The Effect of Kind of Dietary Carbohydrate upon the Composition of Liver Fatty Acids of the Rat[1]

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Abstract

Weanling male rats were fed fat-free diets containing either starch, sucrose, glucose or maltose as principal ingredient. One fourth of each group was fed oral supplements of linoleate. Subgroups of the fat-free groups were killed at 1, 3 and 6 weeks and the linoleate supplemented group also at 6 weeks. Liver fatty acids were analyzed by gas chromatography. All groups showed changes in fatty acid composition characteristic of essential fatty acid deficiency, and linoleate reversed these changes. The three sugars induced similar compositions of liver fatty acids, but starch stimulated appearance of higher proportions of several odd-chain fatty acids. These included 15:0, 15:1, 17:0, 17:1, 17:2, 19:0, 19:1, 19:2, 19:3 and 19:4. These changes could not be accounted for by their content in the dietary starch.

Introduction

THE CONVERSION of carbohydrates into fats is a well-known phenomenon, and it is generally supposed that the composition of fats is independent of the type of carbohydrate fed. However, recent reports show that changes in the quantity and kind of carbohydrate can affect lipids of tissue. MacDonald (1) found that diets with relatively large quantities of carbohydrates and a low content of protein produced changes in the fatty acid composition of the adipose tissue of children. Ahrens et al. (2,3) reported a type of lipemia induced by carbohydrates, and mentioned some changes in lipid composition of human blood serum as a consequence of a high carbohydrate diet. De Somer, Eyssen and Evrard (4), working with chicks, found that both the absorption of fats and the growth-promoting effect of antibiotics were influenced by the type of carbohydrate used in the basal diet. Differences were most pronounced when sucrose, glucose or fructose were fed as the sole source of carbohydrate.

Anderson et al. (5) have studied the effect of glucose, sucrose and lactose on the serum lipid response, and they reported an increase of serum triglycerides with the sucrose but not with the other two sugars. Recently MacDonald and Braithwaite (6) reported different responses in the fatty acid composition in serum and adipose tissue when they fed starch or sucrose with a low fat diet to seven adult males.

The fat-free diet customarily used for study of essential fatty acid deficiency is a high carbohydrate diet. Sucrose has been consistently used for this purpose since the initial demonstration by Burr and Burr (7) that when starch was replaced by sucrose in a fat-free diet, a new deficiency syndrome was produced. Starch is usually avoided for this purpose because of the difficulty of removing traces of lipids which are probably held in the form of inclusion compounds. If the type of dietary carbohydrate influences lipid metabolism, this could be ideally demonstrated in experiments which employ fat-free high carbohydrate diets, for the influence of carbohydrate would be maximal and uncomplicated by dietary fat.

Our purpose in the present experiment was to study the effect of four different carbohydrates upon the composition of liver fatty acids of rats when they were fed a fat-free diet alone or with a methyl linoleate supplement. Effects of type of carbohydrate upon the metabolism of lipids may well differ in the EFA-deficient and linoleate-supplemented condition, for the metabolisms of carbohydrate and of fatty acids are interrelated. The study therefore includes EFA-deficient animals in which possible effects of different dietary carbohydrates would be superimposed upon EFA deficiency, as well as animals which received adequate quantities of linoleate.

Experimental

Seventy-five weanling, 24-day-old male rats of the Sprague-Dawley strain were maintained on a purified diet (8) of the following composition: vitamin-free casein, 18%; cellulose, 4%; carbohydrate, 74%; Wesson salt mixture, 4%. Each kilogram of diet contained the following, in milligrams: thiamin HCl, 30; riboflavin, 30; pyridoxine HCl, 8; dl-calcium pantothenate, 100; niacin, 100; i-inositol (meso), 220; p-aminobenzoic acid, 75; folic acid, 1; biotin, 0.2; vitamin B_{12}, 0.05; vitamin D_2, 4; vitamin K_5, 2; dl-α-tocopherol, 280; vitamin A acetate, 4; and choline chloride, 1000. Sucrose, glucose, maltose and starch were the carbohydrates used in the experiment. Lactose was not included in this study because rats cannot tolerate it in the high levels in a fat-free diet.

Commercial sucrose, analytical reagent grade D (+) glucose and D (+) maltose were used as simple carbohydrates. The starch used was soluble corn starch from Nutritional Biochemical Corp., free of dextrins, and containing 1% of ash. The content of lipids in the carbohydrates used in the experiment was estimated by cold extraction followed by refluxing for 1 hr with a mixture of chloroform:methanol (2:1). A continuous extraction under nitrogen with anhydrous ether also was performed. Both procedures were followed by transesterification of the extract with 5% HCl in methanol, weighing and analysis by gas-liquid chromatography (GLC). The content of lipid in the carbohydrates was found to be 0.03% in sucrose, 0.04% in maltose, 0.03% in glucose and 0.50% in starch by chloroform:methanol extraction. By extraction with ethyl ether all sugars contained less than 0.01% and starch contained less than 0.02% lipid. Extracts were found by thin-layer chromatography to be highly contaminated with sugars.

The composition of the fatty acids as their methyl esters from both the chloroform:methanol (2:1) and anhydrous ether extractions were measured by GLC

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on ethylene glycol succinate (EGS) and on Apiezon L columns, but many of the peaks from the starch sample could not be identified. The methyl esters of the fatty acids of the ether extract from the starch were also hydrogenated and examined by GLC. These results are shown in Table I.

The rats were divided into four groups according to the type of carbohydrate fed—starch, sucrose, glucose and maltose—and in all diets the carbohydrate contents were the same and all diets were fed ad-libitum. Each group was divided into four subgroups. Three subgroups were fed a fat-free diet and the other fed with supplementary methyl linoleate in daily oral doses by microsyringe in amounts equal to 1% of total calories estimated from measured food intake. Linoleate was obtained from The Hormel Institute and was found to have a purity greater than 99%. The principal impurity was oleate.

One subgroup of the fat-deficient animals from each main diet group was killed after 1, 3 and 6 weeks. The linoleate-fed rats were all killed after 6 weeks. All animals were killed by ether anaesthesia and the livers were quickly removed and kept in saline solution at −20C until analyzed.

The tissues were homogenized and the lipids extracted with chloroform:methanol (2:1) according to Folch et al. (9). The lipids were transesterified by refluxing with thirty volumes of 5% HCl in methanol and the methyl esters analyzed by GLC using a Barber-Coleman Model 10 instrument with an argon ionization detector and a column 210 cm by 5 mm I.D. packed with 20% EGS with 2% phosphoric acid coated on Gaschrom P, 80–100 mesh. An F & M Model 810 chromatograph with a hydrogen flame detector was also used, the column being 350 cm by 2 mm I.D. packed with 20% Apiezon L coated on Gaschrom P, 100–120 mesh.

TABLE I

Fatty Acid Composition of Lipid Extracts of Carbohydrates and of Hydrogenated Methyl Esters of Liver Fatty Acids from Rats Fed Starch
Values are Given as Percent of Total Fatty Acids

Fatty Acid	Lipid extracts from carbohydrates				Liver fatty acids	
	Sucrose	Glucose	Maltose	Starch, hydrogenated	Starch, fat-free	Starch-linoleate
12:0	3.2		2.0	3.6		
A[a]				1.2		
14:0	5.4	5.0	3.7	5.8	0.4	0.3
14:1+15:0	3.8	3.5	2.3			
15:0				2.6	1.0	0.8
B[a]				2.3		
16:0	31.6	32.0	23.3	35.9	22.3	18.6
16:1	8.1	9.6	5.4			
16:2+17:0	4.0	3.5	7.2			
17:0				3.5	4.5	2.2
C[a]				1.7		
D[a]						0.7
E[a]						0.8
18:0	12.7	13.6	7.8	35.5	41.5	38.3
18:1	21.2	20.7	15.0			
18:2	7.1	10.0	33.3			
F[a]				8.0		
G[a]					0.3	
19:0					2.3	0.8
20:0					22.9	28.6
21:0					0.6	
22:0					4.0	9.1

[a] Unidentified substances are indicated by letters. C is probably 17:1, and the others are probably branched fatty acids.

The individual esters were identified by equivalent chain length before and after hydrogenation (10). The presence of odd-chain fatty acids in the animals fed with starch was confirmed after separation of the methyl esters according to their chain length by preparative GLC using a Beckman GC-2 chromatograph with a column 240 cm by 7.3 mm I.D. packed with 20% β-cyclodextrin valerate on Chromosorb P, 30–60 mesh. This allowed separation of 40 mg of total esters, collected using the technique of Schlenk and Sand (11). Each fraction was analyzed by GLC on EGS and on Apiezon L columns and the chain length of each fraction was checked by hydrogenation. All

TABLE II

Fatty Acid Composition of Total Liver Lipids of Rats Fed Different Carbohydrates
Values Given as Percent of Total Fatty Acids with Standard Deviations

Fatty acid	ECL on EGS	Starch		Sucrose		Glucose		Maltose	
		Fat-free 6 Wk (3 Rats)	+18:2 6 Wk (5 Rats)	Fat-free 6 Wk (6 Rats)	+18:2 6 Wk (6 Rats)	Fat-free 6 Wk (6 Rats)	+18:2 6 Wk (5 Rats)	Fat-free 6 Wk (6 Rats)	+18:2 6 Wk (6 Rats)
12:0		0.09 ±.04	0.05 ±.05	0 08 ±.07	0.13 ±.21	0.02 ±.05	0.06 ±.02	0.02 ±.03	0.08 ±.04
14:0		0.73 ±.15	0.51 ±.21	0.89 ±.29	0.63 ±.15	0.49 ±.08	0.81 ±.58	0.47 ±.08	0.66 ±.23
15:0		1.55 ±.24	1.01 ±.80	0.39 ±.54	0.18 ±.05	0.10 ±.02	0.22 ±.05	0.12 ±.06	0.23 ±.09
16:0		12.5 ±2.6	15.0 ±2.8	19.7 ±4.0	16.0 ±.94	18.3 ±2.0	15.6 ±.92	16.3 ±.78	17.3 ±2.7
16:1ω7	15.64	7.5 ±.51	3.3 ±.47	9.0 ±2.0	6.6 ±1.5	7.2 ±1.0	5.9 ±.50	7.74 ±1.3	6.44 ±1.6
17:0		2.63 ±.07	2.08 ±.88	0.26 ±.03	0.37 ±.09	0.31 ±.04	0.44 ±.17	0.30 ±.10	0.43 ±.12
17:1	17.53	3.27 ±.39	0.83 ±.27	0.36 ±.12	0.42 ±.05	0.37 ±.06	0.47 ±.11	0.41 ±.08	0.43 ±.14
18:0		13.4 ±1.3	16.4 ±3.1	14.1 ±2.6	16.4 ±1.5	15.5 ±.55	17.0 ±1.4	16.5 ±1.7	15.3 ±1.6
18:1	18.52	24.4 ±1.5	16.9 2.5	30.2 ±3.7	21.5 ±2.2	26.3 ±2.1	22.8 ±1.6	26.8 ±2.0	22.7 ±3.9
19:0?	18.96	0.55 ±.07	0.23 ±.13	0.26 ±.30	0.23 ±.12	0.77 ±.21	0.41 ±.13	0.73 ±.11	0.26 ±.07
18:2ω6	19.28	3.09 ±1.7	8.58 ±2.4	1.69 ±.24	5.26 ±.72	2.19 ±.46	4.78 ±.30	2.15 ±.23	0.05 ±1.3
19:1	19.67	0.37 ±.06	0.05 ±.12	0.35 ±.21	0.46 ±.60	0.60 ±.18	0.15 ±.04	0.45 ±.13	0.18 ±.11
19:2?	19.93	0.21 ±.12	0.34 ±.18	—	0.19 ±.05	0.12 ±.11	0.21 ±.06	0.08 ±.09	0.22 ±.08
19:3	20.49	1.96 ±.36	0.40 ±.20	0.44 ±.12	0.70 ±.30	0.67 ±.18	0.56 ±.18	0.66 ±.10	0.52 ±.12
19:4	20.94	0.23 ±.20	0.02 ±.03	0.24 ±.07	0.32 ±.18	0.37 ±.15	0.09 ±.12	0.32 ±.19	0.11 ±.12
20:3ω9	21.47	9.5 ±1.4	0.89 ±.57	10.7 ±3.0	5.15 ±.94	12.7 ±1.7	4.70 ±.57	13.5 ±1.2	4.46 ±2.2
20:3ω6	21.74	1.28 ±.11	0.67 ±.08	1.07 ±.29	1.44 ±.26	2.40 ±.35	1.11 ±.31	1.85 ±.87	1.23 ±.26
20:4ω6	22.27	9.9 ±4.4	25.3 ±3.1	6.43 ±1.8	17.1 ±2.4	7.5 ±2.1	18.2 ±2.4	6.8 ±1.5	16.9 ±5.0
22:4ω6	24.26	0.71 ±.25	1.95 ±.81	0.49 ±.37	0.92 ±.18	0.75 ±.23	1.08 ±.39	0.89 ±.30	0.84 ±.18
22:5ω6	24.69	1.63 ±.17	3.89 ±1.8	1.59 ±.76	4.13 ±.90	1.17 ±.25	3.84 ±.83	1.26 ±.33	3.53 ±.46
22:6ω3	25.70	3.83 ±.51	3.41 ±1.3	1.99 ±.61	2.17 ±.70	2.00 ±.43	2.12 ±.44	3.20 ±1.4	1.98 ±.61
22:3ω9	23.17	0.96	0.06	1.7	0.30	1.7	0.26	2.2	0.26

liver samples were analyzed using EGS columns, quantification was by triangulation and the results are expressed as area percent.

In the abbreviated notation used in the tables, fatty acids or their methyl esters are designated by chain length and number of double bonds. For example, octadecadienoic acid is 18:2. When double bond position is to be designated, and when double bonds are methylene-interrupted, the position of the first double bond counting from the terminal (ω) methyl group is indicated as a suffix. Thus, linoleate is 18:2ω6.

Results and Discussion

The weight gain of the several groups of rats showed striking differences. The average total weight gain over the six weeks period was 31.7 g for fat-free starch diet, 65.6 g for starch plus linoleate, 78.8 g for fat-free sucrose diet, 104.2 g for sucrose plus linoleate, 121.5 g for fat-free maltose, 141.5 for maltose plus linoleate, 122.1 g for fat-free glucose and 137.3 g for glucose plus linoleate. With all carbohydrates the addition of linoleate increased the weight gain. The starch diet supported the least growth, and mortality was highest in the starch, fat-free diet group. The duration of the experiment was too short to induce measurable dermatitis of essential fatty acid deficiency.

The fatty acid composition of the liver lipids is shown in Table II. Although the fatty acid compositions at 1 and 3 weeks showed progressively the same effects indicated at 6 weeks, the magnitude of change was less, and these data have therefore not been included in the table. The liver fatty acids of rats fed glucose, maltose or sucrose were quite similar in composition. The animals fed with starch, both fat-free and linoleate-supplemented, showed considerable differences from the other groups. Their liver fatty acids contained larger proportions of 18:2ω6, 20:4ω6 and 22:6ω3, and smaller proportions of 16:0, 18:1ω9 and 20:3ω9. Starch-fed groups also showed a significant increase in odd-chain fatty acids. From those animals fed a fat-free starch diet, 8.5% of odd-chain fatty acids was found in the liver fatty acids after hydrogenation. Not only saturated odd-chain fatty acids but also highly unsaturated odd-chain fatty acids were found in the liver lipids. The equivalent chain lengths on EGS and Apiezon L columns and contents of the components of the odd-chain fractions obtained by preparative GLC are shown in Table III. The chain lengths of these fractions were checked after hydrogenation, revealing that the C_{15}, C_{17} and C_{19} fractions were principally of the designated chain lengths, with minor proportions of shorter and longer homologs. The tentative identifications are based upon GLC comparisons with authentic standards of 15:0, 15:1, 15:2, 17:0, 17:1, 17:2, 19:0, 19:1, 19:2, 19:3 and 19:4. The differences in the equivalent chain lengths measured on EGS and Apiezon L (10) revealed the number of double bonds present.

The analysis of the hydrogenated total methyl esters (Table I) from pools of liver lipids from the starch-fed animals killed after six weeks showed less C_{19} and C_{21} acids from the linoleate-supplemented rats than from those of the fat-free group. The principal difference in the C_{17} acids was with 17:1.

The differences in liver fatty acid composition among groups fed mono- and di-saccharides were minor. The main differences were that rats fed sucrose only had somewhat larger amounts of 14:0, 16:0 and 18:1, and smaller quantities of 18:2ω6. The glucose-fed rats had a smaller content of 16:0 in the linoleate-supplemented group and the animals fed with maltose only had a larger proportion of 20:3ω9. Most of the odd-chain fatty acids discussed above were found in low but measurable proportions in liver fatty acids from rats fed the mono- and di-saccharides. This suggests that they were normal constituents which were increased when starch was the dietary carbohydrate. For all carbohydrate-fed groups the chain length of suspected odd-chain acids was verified by hydrogenation followed by GLC analysis. The content of odd-chain fatty acids in livers of rats fed sugars was consistently smaller when analyzed by hydrogenation-GLC than by GLC analysis on EGS, suggesting that in the latter some of the odd-chain components coincide with even-chain components of unknown structure.

TABLE III

Characterization of Odd-Chain Fatty Acids from the Livers of Starch-Fed Rats

	Tentative identification	Relative area %	ECL on EGS column	ECL on Apiezon L column
	15:0	74.7	15.03	15.00
C_{15}	15:1	19.9	15.62	14.75
Fraction	br16:0	4.2	15.97	15.12
	br16:1	1.1	16.60	——
	17:0	44.3	17.04	17.00
C_{17}	17:1	54.4	17.56	16.71
Fraction	17:2	1.4	18.38	16.60
	br19:0	5.9	18.77	——
	19:0	8.0	19.03	18.99
	19:1	24.4	19.51	18.65
	?	7.8	19.85	——
C_{19}	19:2	3.5	20.05	18.36
Fraction	19:2	2.7	20.29	——
	19:3	40.6	20.53	18.19
	19:3	3.8	20.76	——
	19:4	3.3	20.93	——

In general, all the experimental diets produced the fatty acid compositional changes that could be expected from fat-free diets and linoleate supplements, regardless of dietary carbohydrate. This was indicated by the increase in the ratio of 20:3ω9/20:4ω6 with time in all the groups fed fat-free diet and the small value for those rats supplemented with methyl linoleate (Table II). The principal differences in fatty acid composition observed in this experiment were those from animals fed with starch, and they are to some extent in agreement with the results reported by MacDonald and Braithwaite (6) in human serum lipids, but not with their results obtained in human adipose tissue. The results presented here are difficult to interpret because the starch was more contaminated by lipids than were the sugars. In our study we used highly purified starch, because commercial corn starch contained at least four times more lipids in which the principal fatty acid present was 18:2ω6. In our experiment the rats fed with starch received an extra 0.03% of total calories (approximate) as total lipids of which 18:2 was not more than 10%. Thus the linoleate contribution by the starch is in the order of 0.003% of calories. The maximum amount of total C_{18} fatty acids fed per rat for the entire 6 weeks period was 23 mg, whereas each animal received 1.68 g of linoleate as oral supplement. Thus, it seems unlikely that the fatty acid changes induced by the starch diet were induced by the linoleate content of the starch.

Similar calculations of the odd-chain fatty acid content of starch reveal that each rat received during the six weeks period 1.7 mg C_{15} acids, 2.3 mg C_{17} acids and no measurable C_{19} acids. The liver of one animal fed starch was found to contain 1.5 mg of 15:0, 2.3 mg 17:0, 2.7 mg 17:1, 2.5 mg of C_{19} acids, and 0.6 mg of C_{21} acids. Thus, the odd-chain content of the ani-

mal's liver alone exceeds the amount he ingested, and if whole body calculation were possible it might be at least one order of magnitude larger still. Therefore, it seems unlikely that the odd-chain acids detected in the animal arose only from the lipids of the starch ingested. The starch diet caused diarrhea in the rats, suggesting a change in intestinal flora. One explanation for the increased content of odd-chain fatty acids (8.5%) in the livers of these animals may thus be an increased bacterial synthesis of odd-chain acids in the intestinal lumen. Another might be alteration of lipid synthesis by the kind of carbohydrate fed. However, the mechanism of increased synthesis of odd-chain fatty acids is not known or suggested.

In conclusion, the kind of dietary carbohydrate has been shown to influence the fatty acid composition of liver lipids. Dietary sucrose, maltose and glucose produce minor differences, but starch causes a larger proportion of the liver fatty acids to be odd-chain fatty acids. All of the carbohydrate diets permitted induction of fatty acid compositions characteristic of essential fatty acid deficiency, indicating that the development of essential fatty acid deficiency on the traditional high sucrose diets has not been an artifact related to sucrose, but that it is a deficiency observable irrespective of type of dietary carbohydrate. Although the metabolism of carbohydrate and the metabolism of fatty acids are interrelated, high levels of different dietary sugars caused only minor differences in fatty acid composition in both linoleate-supplemented and linoleate-deficient rats. On the other hand, dietary starch induced higher levels of odd-chain fatty acids than did the dietary sugars in both deficient and supplemented rats.

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Competitive Inhibitions in the Metabolism of Polyunsaturated Fatty Acids Studied via the Composition of Phospholipids, Triglycerides and Cholesteryl Esters of Rat Tissues[1]

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Abstract

Male rats which had been kept on fat-free diet and which were deficient in essential fatty acids were divided into ten groups. All ten groups received 0.8% of calories of linolenate, and nine received one of three levels of either linoleate, γ-linolenate or arachidonate for a period of six days. The rats were sacrificed, the livers, kidneys and testes were extracted, and the phospholipids, triglycerides and cholesteryl esters were separated by thin-layer chromatography. The fatty acid composition of each was determined by gas-liquid chromatography. The inhibition of the metabolism of linolenic acid by linoleate, γ-linolenate and arachidonate was evidenced in all three lipid classes and in all tissues. The activities in suppressing linolenate metabolism were in the order 20:4 > 18:3 > 18:2.

Introduction

SEVERAL STUDIES in this laboratory have confirmed conclusively that there is no metabolic crossover between the different families of polyunsaturated fatty acids. The studies were made with rats which had been maintained on fat-free diets supplemented with highly unsaturated fatty acids of different families (1–4). These conclusions are in accord with those of Mead et al. (5) and Klenk and Mohrhauer (6) who also found via tracer experiments no interconversion between fatty acids from different families in mammalian tissue. But although no conversion takes place between families of fatty acids, inhibitions and competitions between fatty acids from different families have been amply demonstrated (7,8). The decrease of the trienoic acid content of tissues of rats fed a fat-free diet plus linoleic and linolenic acids was observed by Rieckehoff et al. (7). Fulco et al. (9) proved that the triene was 5,8,11-eicosatrienoic acid and the inhibition of the synthesis of the oleic family (ω9) by linoleate (ω6) and linolenate (ω3) was clear.

The decreased proportion of tissue oleic acid with increasing amounts of dietary linoleic acid was observed by Dopeshwarkar and Mead (10) and they suggested that oleate and linoleate compete as substrate for the enzymes involved in the linoleic ⟶ arachidonic acid conversion when dietary oleate is abundant compared with dietary linoleate. These suggestions are supported by the work of Tinsley (11), Bozian

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