-cold 0.25 M sucrose buffer, pH 7.4. The organs were weighted and portions stored at −20°C. Samples were removed for the preparation of 10% (wt/vol) liver and kidney homogenates and 0.5% (wt/vol) heart homogenate in 0.25 M sucrose buffer, pH 7.4.[20] Homogenates were stored in 4 to 5 mL aliquots at −20°C.

Organ and plasma protein levels were measured according to the method of Lowry et al.[21] while organ copper levels were determined by AAS following nitric acid digestion.[20] Glutathione peroxidase (EC 1.11.1.9, GSH-Px) activity was determined by a modification[20] of the method of Flohe and Gunzler.[22] The method of Kornberg et al.[23] was employed to determine the activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PDH). A modified[17] method of Jones and Suttle[24] was employed to measure the activities of copper zinc superoxide dismutase and manganese superoxide dismutase (EC 1.15.1.1, CuZnSOD and MnSOD, respectively). Catalase (EC 1.11.1.6, CAT), cytochrome c oxidase (EC 1.9.3.1, CCO), glutathione S-transferase (EC 2.5.1.18, GST), and plasma ceruloplasmin (EC 1.16.3.1, CPL) were measured by standard procedures.[25–28] Plasma glucose and cholesterol levels were determined by the Cobas Fara automatic analyzer using enzymatic kits (Roche Diagnostic Systems, Hoffmann-LaRoche Ltd., Basel, Switzerland).

Results were analyzed for statistical significance by two-way analysis of variance (ANOVA) to assess the effects of diabetes and carbohydrate and the interactive effect of the two variables on antioxidant enzyme activities. Results are given as mean values ± SE.

Results

The diabetic state resulted in significantly ($P < 0.001$) increased plasma glucose concentrations while plasma cholesterol levels were significantly ($P < 0.01$) decreased in diabetic rats compared with controls (*Table 1*). Two way ANOVA indicated that this reduction was more significant in diabetic rats consuming lactose compared with sucrose resulting in a significant ($P < 0.05$) carbohydrate-diabetes interaction.

The use of pair-feeding throughout this study ensured that there was no significant difference in body weights between the lactose- and sucrose-fed animals. However, lactose consumption compared with sucrose resulted in significant ($P < 0.05$ and $P < 0.001$, respectively) increases in liver and spleen weight expressed per 100 g of body weight (BW). Diabetes significantly ($P < 0.001$) decreased total body weight while the weight of the kidney, expressed per 100 g of BW, was significantly ($P < 0.001$) increased in diabetic rats compared with controls. In addition, significant ($P < 0.001$ and $P < 0.01$) carbohydrate-diabetes interactions occurred for liver and spleen weights expressed per 100 g of BW, respectively.

The type of carbohydrate consumed did not significantly influence the measured indices of copper status aside from the significant ($P < 0.05$) increase in renal CCO activity (*Table 2*). Diabetes, however, significantly increased the activity of the copper-dependent enzyme CuZnSOD ($P < 0.01$) in the heart and hepatic ($P < 0.01$) and renal ($P < 0.001$) copper concentration, while significant carbohydrate-diabetes interactions were observed for cardiac ($P < 0.01$) and renal ($P < 0.05$) copper levels.

Table 1 Total body weight, organ weights expressed per 100 g of body weight, plasma glucose, and cholesterol levels in copper-deficient control and diabetic rats fed either lactose or sucrose

	Lactose				Sucrose						
	Control		Diabetic		Control		Diabetic		Statistical analysis (ANOVA)		
Measure	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Carbohydrate	Diabetes	Interactive effect
Plasma glucose (mg/dL)	139	15.4	443	13.9	127	14.6	405	42.1	NS	‡	NS
Plasma cholesterol (mmol/L)	1.61	0.11	1.03	0.14	1.37	0.07	1.27	0.06	NS	†	*
Body weight (g)	279	8.6	207	6.3	282	10.8	196	7.2	NS	‡	NS
Liver weight/100 g of body weight	4.01	0.09	3.74	0.09	3.36	0.14	3.89	0.08	*	NS	‡
Heart weight/100 g of body weight	0.53	0.03	0.49	0.02	0.54	0.02	0.51	0.01	NS	NS	NS
Kidney weight/100 g of body weight	0.74	0.02	1.09	0.04	0.76	0.03	1.14	0.02	NS	‡	NS
Spleen weight/100 g of body weight	0.25	0.02	0.19	0.01	0.16	0.00	0.18	0.01	‡	NS	†

*$P < 0.05$; †$P < 0.001$; ‡$P < 0.0001$; NS = not significant; SE = standard error; ANOVA = two-way analysis of variance.

Table 2 Hepatic, cardiac, renal, and plasma indices of copper status in copper-deficient control and diabetic rats fed either lactose or sucrose

	Lactose				Sucrose						
	Control		Diabetic		Control		Diabetic		Statistical analysis (ANOVA)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Carbohydrate	Diabetes	Interactive effect
Hepatic											
Cu (μg/g)	2.30	0.29	4.85	0.42	3.44	0.72	6.75	1.10	NS	†	NS
Cytochrome c oxidase (U/mg of protein)	1.07	0.21	0.88	0.21	0.84	0.13	1.05	0.13	NS	NS	NS
CuZn superoxide dismutase (mU/mg of protein)	515	197	697	9	512	63	527	56	NS	NS	NS
Cardiac											
Cu (μg/g)	8.48	1.64	6.36	0.37	7.51	0.85	14	1.81	NS	NS	†
Cytochrome c oxidase (U/mg of protein)	1.52	0.16	1.52	0.13	1.41	0.25	1.83	0.23	NS	NS	NS
CuZn superoxide dismutase (mU/mg of protein)	220	15	340	17	190	20	262	47	NS	†	NS
Renal											
Cu (μg/g)	9.11	0.93	10.6	0.19	8.92	0.58	13.7	1.81	NS	‡	*
Cytochrome c oxidase (U/mg of protein)	1.33	0.14	1.23	0.01	1.47	0.11	1.63	0.06	*	NS	NS
CuZn superoxide dismutase (mU/mg of protein)	457	13	466	12	469	35	420	25	NS	NS	NS
Plasma											
Ceruloplasmin (U/L)	ND	—	ND	—	ND	—	13.5	8.26	NS	NS	NS

*$P < 0.05$; †$P < 0.001$; ‡$P < 0.0001$; ND = not detectable; NS = not significant; SE = standard error; ANOVA = two-way analysis of variance.

The activities of antioxidant and related enzyme activities that were significantly affected by diabetes and/or carbohydrate intake are outlined in *Table 3*. G6PDH activity in the liver was significantly ($P < 0.01$) higher in the sucrose-fed animals compared with those consuming the lactose-based diet. Diabetes significantly reduced the activities of hepatic GST ($P < 0.05$), G6PDH and CAT ($P < 0.01$). While the type of carbohydrate consumed did not significantly influence the activities of any of the cardiac antioxidant or related enzymes, the diabetic state significantly ($P < 0.001$) increased cardiac CAT activity. However, significant ($P < 0.01$) carbohydrate-diabetes interactions occurred for both cardiac CAT and MnSOD. Rats consuming the sucrose-based diet had significantly ($P < 0.05$) increased activity of renal GSH-Px and significantly ($P < 0.001$) decreased G6PDH activity compared with those rats consuming the lactose-based diet. Diabetes significantly ($P < 0.001$) decreased CAT activity in this organ while significant carbohydrate-diabetes interactions were also noted for renal G6PDH ($P < 0.001$) and MnSOD ($P < 0.05$) activities.

Discussion

Hyperglycemia is indicative of the diabetic state and the presence of this condition was confirmed by the increase in plasma glucose levels in diabetic animals compared with controls. The observed growth retardation in diabetic rats has been documented[29,30] with markedly reduced liver, heart, and spleen weight observed in this instance. However, kidney weight was not affected by the diabetic state, a feature previously reported in diabetic rats.[30,31]

Hypercholesterolemia is typically associated with diabetes[32]; however, in the current trial plasma cholesterol levels were significantly decreased in diabetic rats compared with controls. This may be due to growth retardation because the lowering of serum cholesterol levels has previously been documented with the restriction of calorie intake in humans.[33] This reduction in the plasma cholesterol levels was more marked in diabetic rats consuming lactose compared with sucrose-based diets. While lactose-containing diets have successfully been used to lower plasma cholesterol levels in humans,[34] a number of other studies have shown

Table 3 Hepatic, cardiac, and renal antioxidant and related enzyme activities in copper-deficient control and diabetic rats fed either lactose or sucrose

	Lactose				Sucrose						
	Control		Diabetic		Control		Diabetic		Statistical analysis (ANOVA)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Carbohydrate	Diabetes	Interactive effect
Hepatic											
Catalase (U/mg of protein)	0.19	0.02	0.13	0.02	0.21	0.03	0.12	0.02	NS	†	NS
Glutathione S-transferase (U/mg of protein)	0.24	0.02	0.15	0.02	0.18	0.02	0.16	0.03	NS	*	NS
Glucose-6-phosphate dehydrogenase (mU/mg of protein)	5.38	0.43	2.41	0.03	8.24	1.62	4.02	0.58	*	†	NS
Cardiac											
Mn superoxide dismutase (U/mg of protein)	1.17	0.05	1.46	0.07	1.30	0.08	1.18	0.13	NS	NS	*
Catalase (mU/mg of protein)	3.37	0.16	7.80	0.60	4.38	0.55	5.92	0.90	NS	‡	*
Renal											
Mn superoxide dismutase (U/mg of protein)	0.96	0.03	1.12	0.05	1.06	0.08	0.97	0.03	NS	NS	*
Catalase (U/mg of protein)	0.20	0.01	0.07	0.13	0.21	0.02	0.17	0.01	NS	‡	NS
Glutathione peroxidase (mU/mg of protein)	9.76	2.47	6.97	0.61	13.9	3.63	24.4	6.43	*	NS	NS
Glucose-6-phosphate dehydrogenase (mU/mg of protein)	6.80	0.46	10.2	0.69	6.79	0.29	5.85	0.38	‡	NS	‡

*$P < 0.05$; †$P < 0.001$; ‡$P < 0.0001$; NS = not significant; SE = standard error; ANOVA = two-way analysis of variance.

the opposite effect in both animals[14–17] and humans.[18] This inconsistency may be due to the differences in dietary composition and the use of the pair-feeding regime.

Increases in hepatic[30,35,36] and renal[30,37–39] copper levels have previously been noted in experimentally induced diabetic rats. Similar results were observed in both the liver and kidney in this study. While there was a small difference in the copper content of the two diets employed, this appeared to be without effect because there was no significant difference in the organ copper content of the control rats consuming each diet. However, the combination of sucrose feeding and diabetes resulted in significant carbohydrate-diabetes interactions for cardiac and renal copper levels, indicating marked increases in the copper content of these organs in the sucrose-fed diabetic animals only. Elevated renal copper levels have previously been linked to diabetic nephropathy[37–39] and complications of diabetes such as retinopathy and nephropathy can be produced in animals by feeding dietary sucrose.[40,41] These interactive effects suggest that the consumption of a sucrose-based compared with a lactose-based diet may accelerate the onset of complications such as nephropathy which are associated with the diabetic condition.

The heart and pancreas, relative to the liver and kidney, contain low levels of radical scavenging enzymes in rats[29,42] and mice.[43] However, in general this situation is reversed in diabetes.[29] This observation is consistent with findings reported here where significant decreases were observed for the activities of hepatic GST, G6PDH, and CAT, and renal CAT and significant increases were observed for the activities of cardiac CuZnSOD and CAT in diabetic rats compared with controls. The changes in antioxidant enzyme activity in the liver and kidney have previously been attributed to radical-induced inactivation[29] while the observed decrease in hepatic G6PDH activity has previously been documented in experimentally induced diabetic mice,[44] rats,[30,45,46] and humans.[47] The increase in cardiac CuZnSOD activity may be a compensatory response to an in-

crease in free radical generation. SOD is responsible for the dismutation of the superoxide radical, a reaction that results in the production of hydrogen peroxide.[48] Higher cardiac CAT activity in diabetes has been reported previously.[29] It is thought to be in response to increased peroxisomal production of hydrogen peroxide in diabetes[49] but may also be partly attributed to the production of hydrogen peroxide by the dismutation of the superoxide radical. Increased production of reactive oxidant species such as hydrogen peroxide and the superoxide radical have been implicated in the long-term complication of diabetes[50] such as angiopathy.[51]

The combination of the lactose-based diet and diabetes resulted in significant carbohydrate-diabetes interactions occurring for cardiac MnSOD and CAT, and renal MnSOD and G6PDH with the activity of these enzymes significantly higher in diabetic rats consuming the lactose compared with the sucrose-based diet. These interactions may reflect a compensatory increase in antioxidant enzyme activity in response to lower GSH-Px activity with lactose feeding compared with sucrose.

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