Description of the Long-Term Lipogenic Effects of Dietary Carbohydrates in Male Fischer 344 Rats[1]

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ABSTRACT The introduction of high fructose corn syrup as a substitute sweetener for sucrose in the mid-1970s has contributed to a general increase in fructose consumption in the U.S. diet. Although several previous investigations suggested that dietary fructose increases serum triglyceride concentration and body fat, these studies have, in general, evaluated this effect in young rats fed the experimental diets for a relatively short period of the life span of the animals. Moreover, these investigations did not control for the possible effects that increased adiposity due to fructose feeding may have on serum triglyceride concentration. The purpose of the current investigation was to describe the long-term effects of specific dietary carbohydrates on serum lipid concentrations and body composition. To this end, we measured serum triglyceride, total cholesterol and HDL cholesterol concentrations and body composition of rats aged 9, 18 and 26 mo that had free access to or were restricted to 60% of free access intake of one of five diets that varied in carbohydrate source (cornstarch, sucrose, glucose, fructose or equimolar fructose plus glucose) starting at 3 mo of age. Dietary fructose significantly increased serum triglyceride concentration across the life span in rats that had free access to food or were calorie restricted. The source of dietary carbohydrate did not have a significant effect on body composition, total cholesterol or the distribution of the cholesterol fractions. These data suggest that dietary fructose per se and not the interaction between fructose and the energy content of the diet increases serum triglyceride concentration in rats. J. Nutr. 130: 3077–3084, 2000.

KEY WORDS: • *aging* • *body composition* • *calorie restriction* • *cholesterol* • *fructose* • *triglyceride* • *rats*

The introduction of high fructose corn syrup as a substitute sweeter for sucrose in the mid-1970s has contributed to a general increase in fructose consumption in the U.S. diet. Per capita disappearance data, an estimate of the availability of specific components for use in the food supply, indicate that the use of corn sweeteners has increased from ~13% of total sugars in the food supply in 1965 to 47% in 1985 (Glinsmann et al. 1986). The increase in the use of high fructose corn syrup parallels a decline in the use of sucrose. The overall effect of this change is that the mean consumption of fructose has increased 18% and that the glucose-to-fructose ratio has decreased from 1.4 to 1.0 between 1977 and 1987 (Gibney et al. 1995). Data from a recent investigation that evaluated the food sources of added sweeteners in the U.S. diet are consistent with these early observations and suggest that the percentage of fructose in the diet continues to increase (Guthrie and Morton 2000). The potential health importance of increased dietary fructose consumption has been a topic of recent research.

Several investigations suggested that diets high in fructose, supplied either as a monosaccharide or as a component of the disaccharide sucrose, increases serum triglyceride concentrations (Albrink and Ullrich 1986, Lock et al. 1980, Macdonald 1966, Reaven et al. 1979 and 1990, Reiser et al. 1979, Waddell and Fallon 1973), increases total cholesterol concentrations and increases the LDL-to-HDL ratio (Albrink and Ullrich 1986, Cybulska and Naruszewicz 1982, Lock et al. 1980, Macdonald 1966, Reiser et al. 1981, 1979 and 1989) and may promote adiposity (Barnard et al. 1993, Hallfrisch et al. 1981, Reiser and Hallfrisch 1977, Rizkalla et al. 1992). In vitro studies investigating the effect of sucrose on lipogenic enzyme activity, hepatic synthesis and secretion and the removal rates of triglycerides have offered further support to the hypothesis that the consumption of a diet high in sucrose or fructose has an effect on serum triglyceride and cholesterol concentrations (Bacon et al. 1984, Kazumi et al. 1989 and 1991, Kok et al. 1996a and 1996b, Mamo et al. 1991, Nassir et al. 1993, Park et al. 1992, Yoshino et al. 1989, 1992 and 1997). Most of these investigations, however, have been of relatively short duration, with the subjects or animals being fed the experimental diets for only a few day or weeks. It is unclear whether the results of these studies more closely reflect a short-term feeding phenomenon. Moreover, studies in mice and rats that investigate the lipogenic effects of dietary carbohydrates use very young animals, typically <6 mo old. Because these animals are still in the developmental stage of life, the results of these studies may reflect more closely developmental changes rather than those associated with the adult aging process.

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Data describing the long-term effects of specific dietary components on normal aging are seriously limited. Understanding these effects is important, because the use of long-term feeding protocols in research to investigate the impact of nutrition on the aging process is becoming common. The purpose of this investigation was to describe the long-term effects of specific dietary carbohydrates on serum lipid concentrations and body composition. It was our specific aims to *1*) establish baseline data on the effects of particular carbohydrates for use by other investigators who are interested in long-term feeding investigations in rats and *2*) to evaluate the impact of long-term high fructose diets on lipogenesis. To this end, we measured serum triglyceride, total cholesterol, HDL cholesterol concentrations and the body composition of rats aged 9, 18 and 26 mo that had free access to or were restricted to 60% of mean intake of rats with free access to one of five diets that varied in carbohydrate source starting at the age of 3 mo.

MATERIALS AND METHODS

Animals and animal care. Two-hundred-eighty male Fischer 344 rats (3 mo old) were obtained from the National Institute on Aging animal colony maintained at Harlan Sprague-Dawley Laboratory (Indianapolis, IN) and housed in clean facilities, as described previously (McDonald et al. 1988). Briefly, rats were housed individually in wire-bottom hanging cages within laminar flow units (Duo-Flow; Laboratory Products, Maywood, NJ). The rooms were maintained at a temperature of 25–26°C with 50% humidity and a 12:12 light/dark cycle (lights on at 0600, lights off at 1800). Rats had free access to distilled, autoclaved water (pH 3.5). A periodic systemic necropsy was performed on a rat chosen at random every 3 mo throughout the duration of the experiment. On necropsy, the rats were evaluated for gross lesions that might result from environmental contamination. Sera from the animals were monitored for specific antibodies that would indicate disease. All results were negative.

Diet and feeding protocol. After 1 wk of acclimation to the housing facility (i.e., light cycle, temperature and so on), rats were assigned to be fed one of five isocaloric semipurified diets that differed in carbohydrate source (**Table 1**). The carbohydrate source was either cornstarch, sucrose, glucose, fructose or equimolar fructose plus glucose. Rats were assigned to their diet group so that initially there were no significant differences in mean body weight between diet groups.

TABLE 1

Composition of diets

Component	Free access	Calorie restricted
	g/100 g	
Carbohydrate[1]	66.5	63.4
Soy protein	13.9	13.9
Hydrogenated oil[2]	10.0	10.0
α-Cellulose	1.8	1.8
Vitamin mix[3]	1.5	2.1
Salt mix[3]	6.0	8.4
dl-Methionine	0.2	0.3
	kJ/g	
Gross energy[4]	17.6	17.6

[1] Carbohydrate is either cornstarch, sucrose, glucose, fructose or equimolar amounts of fructose plus glucose.
[2] Crisco (Procter & Gamble, Cincinnati, OH).
[3] McDonald et al. 1989.
[4] Gross energy content of the diet was determined with bomb calorimetry.

After 2 wk of free access to the semipurified diets, half of the rats per carbohydrate group (total of 28 per diet group) were restricted to 60% of the mean caloric intake of their carbohydrate-matched free access–fed rats. Vitamin and salt contents of the restricted diets were increased by 40% to ensure proper nutrient density. The average daily food intake of rats fed with free access (n = 5/carbohydrate group) was determined during a 7-d period 1 wk before the start of caloric restriction, 1 wk after the initiation of calorie restriction and periodically between wk 15 and 25. The food intake was measured as the difference in the weight of the food cup, corrected for spillage, at the beginning and end of a 24-h period. There was some variability between the free access–fed carbohydrate groups in the first 2 wk, with the glucose- and fructose plus glucose–fed rats being fed ~3 g/d more than the fructose-fed rats. By wk 25, the variability between carbohydrate groups decreased. All of the rats with free access to food were fed a similar amount of grams of food per day. The amount of food fed to the calorie-restricted rats was adjusted accordingly. The body weight of each rat was measured once a week for the first 10 wk of the experiment and then once a month for the remainder of the life of the rat.

The primary purpose of this investigation was to describe possible differences in various lipid variables during long-term feeding of different carbohydrates. The effect of carbohydrate on survival rate was not a specific objective of this investigation; such an investigation has been reported previously (Murtagh-Mark et al. 1995). Nonetheless, it is important to show the effect of diet composition on life-span characteristics. Thus, after removing a total of 150 rats at 9, 18 and 26 mo of age (n = 5 per age, carbohydrate and calorie-intake group), 112 rats (n = 10–16 per diet group) were allowed to live out their natural life span or were killed when they became moribund. The survival analysis presented here is calculated for these 112 rats. To increase the number of rats per group, we combined groups according to the feeding paradigm (free access or calorie restricted) and whether the diet contained fructose (fructose, sucrose or fructose plus glucose) or did not contain fructose (glucose or starch). We recognize, however, that a group size of 24–32 per group is insufficient for accurate calculation of survival characteristics. Thus, these data are presented only to demonstrate that trends in survival characteristics in our rat population are similar to accepted norms previously reported (Murtagh-Mark et al. 1995, Yu et al. 1982). In general, the calorie-restricted rats lived significantly longer than did the rats with free access to food (**Table 2**), as indicated by greater mean, median and upper 10% survival rates for the calorie-restricted group. Survival characteristics were not significantly affected by the source of dietary carbohydrate when rats had free access to food. However, when calories were restricted, the rats fed a diet containing fructose had a significantly greater maximum life span than did the calorie-restricted rats fed a diet that did not contain fructose.

Blood and tissue collection. Food was removed at 0800 h, and 500 μL of blood was taken from the tail at 1100 h in rats aged 9, 18 or 26 mo (n = 5 rats per diet group/age). The serum was separated by allowing the blood to clot at room temperature and then frozen at −20°C until analysis of glucose concentration. One week after the collection of tail blood, 3-h food-deprived, halothane-anesthetized rats were killed by pneumothorax. Approximately 5 mL of whole blood was collected via cardiac puncture and allowed to clot on ice. An additional 100 μL of cardiac blood was collected in heparinized tubes for analysis of hemoglobin and glycated hemoglobin concentration. After blood collection, the carcass was eviscerated, weighed and stored at −20°C until analysis of body composition was performed. The data for serum glucose, hemoglobin and glycated hemoglobin are reported elsewhere (Lingelbach et al. 2000).

Body composition. The composition of eviscerated carcasses was assessed using the method developed by Bell and Stern (1977). Briefly, carcasses were freeze dried, and the water content was determined as the difference between wet weight and the weight after freeze drying. Fat was extracted from the freeze-dried carcasses via ether/methanol extraction. The fat mass was calculated as the difference between the dried carcass and the postextraction weight. Carcasses were then incinerated at 260°F, and the remaining ash weight was recorded. We could not calculate carcass protein and ash weight

TABLE 2

Estimated survival time of male Fischer 344 rats with free access to food or calorie restricted to 60% of amount fed rats with free access to food

Diet group[1]	*n*	Means ± SEM (range)	Median	Lower 10th percentile	Upper 10th percentile
				d	
Free access (+) fructose	32	712 ± 21[a] (248–880)	724[a]	611[a]	834[a]
Free access (−) fructose	22	673 ± 35[a] (263–954)	723[a]	436[a]	821[a]
Calorie restricted (+) fructose	34	802 ± 21[b] (444–1063)	821[b]	600[a]	905[b]
Calorie restricted (−) fructose	24	741 ± 31[ab] (362–887)	792[b]	390[a]	873[a]

[1] Diets that that are (+) fructose have sucrose, fructose or fructose plus glucose as the carbohydrate source. Diets that are (−) fructose have cornstarch or glucose as the carbohydrate source. Within a column, values sharing a superscript letter do not differ significantly ($P > 0.05$).

because the incinerated carcasses were accidentally discarded before post-ashing weight was determined.

Serum analysis. Serum triglyceride concentration was measured in 20 μL of serum according to an enzymatic procedure (procedure 334-UV; Sigma Diagnostics, St. Louis, MO). Total serum cholesterol concentration was measured in 10 μL of serum according to an enzymatic method (procedure 352; Sigma Diagnostics). HDL cholesterol concentration was isolated from 500 μL of serum using HDL ISOSPIN reagents that precipitate the LDL fraction of the sera (procedure 352-5; Sigma Diagnostics). The HDL concentration in the resulting supernatant was then measured with the same method used to measure total cholesterol concentration.

Statistical analysis. Repeated measures ANOVA was used to determine possible differences in mean body weights. Differences in the main effects and the interactions between effects were determined by ANOVA using dietary fructose, calorie restriction and age as independent variables. Possible differences in mean body composition variables and triglyceride, total cholesterol and HDL concentrations were determined from post hoc comparison with Fisher's PLSD. Differences were considered significant at $P < 0.05$. Possible differences in survival characteristics (median and percentile groups) attributed to dietary fructose and/or calorie restriction were analyzed with a χ^2 test to determine whether the groups were from the same distribution. Survival means were compared by one-way ANOVA with post hoc comparison with Fisher's PLSD.

A specific aim of this study was to establish baseline data on the effects of particular carbohydrates for use by other investigators who are interested in long-term feeding investigations in rats. This investigation is primarily a descriptive evaluation. With this specific aim, we provided means and SEM in the tables, with post hoc comparisons, and results of the higher order three-way design described in the text.

Another specific aim of this research was to determine whether dietary fructose alters lipid metabolism. The means of all five carbohydrate groups that are given in the tables do not, we believe, provide an adequate portrayal of the impact that dietary fructose has on measures of lipid metabolism. To more clearly illustrate the effect of dietary fructose on the measured variables, we also performed analyses after the diets were grouped into those that contained fructose (sucrose, fructose and fructose plus glucose) and those that did not (starch and glucose).

RESULTS

Body weight. Rats with free access to food were significantly heavier than their carbohydrate-matched, calorie-restricted counterparts (**Fig. 1**). There was, however, a significant interaction ($P = 0.0046$) between the source of dietary carbohydrate and the amount of calories consumed. Calorie-restricted rats fed a diet that contained fructose (sucrose, fructose and fructose plus glucose) were significantly heavier than calorie-restricted rats fed a diet that did not contain fructose (cornstarch and glucose) (**Fig. 2**). Conversely, the mean body weight of rats with free access to diets containing fructose was significantly less than that of rats with free access to diets that did not contain fructose.

The slope of the regression lines for body weights (used as a measure for rate of weight gain) of rats with free access to food did not differ significantly ($P = 0.9350$) among the five dietary groups on d 0–140 (rapid weight gain) or d 141–794 ($P = 0.1181$) (slow weight gain; Figs. 1, 2). Mean maximal body weight of the cornstarch-, glucose-, sucrose-, fructose- and fructose plus glucose–fed rats was achieved at 83, 81, 78, 76 and 76% of the group maximal life span, respectively.

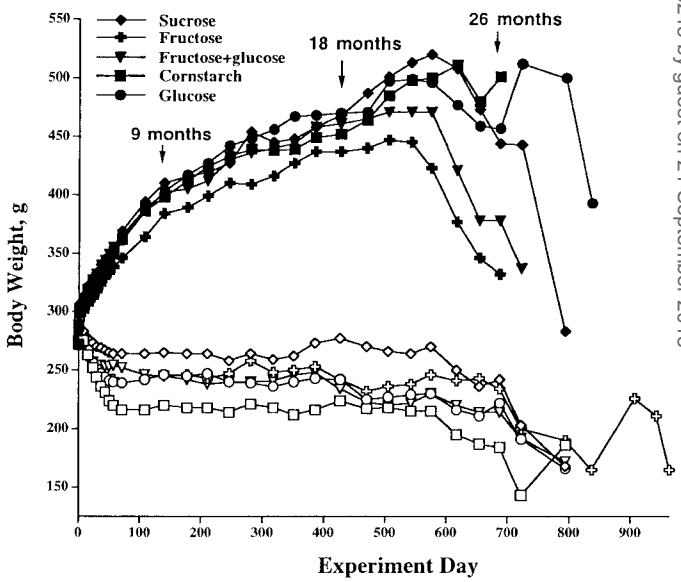

FIGURE 1 Mean body weights of male Fischer 344 rats given free access (filled symbols) or calorie-restricted to 60% of free access (corresponding open symbols) to five carbohydrate-specific diets. For ease of reading, standard error bars have been omitted. The standard error was generally 1–3% of the mean in all groups. Rats were 83 d at experiment day 0.

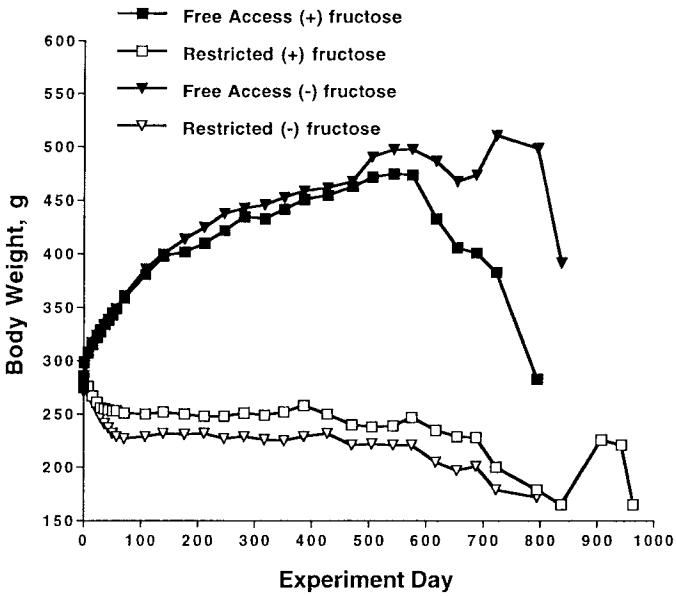

FIGURE 2 Mean body weights of male Fischer 344 rats given free access or calorie-restricted to 60% of free access to diets containing (+) fructose (sucrose, fructose and fructose + glucose) or (−) no fructose (cornstarch and glucose). The analysis comparing fructose with no fructose in the diet was performed to more clearly illustrate the impact of fructose on body weight. For ease of reading, standard error bars have been omitted. The standard error was generally 1–3% of the mean in all groups. Rats were 83 d at experiment day 0.

Carcass composition. In general, mean values for total carcass water, fat and fat-free weight (FFW)[3] were a function of total carcass weight (**Table 3**); significant differences in the total amounts of carcass water, fat and FFW reflected differences in carcass weight. Although several significant differences were noted among the age and carbohydrate groups for total carcass water, fat and FFW, no consistent general pattern was observed. The notable exception to this was the observation that FFW declined significantly in all 26- versus 9- and 18-mo-old rats that were calorie restricted. FFW of 26-mo-old rats that had free access to food significantly increased (cornstarch), did not differ (sucrose, glucose and glucose plus fructose) or declined significantly (fructose) compared with the 9- and 18-mo-old rats.

As expected, there was a significant main effect of calories consumed and of age on the percentage of carcass fat and FFW (**Fig. 3**). Rats with free access to food had significantly greater percentages of carcass fat than did calorie-restricted rats. The main effect of age reflected a primarily significantly greater percent fat of the 18-mo-old rats with free access to food compared with similarly fed 9- and 26-mo-old rats. Percent carcass fat of 26-mo-old rats with free access to diets containing fructose was significantly less than that of the other age and carbohydrate groups with free access to food. Percent carcass fat of 26-mo-old, calorie-restricted rats was significantly less than similarly fed 9- and 18-mo-old rats regardless of the type of carbohydrate in the diet. Percent carcass fat did not differ between calorie-restricted 9- and 18-mo-old rats in either the fructose-containing or fructose-free groups. Percent carcass fat of the 9-mo-old calorie-restricted rats fed diets containing fructose was significantly greater than their age-matched counterparts not fed fructose. Due to the two-compartmental model for body composition (i.e., fat and FFW), significant differences among the groups in percent carcass FFW weight were identical to those observed for percent carcass fat. The percentage of total body water in FFW, a measure of dehydration, did not differ significantly among the age, feeding and carbohydrate groups (mean 69.2–70.1%).

Serum lipids. There was a significant main effect on triglyceride concentrations of consumed calories and a significant interaction between age and consumed calories (**Table 4**). In all carbohydrate groups and at all ages, calorie restriction was associated with significantly lower serum triglyceride concentrations. The significant interaction between age and consumed calories reflected the observation that serum triglyceride concentration increased significantly with age in the calorie-restricted rats, but no trend was evident in the rats with free access to food. Several individual differences among the age and carbohydrate groups in rats with free access to food were observed, but no consistent trend was noted. Conversely, serum triglyceride concentration of calorie-restricted rats was significantly greater in 26- versus 9-mo-old rats regardless of the carbohydrate source.

A significant main effect of dietary carbohydrate on serum triglyceride concentration was observed and reflected, in general, significantly greater serum triglyceride concentrations in rats fed diets containing fructose (**Fig. 4**). This effect was most apparent in the calorie-restricted animals, in which serum triglyceride concentrations of 9-, 18- and 26-mo-old rats fed fructose are significantly greater than those of their age-matched fructose-free counterparts.

A significant main effect of dietary fructose, calorie restriction and age was observed on serum total cholesterol concentration (**Fig. 5**). In all groups, there was a significant age-associated increase in serum cholesterol concentrations. Calorie-restricted rats had cholesterol concentrations similar to those of diet-matched rats with free access to food at 9 and 18 mo of age, but at 26 mo of age, calorie restriction was associated with significantly lower cholesterol concentrations. In the groups with free access to food, but not the calorie-restricted group, a diet that contained fructose was associated with significantly greater cholesterol concentrations in the 26-mo-old rats compared with age-matched rats fed diets that did not contain fructose.

The amount of calories consumed and age, but not dietary fructose, had significant main effects on cholesterol composition. In all diet groups, the percentage of cholesterol found in the HDL fraction was significantly greater in the 9-mo-old rats (79 ± 7 free access to fructose, 82 ± 3 restricted access to fructose, 83 ± 4 free access to nonfructose, 85 ± 3 restricted access to nonfructose) compared with the 18-mo-old rats (44 ± 2, 52 ± 2, 42 ± 2 and 51 ± 2, respectively) and the 26-mo-old rats (57 ± 2, 66 ± 1, 60 ± 2 and 63 ± 1, respectively). The calorie-restricted rats had a significantly greater percentage of HDL cholesterol at 18 and 26 mo of age compared with age-matched rats with free access to food.

DISCUSSION

One aim of the current investigation was to describe the long-term effects of specific dietary carbohydrates, particularly fructose, on serum lipid levels and body composition in rats throughout the life span. To this end, 3-mo-old male Fischer 344 rats were fed one of five different carbohydrate diets with free access to food or restriction to 60% of free access intake until 9, 18 or 26 mo of age. In general, rats fed a diet containing fructose had significantly greater serum triglyceride concentrations than did rats fed a diet that did not contain

[3] Abbreviation used: FFW, fat-free weight.

TABLE 3

Total carcass, water, fat and fat free weight of male Fischer 344 rats fed various dietary carbohydrates and with free access to food or calorie restricted to 60% of rats with free access to food[1]

	Free access			Calorie restricted		
Diet	9 mo old	18 mo old	26 mo old	9 mo old	18 mo old	26 mo old
			g			
Carcass						
Cornstarch	327.8 ± 13.1^{Aa}	401.7 ± 8.5^{Ba}	403.9 ± 14.3^{Ba}	179.8 ± 11.1^{Aa}	186.0 ± 9.3^{Aa}	144.2 ± 9.5^{Ba}
Sucrose	344.4 ± 7.6^{ABa}	385.3 ± 16.6^{Aa}	311.6 ± 26.3^{Bb}	226.0 ± 11.1^{Ab}	232.8 ± 7.7^{Ab}	165.8 ± 16.9^{Bab}
Glucose	351.5 ± 8.1^{Aa}	402.5 ± 23.9^{Aa}	331.7 ± 43.2^{Aab}	210.0 ± 5.4^{Ab}	214.9 ± 6.2^{Ac}	174.4 ± 9.0^{Bb}
Fructose	339.3 ± 11.6^{Aa}	372.0 ± 10.5^{Aa}	224.3 ± 30.9^{Bc}	211.8 ± 1.6^{Ab}	191.9 ± 8.2^{Aac}	173.7 ± 7.4^{Bb}
Glucose + fructose	335.9 ± 5.1^{Aa}	383.5 ± 10.3^{Aa}	298.3 ± 23.3^{Bb}	225.6 ± 8.2^{Ab}	208.6 ± 7.4^{Aac}	154.1 ± 1.6^{Bab}
Water						
Cornstarch	172.1 ± 5.4^{Aa}	186.4 ± 6.8^{Ba}	193.4 ± 6.1^{Ba}	113.1 ± 5.0^{Aa}	117.9 ± 5.0^{Aa}	95.5 ± 5.7^{Ba}
Sucrose	177.1 ± 5.4^{ABa}	184.9 ± 4.7^{Aa}	162.4 ± 6.7^{Bb}	130.3 ± 3.6^{Ab}	136.1 ± 3.8^{Ab}	108.5 ± 10.4^{Bab}
Glucose	176.6 ± 1.9^{Aa}	191.3 ± 4.4^{Ba}	177.1 ± 16.1^{Aab}	128.5 ± 2.2^{Ab}	133.9 ± 2.8^{Ab}	113.5 ± 5.2^{Bb}
Fructose	181.6 ± 3.2^{Aa}	186.6 ± 5.9^{Aa}	134.5 ± 13.3^{Bc}	129.4 ± 2.3^{Ab}	119.9 ± 3.9^{Bac}	110.5 ± 3.8^{Cab}
Glucose + fructose	176.9 ± 3.7^{Aa}	178.8 ± 4.2^{Aa}	169.1 ± 12.3^{Ab}	140.5 ± 7.9^{Ab}	126.7 ± 2.7^{Bbc}	101.5 ± 1.4^{Cab}
Fat						
Cornstarch	80.5 ± 5.9^{Aac}	131.9 ± 7.6^{Ba}	114.2 ± 12.0^{Ba}	18.1 ± 4.9^{Aa}	18.2 ± 6.0^{Aa}	5.1 ± 1.5^{Ba}
Sucrose	89.8 ± 2.8^{Aab}	117.2 ± 11.6^{Ba}	71.8 ± 13.4^{Ab}	39.0 ± 5.9^{Ab}	37.8 ± 4.8^{Ab}	8.1 ± 3.1^{Bab}
Glucose	95.0 ± 4.8^{Abd}	127.4 ± 16.7^{Aa}	74.2 ± 19.5^{Bb}	26.4 ± 2.5^{Aa}	24.2 ± 2.9^{Aa}	9.8 ± 2.3^{Bab}
Fructose	77.3 ± 8.0^{Ac}	103.0 ± 3.9^{Bb}	29.5 ± 12.6^{Cc}	25.7 ± 1.9^{Aa}	18.9 ± 3.2^{Ba}	13.0 ± 2.4^{Cb}
Glucose + fructose	99.5 ± 5.0^{Ad}	124.3 ± 5.5^{Ba}	56.2 ± 9.4^{Cd}	27.9 ± 1.5^{Aa}	27.2 ± 4.8^{Aab}	6.5 ± 1.8^{Ba}
Fat-free weight						
Cornstarch	247.3 ± 7.5^{Aa}	269.7 ± 10.2^{ABa}	289.7 ± 5.7^{Ba}	161.7 ± 6.9^{Aa}	167.7 ± 3.9^{Aa}	139.1 ± 8.0^{Ba}
Sucrose	254.4 ± 7.9^{Aa}	268.1 ± 7.5^{Aa}	239.7 ± 12.7^{Abc}	186.9 ± 5.4^{Ab}	194.9 ± 5.3^{Ab}	157.7 ± 14.2^{Bb}
Glucose	256.5 ± 4.5^{Aa}	275.1 ± 7.4^{Aa}	257.4 ± 24.0^{Aab}	183.6 ± 2.9^{Ab}	190.6 ± 4.2^{Ab}	164.6 ± 6.9^{Bb}
Fructose	262.0 ± 4.5^{Aa}	269.6 ± 8.7^{Aa}	194.7 ± 18.7^{Bc}	186.0 ± 3.1^{Ab}	173.0 ± 5.9^{ABac}	160.7 ± 5.0^{Bb}
Glucose + fructose	256.4 ± 5.3^{Aa}	259.1 ± 5.3^{Aa}	242.1 ± 17.5^{Bab}	197.6 ± 7.8^{Ab}	181.3 ± 3.8^{Ac}	147.5 ± 1.9^{Bab}

[1] Values are means ± SEM, $n = 5$. Within a row and feeding treatment (i.e., Free access or Calorie restricted), values not sharing an uppercase superscript letter are significantly different, $P < 0.05$. Within a column and feeding treatment (i.e., Free access or Restricted), values not sharing a lowercase superscript letter are significantly different, $P < 0.05$.

fructose. This effect was observed throughout the life span and in rats with free access to food or restricted to 60% of free access intake. Dietary fructose increased total cholesterol concentration only in the oldest rats with free access to food; fructose did not significantly alter the cholesterol fraction distribution. Dietary fructose did not significantly increase the deposition of body fat.

Previous investigations in humans and mice and rats that evaluated the effect of dietary carbohydrates on serum triglyceride concentrations suggest that diets high in fructose significantly increase serum triglyceride levels (Albrink and Ullrich 1986, Lock et al. 1980, Macdonald 1966, Reaven et al. 1979 and 1990, Reiser et al. 1979, Waddell and Fallon 1973). Although these investigations provide a general consensus that increasing levels of dietary fructose result in elevated serum triglyceride levels, all of these studies used young animals that were fed the various diets for a specific period of the life span, usually 3–12 wk. No previous investigation evaluated the effect of dietary fructose on indices of lipogenesis throughout the life span. This fact is important because some of these investigations found that serum triglyceride concentration increases during the first few days of the diet and then plateaus and declines after 2–3 wk. Moreover, the interaction between dietary fructose and the energy content of the diet remains unclear because previous investigations of the effect of carbohydrate on serum triglyceride concentration provided the study animals with free access to food.

Our data clearly show that diets high in fructose increase serum triglyceride concentrations in male Fischer 344 rats and that this effect is evident after 6, 15 or 23 mo of feeding (i.e., the effect is not transitory). Most important, however, is our finding that calorie-restricted rats fed diets containing fructose had significantly greater serum triglyceride concentrations than did rats not fed dietary fructose. We found that fructose per se rather than the interaction between energy intake and fructose is the factor responsible for the increase in serum triglyceride concentration.

This investigation was designed simply to describe the long-term feeding effects of various dietary carbohydrates on variables of lipogenesis to establish some baseline data for other investigators who are interested in long-term feeding studies. An evaluation of mechanisms that underlie the effect of fructose on variables of lipogenesis was not our intent, and thus our discussion on such mechanisms is speculative. Nonetheless, it is interesting to note that there was no significant difference in serum triglyceride concentrations among rats fed the high fructose diet and rats fed the sucrose or fructose plus glucose diet, despite the fact that the high fructose diet contained twice the amount of fructose. This observation is in contrast to the dose response observed by others in humans (Hallfrisch et al. 1983, Reiser et al. 1981). It has been proposed that the lipogenic effect of fructose reflects differences in the hepatic metabolism of fructose and glucose. Glucose metabolism in the liver has the potential to produce glucose or fatty acids, whereas the metabolism of fructose has the potential to produce glucose and both fatty acids and glycerol, the two components of triglycerides. In a crossover design study in which male and female subjects ingested controlled diets containing 44% of total energy as carbohydrate with increasing proportions provided as sucrose (2, 15 and 30% of total en-

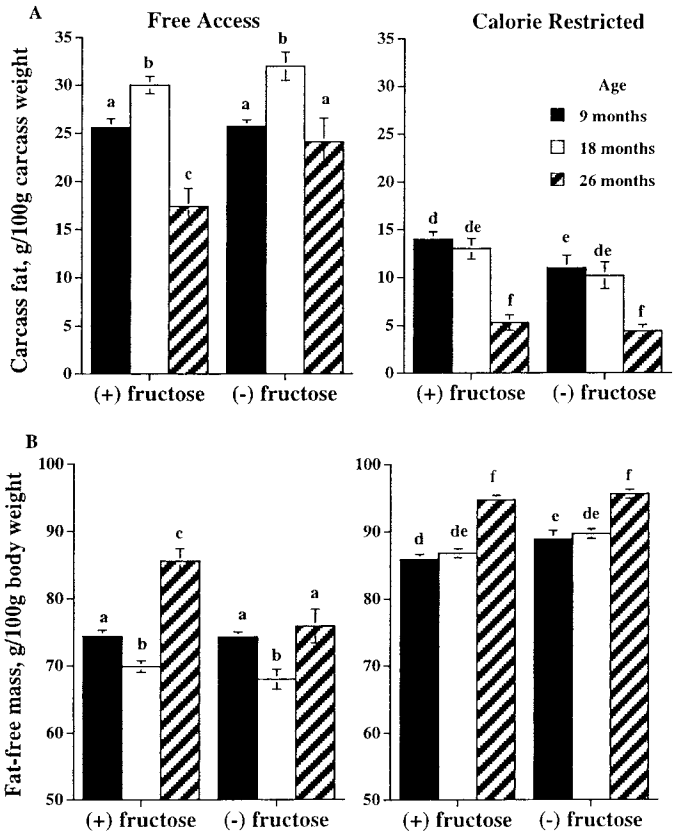

FIGURE 3 Percent carcass fat (*A*) and fat-free mass (*B*) of rats with free access, or calorie-restricted to 60% of free access to diets containing (+) fructose (sucrose, fructose and fructose plus glucose) or (−) no fructose (cornstarch and glucose). Within a graph and body composition variable (Free Access or Calorie Restricted; carcass fat or carcass fat-free mass), values with the same letter above them do not differ significantly ($P > 0.05$). The analysis comparing fructose with no fructose in the diet was performed to more clearly illustrate the impact of fructose on percent carcass fat and fat-free mass.

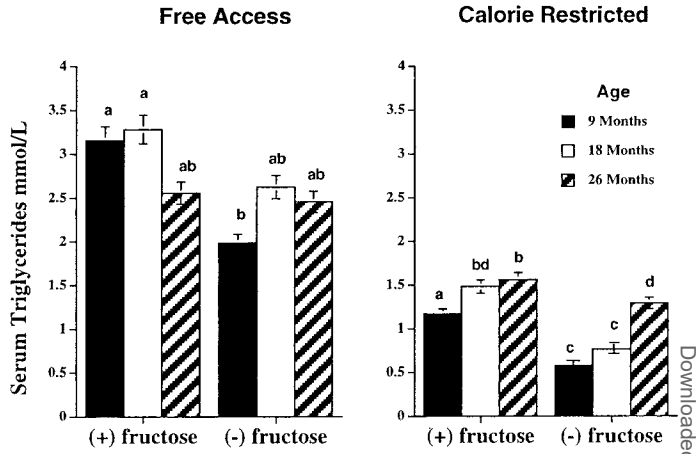

FIGURE 4 Serum triglyceride concentrations of rats with free access or calorie-restricted to 60% of free access to diets containing (+) fructose (sucrose, fructose and fructose plus glucose) or (−) no fructose (cornstarch and glucose). Within a graph (Free Access or Calorie Restricted), values with the same letter above them do not differ significantly ($P > 0.05$). Serum triglyceride concentrations of all calorie-restricted groups differ significantly from all groups with free access to food. The analysis comparing fructose with no fructose in the diet was performed to more clearly illustrate the impact of fructose on serum triglyceride concentrations.

ergy), Reiser et al. (1979) observed a positive correlation between serum triglyceride concentrations and the amount of sucrose in the diet in the male but not in the female subjects after 6 wk on the experimental diets. A similar dose response was observed in hyperlipidemic men who ingested for a 5-wk period diets that contained increasing amounts of fructose (0, 7.5 and 15% of total energy) and 43% of total calories as carbohydrate (Hallfrisch et al. 1983). Our data indicate that there is no dose response when rats are fed a specific carbohydrate diet for a prolonged period. We believe that the dose response of fructose previously reported by others may be more closely related to a short-term feeding phenomenon than to fructose per se.

Age and energy content of the diet, but not dietary fructose, were associated with an increase in total cholesterol. An age-related increase in total cholesterol concentration in rats with free access to food was observed in both male and female Fischer 344 and Sprague-Dawley rats fed nonpurified diets

TABLE 4

Serum triglyceride concentration in male Fischer 344 rats fed various dietary carbohydrates and with free access to food or calorie restricted to 60% of rats with free access to food[1]

		Age, *mo*		
Feeding group	Carbohydrate source	9	18	26
		mmol/L		
Free access	Cornstarch	1.62 ± 0.28^{Aa}	2.12 ± 0.38^{ABa}	2.62 ± 0.35^{Ba}
	Glucose	2.34 ± 0.30^{Aab}	3.25 ± 0.63^{Bb}	2.28 ± 0.32^{Aa}
	Sucrose	2.85 ± 0.64^{Aab}	3.51 ± 0.91^{Bb}	2.66 ± 0.47^{Aa}
	Fructose	3.48 ± 0.60^{Ab}	3.61 ± 0.58^{Ab}	2.84 ± 0.54^{Ba}
	Fructose + glucose	3.00 ± 0.48^{Ab}	2.56 ± 0.54^{ABa}	2.16 ± 0.20^{Ba}
Calorie restricted	Cornstarch	0.61 ± 0.11^{Aa} (4)	0.79 ± 0.17^{Aa} (4)	1.30 ± 0.03^{Ba}
	Glucose	0.55 ± 0.10^{Aa}	0.73 ± 0.11^{Aa}	1.32 ± 0.02^{Ba}
	Sucrose	1.01 ± 0.11^{Ab}	1.73 ± 0.38^{Bb}	1.83 ± 0.03^{Bb}
	Fructose	1.34 ± 0.10^{Ab}	1.50 ± 0.39^{ABb} (3)	1.62 ± 0.04^{Bb}
	Fructose + glucose	1.06 ± 0.12^{Ab}	1.15 ± 0.20^{Aab}	1.52 ± 0.06^{Bb}

[1] Values are means ± SEM, $n = 5$ unless otherwise noted in parentheses. Within a row and feeding treatment (i.e., Free access or Calorie restricted), values sharing an uppercase superscript letter do not differ significantly ($P > 0.05$). Within a column and feeding treatment (i.e., Free access or Calorie restricted), values sharing a lowercase superscript letter do not differ significantly ($P > 0.05$). Within an age group, all calorie-restricted groups had significantly lower serum triglyceride concentrations than carbohydrate-matched, free access–fed rats.

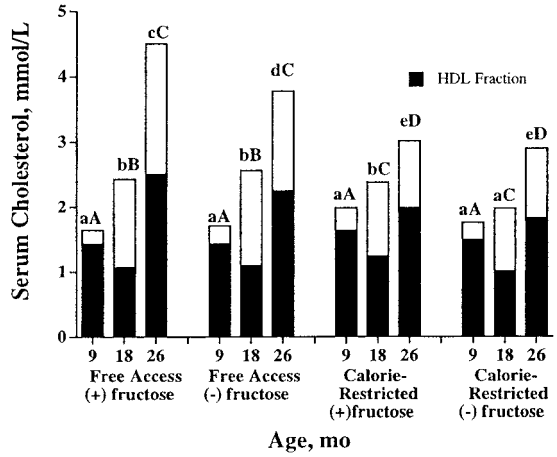

FIGURE 5 Serum cholesterol concentrations of rats with free access to or calorie-restricted to 60% of free access to diets containing (+) fructose (sucrose, fructose and fructose plus glucose) or (−) no fructose (cornstarch and glucose). Total serum cholesterol concentration is represented by the total height of the column. Columns with the same lowercase letter indicate that mean total cholesterol concentrations differ significantly ($P > 0.05$). Columns with the same uppercase letter indicate that HDL/total cholesterol ratios do not differ significantly ($P < 0.05$). There was a significant main effect of age ($P < 0.05$) on mean total cholesterol in all four feeding groups. The analysis comparing fructose with no fructose in the diet was performed to more clearly illustrate the impact of fructose on serum cholesterol concentrations.

(Carlson et al. 1968, Dupont et al. 1972, Lacko and Davis 1979, Liepa et al. 1980, Masoro et al. 1983, Story et al. 1976). Fructose enhances the increase in total cholesterol in older rats when with free access to food. This effect of fructose was not observed in the restricted rats. Restricted calorie intake blunted the age-related increase in total cholesterol in the oldest group observed. This effect of calorie restriction was also observed in diet-fed rats (Liepa et al. 1980, Masoro et al. 1983). Masoro et al. (1983) reported that the age-related increase in total cholesterol reflected an increase in HDL cholesterol. We observed that the age-related changes in total cholesterol concentrations reflect changes in the HDL cholesterol fraction. HDL cholesterol was significantly lower in the 18-mo-old rats than in the younger 9-mo-old rats. Overall, dietary fructose did not affect the total amount or composition of serum cholesterol.

Several investigations in mice and rats fed experimental diets for a period of several weeks suggest that free access to dietary sucrose promotes the deposition of body fat (Barnard et al. 1993, Hallfrisch et al. 1981, Reiser and Hallfrisch 1977, Rizkalla et al. 1992). For example, rats with free access to diets containing 40% (by weight) fat and 30% sucrose for 8–9 wk had greater body weights and greater epididymal and perirenal fat pad weights than rats fed an isocaloric cornstarch diet (Hallfrisch et al. 1981). A similar effect was observed in rats fed a 16% fat and 54% sucrose or starch diet for 7–11 wk, suggesting that the greater fat gain was due to the high fat content of the diet (Reiser and Hallfrisch 1977). The current investigation does not support the hypothesis that diets high in fructose content promote the deposition of body fat in rats. We found that 18- and 26-mo-old rats with free access to diets containing fructose had a similar percent body fat as rats fed fructose-free diets. This effect was not due to differences in the amount of calories consumed, because similar results were noted in the calorie-restricted animals. We did find, however, that 9-mo-old calorie-restricted rats fed fructose had a slight but significantly greater percent body fat than did age-matched restricted rats fed the diets without fructose. This finding supports our suggestion that an increase in body fat associated with dietary fructose previously reported reflects more closely a short-term feeding phenomenon in young animals.

Previous investigations have described a significant decline in the body weight of rats near the end of life, usually beginning at 70–90% of the life span. We have shown than this decline in body weight is concomitant with a significant reduction in food intake (Blanton et al. 1998). The data presented here are consistent with this age-related anorexia/hypophagia. Although the mechanisms causing the body weight loss near the end of life have not been elucidated, it is clear from this and previous investigations that the decline more closely reflects a starvation-like state rather than a wasting disorder such as disease-associated cachexia (Table 3). This suggestion is substantiated by the observation that total FFW (g) of rats with free access to food is conserved or only slightly decreased during weight loss. The decline in carcass weight between 18- and 26-mo-old rats with free access to food primarily reflects a loss in fat weight. That the end-of-life body weight loss is not associated directly with a disease process is supported by the observation that a similar pattern of fat weight loss was observed in the calorie-restricted rats (Fig. 1, Table 3). Calorie-restricted rats have significantly less definable disease at death yet still show a significant loss in body weight near the end of life.

It is interesting to note, however, that the conservation of FFW during body weight loss seen in the rats with free access to food was not observed in the calorie-restricted rats. Although a significant decline in fat weight accounted for the majority of carcass weight loss in the calorie-restricted rats, significant declines in FFW also occurred in all calorie-restricted groups. Mechanisms accounting for the reduction in FFW seen in the 26-mo-old calorie-restricted rats are not known, and we can only offer a speculative proposal. We contend that the calorie-restricted animals did not have sufficient fat reserves available to provide the energy needed for soma maintenance when the inevitable age-related anorexia began. Thus, these animals must turn to protein as an energy substrate. This suggestion is supported by the observation that the percent carcass fat in the 26-mo-old calorie-restricted rat was undoubtedly close to the essential level (i.e., lipid associated with cellular function, not storage, and considered to be <5%; Fig. 3). Moreover, the only rats with free access to food and to show a decline in FFW were the fructose-fed rats, the group that also had the greatest loss in fat weight.

The significant decline in FFW of the oldest calorie-restricted rats is part of a growing body of evidence indicating that although calorie restriction can extend life span and delay or prevent many age-associated diseases, there may be some detrimental consequences in old age. Our current and previous data suggest that at least one of these detrimental consequences is related to protein metabolism. For example, we previously found that wound healing is significantly blunted in calorie-restricted versus rats with free access to food and reflects diminished collagen synthesis at the wound (Reiser et al. 1995). It is possible that as the older calorie-restricted animal enters a period of altered energy balance, such as that seen in age-related anorexia, the total protein content of the diet may fall below levels needed for maintenance of tissue (i.e., protein synthesis). The protein content of the diet used here was 13.9%, an amount that is consistent with helping to reduce kidney disease during long-term feeding experiments. Our data suggest that the protein requirements of the calorie-restricted animal may have to be increased in old age. Regardless, investigators considering studies with calorie-restricted animals

should be aware of the reduction in FFW in the oldest rats and how this reduction may influence their study results.

In summary, the data presented here suggest that feeding rats diets that contain fructose elevates serum triglyceride concentrations and that this effect is consistent over the life span. Moreover, the increased serum triglyceride concentration reflects fructose per se and is not associated with the energy content of the diet. Conversely, dietary fructose did not enhance fat deposition in the rats that either had free access to food or were calorie restricted to 60% of free access.

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