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Effects of Dietary Carbohydrate Source on Growth, Plasma Metabolites and Lipogenesis in Rats, Pigs and Chicks[1] (39006)

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The role of dietary sucrose in the development of cardiovascular diseases and other metabolic disorders in humans has not been unequivocally established (1–3). Substitution of sucrose or fructose for other carbohydrates in the diet does alter lipid metabolism in rats. Plasma triglyceride levels were elevated, the rate of hepatic lipogenesis increased and the rate of lipogenesis in adipose tissue decreased when rats were fed fructose- rather than glucose-containing diets (4–8).

The influence of dietary sucrose or fructose on lipid metabolism in species other than the rat is less well defined. Qualitative differences in carbohydrate metabolism have been reported when birds and mammals were compared (9, 10). The chick and pig represent two animal models previously shown to respond differently than the rat to certain dietary stimuli (11–14). Additionally, carbohydrate conversion to fatty acids occurs primarily in chick liver, whereas adipose tissue is the predominant site for fatty acid synthesis in the pig. Both adipose tissue and liver are important sites for fatty acid synthesis in the rat (see Ref. 15).

The purpose of this investigation was to study the influence of dietary fructose and glucose on lipogenesis in three species, the rat, pig and chick, known to differ with regard to the major site of fatty acid synthesis. Additionally, the influence of these dietary carbohydrates on several plasma metabolites was examined.

Materials and methods. Experimental animals and diets. Male Sprague–Dawley[3] rats were used. Rats were individually housed in stainless-steel cages with raised wire floors. Male crossbred[4] chicks were placed on experimental diets 8–10 days after hatching. Chicks were housed, six birds per pen, in electrically heated pens with raised wire floors. Castrated male Yorkshire–Hampshire crossbred pigs were weaned at 3 weeks of age and fed a commercial diet for 1 week. At this time the 16 pigs were assigned to one of two experimental diets. The pigs were housed in groups of eight in pens with aluminum-slat floors. Diets and water were provided *ad libitum* in all experiments. Individual body weights were recorded weekly. Individual food consumption was recorded weekly for the rat experiments, while group food-intakes were recorded weekly in the chick and pig experiments. The composition of the diets fed is presented in Table I. Because of the prohibitive cost of fructose, diets containing only glucose or sucrose were fed to pigs.

Blood sampling and analyses. Blood was collected in heparinized beakers following decapitation of the rats, by heart puncture of the chicks, and by puncture of the anterior vena cava of the pigs. Plasma fructose (20), glucoses, triglyceride[5] (21), cholesterol (22) and free fatty acid (23) levels were analyzed by the methods indicated.

In vitro lipogenesis. Distal portions of rat epididymal adipose tissue, slices of pig subcutaneous adipose tissue and rat and chick liver-slices were incubated for 2 hr at 37° in 3 ml of Krebs–Ringer bicarbonate buffer. The tissue slices were prepared with a Stadie–

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[5] Glucostat, Worthington Biochemical Corporation.

TABLE I. COMPOSITION OF DIET.[a]

Component	Species		
	Rat	Chick	Pig
Casein	20.0	—	—
Isolated soy protein	—	23.1	26.3
Methionine	0.3	0.4	0.3
Mineral mix[b]	4.0	5.6	2.5
Vitamin mix[c]	0.4	0.6	1.0
Choline chloride	0.2	0.3	—
Fiber	4.0	5.0	3.0
Safflower oil	3.0	5.0	5.0
Glucose, fructose or sucrose	68.1	60.0	61.9
Total	100.0	100.0	100.0

[a] The isolated soy protein and safflower oil were generously supplied by Central Soya, Decatur, IN, and by Pacific Vegetable Oil Corporation, Richmond, CA, respectively.

[b] The compositions of the rat and chick mineral mix have been presented by Leveille and O'Hea (16) and by Velu *et al.* (17), respectively. The mineral mix fed to the pigs contained 20% sodium chloride, 40% dicalcium phosphate and 40% limestone; trace minerals were present in the vitamin mix.

[c] The compositions of the rat and chick vitamin mix (18) and the pig vitamin mix (19) have been presented previously.

Riggs hand microtome. The tissue preparations weighed approximately 150 mg. The incubation buffer contained per milliliter: 0.1 U porcine insulin,[6] 5 μmoles of glucose, 10 μmoles of acetate and 0.1 μCi ^{14}C-acetate. The incubation procedures and methods for isolation and counting the radioactive fatty acids and digitonin-precipitable sterols have been described (24). ^{14}C-acetate incorporation into digitonin-precipitable sterols was assumed to represent rates of cholesterol synthesis.

Enzyme analyses. About 1 g of liver or adipose tissue was weighed, homogenized in 9 ml of 0.15 *M* KCl, 4 m*M* $MgCl_2$, 4 m*M* *N*-acetylcysteine, pH 7.0, and centrifuged at 100,000*g* at 4° for 30 min. The resulting supernatant fluid was used to determine the activities of malic enzyme (EC 1.1.1.40) (25), acetyl-CoA carboxylase (EC 6.4.1.2) (26) and fatty acid synthetase (27). Protein content of the supernatant fluid was determined by the method of Lowry *et al.* (28). Enzyme activities were expressed as units, where a unit is the amount of enzyme that will catalyze the utilization of one nanomole of substrate per minute.

Results. Rats fed diets containing fructose as the carbohydrate source gained less weight than did rats fed diets containing glucose (Table II). Replacement of glucose with sucrose did not influence body weight gain in the rats. In one experiment the addition of fructose rather than glucose to the diet depressed body weight gain in chicks, but in the second experiment the differences were not significant (Table II). In agreement with the influence of dietary sucrose on body weight gain in the rat, replacement of dietary glucose with sucrose did not influence growth in chicks or pigs (Tables II and IV). The influence of these dietary carbohydrates on food intake in rats, chicks, and pigs paralleled the body weight gain response (Tables II and IV).

Plasma fructose levels were elevated in rats and chicks fed fructose rather than glucose (Table II). Sucrose consumption also elevated plasma fructose levels in chicks and pigs but not in rats (Tables II and IV). Whether the low levels of fructose, detected with the colorimetric assay, in the plasma of animals fed glucose actually represented fructose or a nonspecific reaction was not determined. Consumption of fructose-containing diets elevated plasma fructose levels above the basal values observed in the glucose-fed animals. Plasma glucose levels were not influenced by diet in the three species examined (Tables II and IV). As expected and in agreement with previous observations, plasma triglyceride levels were elevated in rats fed fructose- rather than glucose-containing diets (Table II). Plasma triglyceride levels in rats fed sucrose were intermediate between the levels observed in rats fed glucose or fructose. Plasma triglyceride levels in the other two species, chicks and pigs, were not influenced by the source of dietary carbohydrate (Tables III and IV). The source of carbohydrate did not alter plasma cholesterol or free fatty acid levels in the three species studied (Tables II and IV).

The influence of dietary fructose, sucrose and glucose on lipogenesis in rat and chick

[6] Generously supplied by Eli Lilly, Indianapolis, IN.

TABLE II. EFFECTS OF DIETARY CARBOHYDRATE SOURCE ON BODY WEIGHT GAIN AND PLASMA METABOLITES IN RATS AND CHICKS.[a]

	Experiment I Carbohydrate source		Experiment II Carbohydrate source		
	Glucose	Fructose	Glucose	Fructose	Sucrose
			Rats		
Initial weight (g)	104 ± 1	105 ± 1	90 ± 1	87 ± 2	90 ± 1
Daily body weight gain (g)	7.15 ± 0.12	6.44 ± 0.11[b]	7.26 ± 0.22	6.24 ± 0.13[b]	7.43 ± 0.15
Daily food intake (g)	20.1 ± 0.38	18.0 ± 0.26[b]	20.2 ± 0.40	17.9 ± 0.31[b]	19.6 ± 0.46
Plasma (mg/100 ml)					
Fructose	4 ± 1	13 ± 1[b]	4 ± 1	10 ± 1[b]	5 ± 1
Glucose	138 ± 8	139 ± 8	133 ± 11	107 ± 18	123 ± 10
Triglyceride	105 ± 4	203 ± 8[b]	138 ± 9	314 ± 29[b]	194 ± 21[b]
Cholesterol	148 ± 4	150 ± 5	151 ± 5	161 ± 4	173 ± 17
Free fatty acids	5.6 ± 0.7	5.8 ± 0.5	5.0 ± 0.6	4.6 ± 0.3	5.5 ± 0.4
			Chicks		
Initial weight (g)	127 ± 2	126 ± 3	199 ± 3	200 ± 4	200 ± 5
Daily body weight gain (g)	33.7 ± 0.9	30.1 ± 1.4[b]	35.6 ± 1.7	34.9 ± 1.1	36.7 ± 0.7
Daily food intake (g)	52.4	52.9	58.5	58.1	60.4
Plasma (mg/100 ml)					
Fructose	6 ± 1	25 ± 3[b]	7 ± 1	25 ± 2[b]	14 ± 1[b]
Glucose	231 ± 41	244 ± 18	249 ± 19	291 ± 14	293 ± 23
Triglyceride	81 ± 8	92 ± 3	85 ± 7	91 ± 5	92 ± 5
Cholesterol	204 ± 38	200 ± 14	196 ± 5	182 ± 6	191 ± 5
Free fatty acids	8.0 ± 0.8	6.2 ± 0.7	8.3 ± 0.4	9.2 ± 0.4	10.3 ± 0.9

[a] Values represent mean ± SEM for 10 and 12 rats per treatment in Experiment I (24 days) and II (26 days) or mean ± SEM for 6–12 chicks per treatment in Experiment I (22 days) and Experiment II (24 days), respectively.
[b] Glucose vs fructose or sucrose, significantly different, $P < 0.05$ level.

TABLE III. EFFECTS OF DIETARY CARBOHYDRATE SOURCE ON LIPOGENESIS IN RATS AND CHICKS.[a]

	Experiment I Dietary carbohydrate		Experiment II Dietary carbohydrate		
	Glucose	Fructose	Glucose	Fructose	Sucrose
			Rats		
Liver					
Wet weight (g)	12.6 ± 0.3	15.6 ± 0.5[b]	13.0 ± 0.3	14.7 ± 0.3[b]	15.6 ± 0.5[b]
Soluble protein (mg/g)	110 ± 2	108 ± 2	106 ± 2	105 ± 1	103 ± 2
Fatty acid synthetase[c]	8.8 ± 0.4	19.0 ± 0.6[b]	9.9 ± 0.5	22.8 ± 1.1[b]	16.8 ± 1.1
Acetyl CoA carboxylase[c]	11.2 ± 0.4	19.6 ± 0.7[b]	8.0 ± 0.5	10.9 ± 0.9[b]	7.7 ± 0.5
Fatty acid synthesis[c]	1517 ± 142	2465 ± 268[b]	1324 ± 214	2555 ± 316[b]	2119 ± 233[b]
Cholesterol synthesis[c]	38.1 ± 5.3	31.7 ± 3.1	33.5 ± 3.5	41.6 ± 7.2	28.3 ± 1.8
Adipose tissue					
Fat pad weight (g)	3.02 ± 0.15	3.17 ± 0.12	3.42 ± 0.12	2.82 ± 0.32	3.81. ± 0.28
Soluble protein (mg/g)	9.71 ± 0.40	7.97 ± 0.30[b]	8.16 ± 0.22	7.25 ± 0.18[b]	7.38 ± 0.28
Fatty acid synthetase[c]	46.2. ± 3.3	33.2 ± 2.0	46.5 ± 1.8	29.4 ± 3.4	41.2 ± 2.2
Acetyl CoA carboxylase[c]	43.1 ± 3.0	29.3 ± 3.1[b]	33.4 ± 1.9	17.1 ± 0.6[b]	29.3 ± 3.6
Fatty acid synthesis[c]	3153 ± 198	2975 ± 266	3769 ± 211	3139 ± 197[b]	3157. ± 169[b]
			Chicks		
Liver					
Wet weight (g)	23.4 ± 1.0	22.1 ± 1.3	24.9 ± 0.7	23.6 ± 1.1	23.6 ± 1.1
Soluble protein (mg/g)	109 ± 3	107 ± 4	91 ± 2	92 ± 3	90 ± 2
Fatty acid synthethase[c]	32.2 ± 2.0	26.2 ± 2.2	53.1 ± 3.7	54.3 ± 4.1	62.7 ± 4.1
Acetyl CoA carboxylase[c]	23.6 ± 1.6	22.4 ± 1.8	34.2 ± 3.5	40.8 ± 3.2	41.2 ± 2.0
Fatty acid synthesis[c]	13,788 ± 1,120	10,489 ± 531[b]	9,342 ± 591	10,278 ± 706	10,907 ± 490
Cholesterol synthesis[c]	211 ± 25	294 ± 66	166 ± 24	121 ± 33	117 ± 8

[a] See Table II.
[b] See Table II.
[c] Enzyme activity expressed as nanomoles of substrate converted to product per milligram of protein per minute at 37°. Fatty acid and cholesterol synthesis expressed as nanomoles of ^{14}C-acetate incorporated into fatty acids and into digitonin-precipitable sterols per gram of tissue per hour, respectively.

liver was investigated (Table III). The activities of fatty acid synthetase and acetyl-CoA carboxylase were elevated in rats but not in chicks fed fructose rather than glucose (Table III). Fatty acid synthetase activity but not acetyl-CoA carboxylase activity was also increased in livers of rats fed sucrose rather than glucose. Dietary sucrose did not in-

TABLE IV. EFFECTS OF DIETARY CARBOHYDRATE SOURCE ON BODY WEIGHT GAIN, PLASMA LIPIDS AND ADIPOSE TISSUE LIPOGENESIS IN PIGS.[a]

	Dietary carbohydrate			
	Glucose		Sucrose	
	3 Weeks	4 Weeks	3 Weeks	4 Weeks
Daily body weight gain (g)		430 ± 30		460 ± 30
Daily food intake (g)		890		950
Plasma (mg/100 ml)				
Fructose		4 ± 1		7 ± 1[b]
Glucose		99 ± 5		88 ± 6
Triglyceride		31 ± 5		30 ± 5
Cholesterol		106 ± 6		112 ± 4
Free fatty acids		1.2 ± 0.1		1.5 ± 0.2
Adipose tissue				
Soluble protein (mg/g)	6.12 ± 0.34	6.04 ± 0.44	6.58 ± 0.27	5.75 ± 0.17
Fatty acid synthetase[c]	8.3 ± 0.7	6.2 ± 1.6	9.7 ± 1.5	6.2 ± 1.7
Acetyl CoA carboxylase[c]	2.03 ± 0.16	1.07 ± 0.18	2.01 ± 0.25	1.37 ± 0.29
Malic enzyme[c]	194 ± 28	169 ± 28	278 ± 38[d]	238 ± 25[d]
Fatty acid synthesis[c]	7905 ± 890	4946 ± 710	8721 ± 925	6530 ± 535[d]

[a] Values represent mean ± SEM for eight pigs per treatment. The initial weight of the pigs was 8.25 ± 0.26 kg.

[b] See Table II.

[c] See Table II.

[d] Glucose vs sucrose, $P < 0.1$.

fluence the activities of these enzymes in chick liver (Table III). Liver wet weight but not soluble protein concentration was elevated in rats fed fructose or sucrose in place of glucose. However, no differences in liver weight were observed in chicks fed these diets.

In vitro measures of fatty acid and cholesterol synthesis were obtained in livers of rats and chicks fed the various carbohydrates (Table III). In agreement with the observed changes in lipogenic enzyme activities fructose or sucrose consumption elevated ^{14}C-acetate conversion to fatty acids in rat liver but not in chick liver. In fact the rate of fatty acid synthesis in chick liver-slices was depressed in the first but not in the second experiment when fructose replaced glucose in the diet. In both rats and chicks, the source of dietary carbohydrate did not influence the rate of hepatic cholesterol synthesis.

In the rat and pig, adipose tissue is a major organ involved in fatty acid synthesis. The influence of various dietary carbohydrates on lipogenesis in rat and pig adipose tissue was investigated (Tables III and IV). Both fatty acid synthetase and acetyl-CoA carboxylase activities were depressed in adipose tissue preparations of rats fed fructose rather than glucose. The activities of these enzymes were not significantly depressed ($P > 0.05$) when sucrose replaced glucose in the diet of rats. Because the adipose tissue soluble-protein concentration was reduced in rats fed fructose or sucrose, the magnitude of the enzymatic responses observed would have been even greater had the results been expressed per gram of tissue rather than per milligram of protein. In the pig, the activities of fatty acid synthetase and acetyl-CoA carboxylase in the adipose tissue were not significantly changed when sucrose replaced glucose in the diet. The activity of malic enzyme in the adipose tissue was slightly elevated ($P < 0.1$) when sucrose rather than glucose was fed to the pigs.

The rate of conversion of ^{14}C-acetate to fatty acids in adipose tissue preparations from rats and pigs fed the various carbohydrates usually paralleled the observed changes in enzyme activities. The rate of fatty acid synthesis was significantly depressed in adipose tissue preparations from rats fed fructose (Experiment II) or sucrose rather than glucose. ^{14}C-acetate conversion to fatty acids in adipose tissue was slightly increased ($P < 0.1$) in pigs fed sucrose rather

than glucose for 4 weeks, as compared to being decreased in rats.

Discussion. Virtually all species studied can absorb and utilize fructose. The growth rate of rats, chicks and pigs was not influenced by substitution of sucrose for glucose in the diet. However, the growth rate of rats but not chicks was depressed when fructose was fed. It has been demonstrated that fructose is absorbed across the intestinal wall largely unchanged in rats and chicks (29). However, the mode of fructose absorption has not been elucidated in the pig. In the guinea pig and hamster, fructose is largely converted to glucose during transfer across the intestinal wall (29). Because plasma fructose levels were higher in pigs fed sucrose than in those fed glucose, it is suggested that at least a portion of the fructose escaped intestinal metabolism in the pig during absorption.

The plasma triglyceride response of rats, chicks and pigs to various dietary treatments is species specific (11–13). In this study rat plasma triglyceride levels were elevated when the diet contained fructose or sucrose rather than glucose. However, the source of dietary carbohydrate did not influence plasma triglyceride levels in the chicks or pigs. Bruckdorfer *et al.* (4) also observed that chicks fed either sucrose- or starch-containing diets exhibited similar plasma triglyceride levels. It has been reported that plasma triglyceride levels are not elevated in weanling rats (6) and that the plasma triglyceride response to dietary fructose is somewhat transitory (30). Whether the age of the experimental animals or the length of the experiments influenced the results in the present study is not clear. Plasma triglyceride levels in pigs fed the experimental diets for 2 weeks (results not reported) were similar to those obtained after feeding the diets for 4 weeks. The present chick study lasted 3 weeks whereas Bruckdorfer *et al.* (4) fed chicks for 22 weeks. In neither chick experiment did plasma triglycerides respond to source of dietary carbohydrate. Thus, it is possible that dietary carbohydrates have species-specific influences on plasma triglycerides. In earlier reports it was demonstrated that dietary safflower oil and tallow (14) and dietary butanediol (11–13) also have species-specific influences on plasma triglyceride levels in these species.

The liver is the major lipogenic organ in the chick (see Ref. 10, 15); however, few studies have examined the influence of various dietary carbohydrates on the rate of hepatic fatty acid synthesis in this species. It is now well-established that the rate of fatty acid synthesis in the liver is increased in rats fed fructose- or sucrose- rather than glucose-containing diets (Ref. 8 and our present results). In contrast to the observations in rats, dietary fructose or sucrose did not increase lipogenic enzyme activities or rates of fatty acid synthesis in chick liver preparations. In fact, in one experiment, the rate of fatty acid synthesis in chick liver-slices was significantly decreased when dietary fructose replaced glucose. Hepatic fatty acid synthetase activities were significantly increased when sucrose replaced starch in the diet of chicks (4). However, it has also been observed that, in the rat at least, the rate of fatty acid synthesis was significantly lower in animals fed starch rather than glucose (31). Pearce (32, 33) has also fed chicks diets containing glucose or or fructose as the carbohydrate source. The activities of acetyl-CoA carboxylase, citrate cleavage enzyme, malic enzyme, and pyruvate kinase were not influenced by the source of dietary carbohydrate. Pearce (32) did observe that the activity of ketohexokinase (EC 2.7.1.3) in chick liver was increased suggesting that the potential for fructose phosphorylation was increased when fructose rather than glucose was fed. The rapid rate of fructose conversion to fatty acids in rat liver has been attributed to the rapid phosphorylation and conversion to trioses of fructose, effectively bypassing the initial rate-limiting steps of glycolysis. Control of the initial steps of glycolysis in chick liver may differ from that observed in rat liver because chick liver lacks a high K_m hexokinase similar to glucokinase of rat liver (see Ref. 15).

The pig was used to study the influence of various dietary carbohydrates in an animal model where adipose tissue is the major lipogenic organ. In the rat, where both the liver and adipose tissue actively participate in carbohydrate conversion to fatty acids, substitution of dietary fructose for glucose increased the rate of fatty acid synthesis in the liver and decreased rates of fatty acid synthesis in adipose tissue (present study and Ref. 8). In the pig, substitution of dietary

sucrose for glucose did not decrease but rather slightly increased malic enzyme activity and *in vitro* rates of fatty acid synthesis in adipose tissue. Pigs fed sucrose gained weight slightly but not significantly faster than did pigs fed glucose. It has been noted that fructose transport into the adipocyte is mediated by a carrier with a relatively high K_m for fructose and that adipose tissue lacks ketohexokinase (34). These observations coupled with the low circulating plasma fructose levels in the rats and pigs, suggest that dietary fructose was probably largely converted to glucose in the liver prior to metabolism in adipose tissue.

While it has been reported that the rate of fatty acid synthesis is increased in liver and depressed in adipose tissue preparations of rats fed fructose rather than glucose, it is important to note that the total capacity of the animal to synthesize fatty acids was not influenced by the source of dietary carbohydrate (8). The present results suggest that, in species where either the liver (chick) or the adipose tissue (pig) is the primary organ responsible for carbohydrate conversion to fatty acids, replacement of dietary glucose with fructose does not have a major influence on lipogenic enzyme activities or *in vitro* rates of fatty acid synthesis.

Summary. Rats, chicks, and pigs were fed diets containing fructose or glucose. Plasma triglyceride levels were elevated in rats but not in chicks or pigs fed diets containing fructose. The rate of fatty acid synthesis in rat liver but not in chick liver was elevated when fructose-containing diets were fed. Conversely, the rate of fatty acid synthesis in rat adipose tissue but not in pig adipose tissue tended to be depressed when fructose-containing diets were fed. These results indicate that there are species-specific as well as organ-specific metabolic responses to various dietary carbohydrates.

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