Comparison of Copper Status in Rats When Dietary Fructose Is Replaced by either Cornstarch or Glucose (43064)

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Abstract. **The purpose of this study was to determine what levels of starch or glucose replacement for fructose in the copper-deficient diet (copper) can minimize the fructose-copper interaction. Experimental diets contained either 100% fructose as the carbohydrate source, or the fructose was partially replaced with 50% starch, 50% glucose, 75% starch, or 75% glucose. Diets were either copper adequate (7–8 ppm) or inadequate (<1 ppm). Male weanling rats were fed their respective diet for 5 weeks and then fasted overnight. After decapitation, blood was collected and liver and heart were removed. Plasma copper was significantly reduced and ceruloplasmin was not detected in all copper-deficient groups. Copper deficiency increased plasma cholesterol, as well as heart and liver weight in the glucose groups, but not in the starch groups. Those organ weights were heavier in glucose-copper than starch-copper rats. Erythrocyte copper-zinc-superoxide dismutase activity was greater in starch-copper than glucose-copper rats regardless of carbohydrate amount. Hepatic copper concentration of the group fed starch-copper was twice levels observed in glucose-copper. The 50% glucose rats had lower hepatic copper than the 75% glucose rats. Hepatic copper-zinc-superoxide dismutase activity showed patterns similar to hepatic copper. Cardiac copper was greater in starch-copper than glucose-copper rats. Cardiac copper-zinc-superoxide dismutase activity was equally reduced in all copper-deficient groups. The 50% starch-replaced diet was more effective in minimizing copper deficiency than the 75% glucose-replaced diet. This poorer improvement of copper deficiency by glucose than starch may partially be due to a more severe reduction of food intake in glucose than in starch diets.**

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An interaction between carbohydrate and copper status of copper-deficient rats has been reported (1–3). The degree of severity of the signs of copper deficiency depends on the type of dietary carbohydrate: fructose and sucrose producing much more severe signs of deficiency than do glucose and especially starch (4–8). In many of these studies (1–8), fructose at levels contributing 60% of the total calories have been used to study this interaction. However, fructose at these levels is not relevant to human consumption of this sugar (10–12% of total calories) (9) and, thus, it is of nutritional importance to evaluate the relevance of this interaction at levels more closely approximating human consumption. It is also of interest to compare the copper status in rats when dietary fructose is replaced by either starch or glucose.

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Materials and Methods

Weanling male Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) weighing approximately 50–60 g each were housed individually in stainless steel cages with wire mesh bottom in a temperature humidity controlled room with a 12-hr light/dark cycle. The rats were randomly divided into 10 groups. They were fed either sole fructose as the carbohydrate source (100% fructose); 50% starch and 50% fructose (50% starch); 50% glucose and 50% fructose (50% glucose); 75% starch and 25% fructose (75% starch), or 75% glucose and 25% fructose (75% glucose) with or without copper (Table I).

The basal diet contained the following (g/kg diet): 628 carbohydrate, 200 eggwhite, 95 corn oil, 30 cellulose, 35 AIN mineral mix (10) prepared in my laboratory and formulated to omit cupric compounds from

the mineral mix for the copper-free basal diet, 2.7 choline bitartrate, and 10 vitamin AIN-76 (10, 11) supplemented with 2 mg of biotin/kg diet. The basal diets contained 0.79–0.98 μg of copper/g diet. Cupric carbonate (10.5 mg of $CuCO_3$/kg) supplemented diets contained 7.06–7.81 μg of copper/g diet. All animals were provided with distilled, deionized drinking water. Body weight and food intake were measured weekly.

The termination of the experiment was based on the time of death of the first rat. This occurred during the fifth week in the rats fed 100% fructose-copper diet. None of the animals in the other groups died. The study was terminated following an overnight fast. Animals were killed by decapitation and blood was collected into heparinized test tubes. The blood was centrifuged at 2200*g* for 20 min at 4°C and the plasma was analyzed for triglycerides, cholesterol, and ceruloplasmin. Erythrocytes were used to determine copper-zinc-superoxide dismutase (EC 1.15.1.1) activity (12). Livers and hearts were removed from the rats immediately after they were killed and weighed, washed in ice-cold saline solution, and homogenized with five volumes of 0.2% Triton X-100 using a homogenizer equipped with stainless steel blades. Homogenate aliquots were extracted to isolate copper-zinc-superoxide dismutase (13).

Plasma total cholesterol and triglycerides were determined by enzymatic methods using the Centrifichem System (Baker Instrument Co., Allentown, PA). Plasma ceruloplasmin activity (EC 1.16.3.1) was measured by the use of *O*-dianisidine dihydrochloride (14). Copper-zinc-superoxide dismutase activity was determined according to the method of Misra and Fridovich (12). Copper concentration in liver and heart was measured following their digestion by a method combining wet and dry ashing (15, 16). The ashed residue was dissolved in 0.1 *N* HCl (15). Duplicate samples of the tissue homogenates and of plasma were analyzed for copper by flame atomic absorption spectrophotometry (model 5000; Perkin-Elmer, Norwalk, CT) (16). Bovine liver 1577a from the National Bureau of Standards Reference Materials (17) was digested and analyzed along with samples to verify accuracy. The percentage of recoveries for copper was 98.5%.

Table I. Experimental Groups

Carbohydrate in diet			Copper (μg/g)	Sample no.	Group designation
Fructose	Starch (%)	Glucose (%)			
62.8			7.06[a]	8	+Cu 100F[b]
62.8			0.79	13	−Cu 100F[c]
31.4	31.4		7.52	8	+ Cu 50S[d]
31.4	31.4		1.01	8	−Cu 50S[e]
15.7	47.1		7.07	8	+Cu 75S[f]
15.7	47.1		0.98	8	−Cu 75S[g]
31.4		31.4	7.42	8	+Cu 50G[h]
31.4		31.4	0.96	8	−Cu 50G[i]
15.7		47.1	7.81	8	+Cu 75G[j]
15.7		47.1	0.89	8	−Cu 75G[k]

[a] Copper level analyzed by atomic absorption.
[b] One-hundred percent fructose with copper.
[c] One-hundred percent fructose without copper.
[d] Fifty percent fructose replaced by starch with copper.
[e] Fifty percent fructose replaced by starch without copper.
[f] Seventy-five percent fructose replaced by starch with copper.
[g] Seventy-five percent fructose replaced by starch without copper.
[h] Fifty percent fructose replaced by glucose with copper.
[i] Fifty percent fructose replaced by glucose without copper.
[j] Seventy-five percent fructose replaced by glucose with copper.
[k] Seventy-five percent fructose replaced by glucose without copper.

Statistics

Data were analyzed by analysis of variance (ANOVA) using the SAS software system for data analysis (18). In all of the statistical comparisons, difference with $P < 0.05$ were considered to be significant.

Results

The effects of carbohydrate type with or without copper on final body weight, food intake, and relative organ sizes are shown in Table II. The animals fed fructose without copper had significantly lower food intake and lower body weight as compared with those fed fructose with copper. Copper deficiency did not affect food intake in either level of the starch diets, while it significantly reduced food intake in the animals fed glucose as compared with their respective copper-adequate animals. Copper deficiency did not change relative liver weight and relative heart weight in the starch-fed animals. However, copper deficiency significantly increased relative liver weight and relative heart weight in the animals fed 100% fructose as well as those fed glucose. The effect of copper deficiency on food intake and relative liver weight was even greater in the 50% glucose than in the 75% glucose group. Relative heart weight was equally increased by copper deficiency in both glucose groups.

The effects of carbohydrate source with or without copper on blood copper indices, cholesterol, and triglycerides are presented in Table III. Erythrocyte copper-zinc-superoxide dismutase activity was reduced in all copper-deficient groups. However, there was a significant carbohydrate difference in the degree of reduction in enzyme activity. Both starch groups had significantly higher erythrocyte copper-zinc-superoxide dismutase activity than either the fructose or two glucose groups. The enzyme activity in the glucose-fed animals was further reduced when the diet contained more fructose. Regardless of carbohydrate sources and their combination levels, plasma copper was extremely lower in all copper-deficient groups than their comparable

Table II. Effects of Carbohydrate Sources with or without Copper on Final Body Weight, Food Intake, and Relative Organ Weights

Experimental groups	Final body weight (g)	Food intake (g/day)	Relative liver weight (g/100 body wt)	Relative heart weight (g/100 body wt)
−Cu 100 F	258 ± 10.7[a]	12.3 ± 1.1	4.74 ± 0.12	0.56 ± 0.03
−Cu 50 G	288 ± 9.3	15.6 ± 0.9	4.15 ± 0.20	0.49 ± 0.04
−Cu 50 S	296 ± 7.3	18.5 ± 0.8	3.31 ± 0.09	0.38 ± 0.01
−Cu 75 G	293 ± 8.4	17.2 ± 0.7	3.43 ± 0.14	0.50 ± 0.01
−Cu 75 S	305 ± 9.7	19.4 ± 0.6	3.05 ± 0.04	0.37 ± 0.01
+Cu 100 F	314 ± 6.5	20.5 ± 0.9	3.47 ± 0.08	0.37 ± 0.01
+Cu 50 G	303 ± 15.0	17.7 ± 0.9	3.04 ± 0.14	0.37 ± 0.02
+Cu 50 S	294 ± 7.2	16.6 ± 1.3	3.12 ± 0.02	0.36 ± 0.01
+Cu 75 G	307 ± 6.9	20.2 ± 0.3	2.98 ± 0.07	0.37 ± 0.01
+Cu 75 S	294 ± 4.8	19.6 ± 0.6	3.08 ± 0.08	0.35 ± 0.01
Sources			ANOVA	
Carbohydrate	NS	0.0033	0.0001	0.0001
Copper	0.0160	0.0001	0.0001	0.0001
Carbohydrate ratio	NS	NS	0.0001	NS

[a] Mean ± SE.

Table III. Effects of Carbohydrate Sources with or without Copper on Blood Parameters

Experimental groups	Erythrocyte copper-zinc-superoxide dismutase (units/ml)	Plasma			
		Copper (μg/dl)	Ceruloplasmin (units/liter)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
−Cu 100 F	75 ± 9[a]	5 ± 0.9	ND[b]	216 ± 13	146 ± 26
−Cu 50 G	79 ± 9	5 ± 0.5	ND	192 ± 14	87 ± 9
−Cu 50 S	137 ± 6	8 ± 0.7	ND	102 ± 4	52 ± 6
−Cu 75 G	102 ± 16	10 ± 0.4	ND	155 ± 11	68 ± 6
−Cu 75 S	166 ± 15	11 ± 1.5	ND	92 ± 5	47 ± 4
+Cu 100 F	399 ± 48	83 ± 24	204 ± 4.0	128 ± 6	99 ± 8
+Cu 50 G	349 ± 17	98 ± 29	187 ± 13	121 ± 3	64 ± 12
+Cu 50 S	522 ± 54	109 ± 13	312 ± 17	108 ± 5	47 ± 4
+Cu 75 G	375 ± 27	51 ± 6.5	149 ± 30	101 ± 11	61 ± 4
+Cu 75 S	397 ± 15	139 ± 7.6	300 ± 15	101 ± 5	48 ± 5
			ANOVA		
Carbohydrate	0.0003	0.0206	0.0001	0.0001	0.0010
Copper	0.0001	0.0001	0.0001	0.0001	NS
Carbohydrate ratio	NS	0.0282	NS	0.0001	0.0310

[a] Mean ± SE.
[b] Nondetectable.

copper-adequate groups. The ranges of plasma copper in the copper-deficient groups are 5 through 11 μg/dl, whereas they are 51 through 139 μg/dl in the copper-adequate groups. The animals fed 50% glucose-copper had as low plasma copper as those fed 100% fructose-copper. Ceruloplasmin activity was not detected in all copper-deficient groups. Among the copper-adequate groups, both starch groups had the highest plasma copper level and ceruloplasmin activity, which such activity was lowest in the glucose groups.

All copper-adequate rats had similar concentrations of plasma cholesterol. Copper deficiency significantly increased plasma cholesterol in the animals fed fructose as well as glucose, although it did not affect plasma cholesterol in the animals fed starch (Table III). As fructose content increases in the glucose-copper, plasma cholesterol was also increased, i.e., 192 mg/dl in 50% glucose-copper vs 155 mg/dl in 75% glucose-copper. This difference did not exist in the starch groups. Plasma triglycerides were also lower in all four starch groups, while it was greatest in the fructose groups. Plasma triglyceride levels were 1.5 times as high in 100% fructose-copper than in 100% fructose plus copper.

The effects of carbohydrate sources with or without copper on hepatic and cardiac copper and copper-zinc-

superoxide dismutase activity are shown in Table IV. As expected, copper deficiency significantly reduced hepatic copper in all copper-deficient groups. However, there was a significant carbohydrate effect on hepatic copper among the copper-deficient groups. Hepatic copper of the animals fed starch-copper was twice as great as that of those fed either 100% fructose-copper or 50% glucose-copper. Furthermore, hepatic copper was lower in 50% glucose-copper than in 75% glucose-copper rats. Hepatic copper-zinc-superoxide dismutase activity showed a similar pattern. Copper deficiency decreased copper-zinc-superoxide dismutase activity sharply in all copper-deficient groups. However, the degree of reduction was dependent on the carbohydrate source. The enzyme activity was 2- to 3-fold greater in starch-copper than either in glucose-copper or fructose-copper. In the copper-adequate groups, cardiac copper was greatest in the starch groups, while it was lowest in the fructose groups. Cardiac copper in the copper-deficient groups was reduced by approximately 50% compared with copper-adequate groups. Regardless of different cardiac copper levels in the copper-deficient groups, copper-zinc-superoxide dismutase activity was almost equally reduced by copper deficiencies in all of those groups.

Discussion

The rats fed fructose diets deficient in copper exhibited depressed food intake, increased liver and heart weights, plasma cholesterol and triglycerides, decreased erythrocyte copper-zinc-superoxide dismutase activity and plasma copper, undetectable activity of ceruloplasmin, decreased hepatic and cardiac copper and copper-zinc-superoxide dismutase activity. These results are well in agreement with the previous studies (1–6). However, when dietary fructose was partially replaced by starch, these undesirable interactions improved significantly. The striking finding of the present study was that copper levels of tissue and plasma copper and the activity of copper-zinc-superoxide dismutase increased, and plasma lipids decreased to a much greater extent when fructose was replaced by starch rather than glucose. Moreover, the 50% starch-replaced diet was more effective in minimizing copper deficiency than the 75% glucose-replaced diet. The improvement of undesirable interaction between fructose and copper deficiency shows a dose response of glucose or starch replacement.

It has been generally assumed that glucose and starch, a polymer of glucose, behave similarly in the metabolism. However, the statement is not true in the copper-deficient rats fed a mixture of fructose and glucose or a mixture of fructose and starch as the carbohydrate source. Starch feeding improved copper deficiency a greater extent than did glucose feeding. The mechanism of this difference is not clear at this time. However, one possible explanation may be the difference in daily food consumption among the groups. The severest reduction in food intake was obtained in the animals fed 100% fructose-copper who had the severest copper deficiency; followed by the animals fed 50% glucose-copper who had the second severest copper deficiency; and, again, followed by those fed 75% glucose-copper who had the third severest symptoms. This mechanism can be supported by an acute reduction of food intake in the rat who died during the experiment. The mechanism is also supported by the conflict results in hepatic copper content of rats fed copper-adequate diet containing glucose or starch diet between the present study and other studies (2, 3, 19). In the present study, the hepatic copper

Table IV. Effects of Carbohydrate Sources with or without Copper on Hepatic and Cardiac Parameters

Experimental groups	Liver		Heart	
	Copper (μg/g)	Copper-zinc-superoxide dismutase (units/g)	Copper (μg/g)	Copper-zinc-superoxide dismutase (units/g)
−Cu 100 F	1.67 ± 0.07[a]	116 ± 12	2.38 ± 0.18	36 ± 8.5
−Cu 50 G	1.59 ± 0.10	101 ± 18	3.14 ± 0.22	38 ± 10
−Cu 50 S	3.13 ± 0.18	292 ± 48	3.40 ± 0.14	35 ± 6
−Cu 75 G	2.35 ± 0.30	158 ± 34	3.23 ± 0.23	43 ± 10
−Cu 75 S	3.58 ± 0.22	390 ± 98	4.36 ± 0.24	59 ± 11
+Cu 100 F	5.47 ± 0.40	827 ± 109	4.87 ± 0.45	141 ± 20
+Cu 50 G	5.93 ± 0.08	1036 ± 29	6.77 ± 0.31	157 ± 8.3
+Cu 50S	5.80 ± 0.14	1007 ± 29	7.19 ± 0.53	162 ± 10
+Cu 75 G	6.47 ± 0.24	876 ± 98	6.60 ± 0.45	119 ± 11.7
+Cu 75 S	5.94 ± 0.30	991 ± 33	8.21 ± 0.35	107 ± 14
Sources			ANOVA	
Carbohydrate	0.0001	0.0003	0.0001	NS
Copper	0.0001	0.0001	0.0001	0.0001
Carbohydrate ratio	0.0065	0.0492	0.0051	NS

[a] Mean ± SE.

content was greater in the glucose group who consumed more food as compared with those fed starch. However, this was reversed along with food intake in those studies (2, 3, 19). Therefore, the reduction in food intake may be the critical factor in the interaction between fructose and copper deficiency. The reason for reduction of food intake in the copper-deficient rats fed fructose or glucose is not clear at this time.

In a recent study (20), the greater ratio of sorbitol to copper was reported in the liver and kidney of male rats consuming the copper-deficient fructose diet as compared with the copper-deficient starch diet. If sorbitol binds with copper (21) and forms a stable complex, then the complex copper will be unavailable for utilization. The diluting of fructose by starch combined with the positive metabolic changes associated with dietary starch may reduce sorbitol production and its accumulation in vital tissues. If sorbitol does chelate Cu+, its reduction would make more copper available for utilization. However, glucose replacement showed less efficiency when compared with starch replacement. This finding may be supported by the abnormal stimulation of the polyol pathway. The polyol pathway is normally a minor pathway of glucose metabolism (22, 23) which assumes a major role under certain pathologic conditions. The polyol pathway has been reported to be elevated in normal rats consuming fructose (24) and in diabetes (25, 26) where due to hyperglycemia, the flux of glucose through the polyol pathway is greatly augmented, leading to intracellular accumulation of sorbitol (23, 25, 26).

Until recently, free fructose was found in small amounts in the American diet. Due to the introduction of high fructose corn syrup in 1970 (28), fructose is becoming a major dietary ingredient (29, 27), accounting 10–12% of total calories. A recent U.S. diet study also indicated that dietary copper intake was approximately 50% of the Recommended Dietary Allowance (RDA), ranging from 38% through 70% (29).

Due to these dietary patterns, the fructose-copper interaction observed in rats could have a great relevance in human nutrition. The dietary regime in the present study approximates human dietary carbohydrate consumption. In the diet 50% starch with 50% fructose, each carbohydrate provides 30.0% of the total calories. In the 75% starch with 25% fructose, starch accounts 45.0%, and fructose, 15.0%, respectively. The result of this study shows that starch consumption at these levels would ameliorate the fructose-copper interaction in humans.

1. Reiser S, Ferretti RJ, Fields M, Smith JC Jr. Role of dietary fructose in the enhancement of mortality and biochemical changes associated with copper deficiency in rats. Am J Clin Nutr **38**:214–222, 1983.
2. Johnson MA. Interaction of dietary carbohydrate, ascorbic acid and copper with development of copper deficiency in rats. J Nutr **116**:802–815, 1986.
3. Failla ML, Babu U, Seidel KE. Use of immunoresponsiveness to demonstrate that the dietary requirement for copper in young rats is greater with dietary fructose than dietary starch. J Nutr **118**:487–496, 1988.
4. Fields M, Ferretti RJ, Smith JC Jr, Reiser S. The interaction of type of dietary carbohydrate with copper deficiency. Am J Clin Nutr **39**:289–295, 1984.
5. Fields M, Ferretti RJ, Smith JC Jr, Reiser S. The severity of copper deficiency in rats is determined by the type of dietary carbohydrate. Proc Soc Exp Biol Med **175**:530–537, 1984.
6. Fields M, Ferretti RJ, Smith JC Jr, Reiser S. Effect of copper deficiency on metabolism and mortality in rats fed sucrose or starch diets. J Nutr **113**:1335–1345, 1983.
7. Fields M, Lewis CG, Beal T, Scholfield D, Patterson K, Smith JC Jr, Reiser S. Sexual differences in the expression of copper deficiency in rats. Proc Soc Exp Biol Med **186**:183–187, 1987.
8. Fields M, Lewis CG, Beal T. Copper-carbohydrate interaction in maternal, fetal and neonate rat. Neurotoxicol Teratol **10**:555–562, 1988.
9. Sugar and Sweeteners Outlook and Situation Report. United States Department of Agriculture, Economic Research Service, SSRV9N2. Washington, DC 8–9, 1984.
10. American Institute of Nutrition, Report on the AIN ad hoc committee on standards for nutritional studies. J Nutr **107**:1340–1348, 1977.
11. American Institute of Nutrition, Second report of the ad hoc committee on standards for nutritional studies. J Nutr **110**:1726, 1980.
12. Misra HP, Fridovich I. Superoxide dismutase: A photochemical augmentation assay. Arch Biochem Biophys **181**:308–312, 1977.
13. McCord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J Biol Chem **244**:6049–6055, 1969.
14. Schosinsky KH, Lehmann HP, Beller MF. Measurement of ceruloplasmin and its oxidase activity in serum by use of *O*-dianisidine dihydrochloride. Clin Chem **20**:1556–1563, 1974.
15. Hill AD, Patterson KY, Veillon C, Morris ER. Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. Anal Chem **58**:2340–2346, 1983.
16. Anonymous. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer, Norwalk, CT, 1976.
17. U.S. Department of Commerce, NBS Standard Reference Materials Catalog 1988-89. Office of Standard Reference Materials, National Bureau of Standards, Gaithersburg, MD, 1988.
18. SAS Institute, SAS User's Guide: Statistics, 5th ed. Statistical Analysis System (SAS) Institute, Cary, NC, 1985.
19. Landes DR. Influences of dietary carbohydrate on copper, iron, and zinc status of the rat. Proc Soc Exp Biol Med **150**:686–689, 1975.
20. Fields M, Lewis CG, Beal T. Accumulation of sorbitol in copper deficiency: Dependency on gender and type of dietary carbohydrate. Metabolism **38**:371–375, 1989.
21. Briggs J, Finch P, Matulewicz C, Weigel H. Complexes of copper (II), calcium and other metal loss with carbohydrate. Thin-layer ligand-exchange chromatography and determination of relative stabilities of complexes. Carbohydr Res **97**:181–188, 1981.
22. O'Brien MM, Scholfield DJ, Edwards MR. Polyol-pathway enzymes of human brain. Biochem J **211**:81–90, 1983.
23. Jeffery JI, Jornvall M. Enzyme relationship in a sorbitol pathway that bypasses glycolysis and pentose phosphates in glucose metabolism. Proc Natl Acad Sci USA **80**:901–905, 1983.
24. Bellomo G, Comstock JP, Wen D, Hazelwood RL. Prolonged fructose feeding and aldose reductase inhibition: Effect on the polyol pathway in kidneys of normal rats. Proc Soc Exp Biol Med **186**:348–354, 1987.

25. Wang YM, Van EJ. Nutritional significance of fructose and sugar alcohols. Annu Rev Nutr **1**:437–475, 1981.
26. Beyer-Mears A, Cohen MP. Glomerular polyol accumulation in the diabetes and its prevention by oral sorbitol. Diabetes **33**:604–607, 1984.
27. Page L, Friend B. Levels of uses of sugars in the United States. In: Sipple HL, McNutt KW, Eds. Sugar in Nutrition. New York: Academic Press, pp 93–107, 1974.
28. Woteki CE, Welsh SO, Raper W, Marston RM. Recent trends and levels of dietary sugars and other sweeteners. In: Reiser S, Ed. Metabolic Effects of Utilizable Carbohydrates. New York: Mercel Dekker, pp 1–27, 1982.
29. Pennington JAT, Young BC, Wilson DB. Nutritional elements in the U.S. diets: Result from the total diet study, 1982 to 1986. J Am Diet Assoc **89**:659–664, 1989.