Effect of Dietary Carbohydrate on Fatty Acids in the R3230AC Mammary Adenocarcinoma[1] (37193)

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It is now well established that alteration of the diet has a significant effect on lipogenesis. Feeding a diet high in carbohydrate and low in fat content resulted in an increase in lipogenesis in the liver and in adipose tissue in the rat (1). These changes are often accompanied by changes in the activities of a number of enzymes concerned with lipogenesis (2, 3). As a result of earlier studies with the R3230AC mammary tumor, wherein administration of estrogen caused an increase in free fatty acid and triglyceride content in the neoplasm (4), an investigation of the effect of dietary glucose supplementation was conducted. It was found that consumption of approximately 2.5 g of glucose/day in addition to the chow diet produced an increase in the levels of free fatty acids and triglycerides in the mammary adenocarcinoma, but not in the mammary gland of the tumor-bearing animal (5). Since no attempt was made to identify the individual fatty acids in these lipid classes, it seemed logical to explore the effect of diets varying in carbohydrate content on the fatty acid composition of the lipids in this neoplasm. The data indicate that the increased levels of free fatty acids and triglycerides in the tumor, resulting from high carbohydrate feeding, are due primarily to an increase in the shorter chain fatty acids.

Materials and Methods. Female Fischer rats, 80 to 100 g, were transplanted with the R3230AC mammary adenocarcinoma by a sterile trochar technique. Six animals were placed in each group and received the following diets for 3 wk: Group I, Purina laboratory chow; Group II, high carbohydrate diet; and Group III, zero carbohydrate diet.[4]

Animals were sacrificed 21 days after tumor implantation. Tumors and livers were quickly removed, trimmed, weighed and quick-frozen in liquid nitrogen. Samples were stored at —20° until assayed.

Tissues were thawed, homogenized (10 to 20%, w/v) in cold Tris buffer (pH 7.4), and aliquots of the homogenates were extracted for lipid as described previously (5). The lipids were separated by thin layer chromatography utilizing ascending chromatography on silica G precoated plastic sheets (0.25 mm thickness, Brinkmann Co.). Cholesterol, free fatty acids and triglycerides were localized under uv light, after the plates were sprayed with a 0.01% solution of rhodamine 6G in ethanol. The lipids were scraped from the plate and eluted from the gel and a portion of the eluate was taken for colorimetric assay, according to methods described earlier (5). Recoveries were 90% or greater.

An aliquot of the eluates containing either free fatty acids or triglycerides was taken and the fatty acids were converted to their methyl esters using either a BCl_3–methanol mixture (Supelco Inc., Bellefonte, PA) for the free fatty acids or a mixture containing

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[4] The diets contained the following: Purina rat chow, protein, 23%; fat, 4.6%; carbohydrate, 52.7% and ash, 6.3%; high carbohydrate diet (Nutritional Biochemicals), casein, 18%; vegetable oil, 8%; sucrose, 68%; Brewers yeast, 2% and salt mix, 4%; and zero carbohydrate diet (Nutritional Biochemicals); casein, 18%; vegetable oil, 8%; alphacel; 68%; Brewers yeast, 2% and salt mix, 4%.

TABLE I. Effect of Dietary Carbohydrate Levels on Lipid Classes in the R3230AC Mammary Tumor and Liver.

Diet	Tumor wt (g)	Cholesterol		Free fatty acid		Triglyceride	
		Tumor	Liver	Tumor	Liver	Tumor	Liver
Control (chow)	2.66 ± 0.37	1.21 ± 0.05	0.74 ± 0.04	2.77 ± 0.08	1.98 ± 0.23	26.6 ± 2.5	0.8 ± 0.1
High carbohydrate	2.99 ± 0.49	1.35 ± 0.04[a]	0.96 ± 0.08[a]	4.88 ± 0.45[a]	5.07 ± 0.90[a]	49.3 ± 5.0[a]	5.2 ± 0.8[a]
Zero carbohydrate	2.18 ± 0.40	1.38 ± 0.02[a]	0.77 ± 0.06	5.04 ± 0.31[a]	3.06 ± 0.20[a]	13.3 ± 1.8[a]	0.3 ± 0.01[a]

[a] Differs significantly ($p < .05$) from the control (chow-fed) animals.

benzene (7.0 ml), 2,2-dimethoxypropane (0.5 ml) and 2.5 ml methanolic-HCl (10%, w/v) for the triglycerides. Gas–liquid chromatography was performed using the F & M Model 402 gas chromatograph with hydrogen flame ionization detector. The following conditions were used throughout: columns were glass U-tubes, 6 ft × 2 mm, containing 15% diethylene glycol succinate on Chromosorb W (80–100 mesh), operated isothermally at 200° oven temperature, 240° flash heater and 240° detector temperature, with an argon carrier flow rate of 40 ml/min. Identification of individual fatty acids was done by comparison of retention times with known standards and quantitation was achieved by calculation of areas under the peaks. Recoveries were 95% or greater. The data are expressed as moles/100 moles of fatty acid. Data are presented as mean ± SEM and significance was determined by the Student's *t* test; a probability (p) value of 0.05 or less was considered to be significant.

Results. Table I summarizes data on the levels of cholesterol, free fatty acids and triglycerides in the R3230AC tumor and liver from animals receiving the chow diet (control), the high carbohydrate diet or the zero carbohydrate diet. The average daily consumption of the chow diet and of the high carbohydrate diet was approximately 10 g/day/animal, whereas the daily consumption of the zero carbohydrate diet was 55 g/day/animal; body weight gain for the experimental period was 46 g for the animals fed laboratory chow, 51 g for the animals consuming the high carbohydrate diet and 46 g for the animals receiving the zero carbohydrate diet. Tumor growth was not influenced by the carbohydrate content of the diet. Although cholesterol content of the tumors was slightly increased, the ingestion of the diet high in carbohydrate content resulted in a significant increase in the free fatty acid and triglyceride content of the neoplasms. Although the tumors of animals fed the zero carbohydrate diet had an elevated level of free fatty acids, the amount of triglyceride in these carcinomas was reduced compared to neoplasms of animals that consumed laboratory chow. The livers of these animals were also examined

and the changes in lipids were similar in the livers as seen in the tumors. Thus, both an abnormal and normal tissue showed similar alterations in lipids as a result of alteration of dietary carbohydrate.

The effect of diet on the composition of fatty acids in the free fatty acid fraction and in the triglyceride fraction is shown in Table II. The response of the neoplasm in the animal consuming the high carbohydrate diet was reflected by an increase in the shorter chain fatty acids, C_{10}, C_{11}, and C_{12} along with a decrease in the medium length fatty acids, particularly in oleic acid in the free fatty acids fraction and in palmitic and oleic acids in the triglyceride fraction. It is interesting that the composition of the free fatty acid fraction of the tumor from animals ingesting a carbohydrate-free diet was quite similar to that found in the neoplasms of animals receiving the high carbohydrate diet, *i.e.*, increases in shorter chain fatty acids and decreases in longer chain fatty acids. Examination of the fatty acid composition of the triglyceride fraction of tumors from animals deprived of dietary carbohydrate showed few significant changes from those seen in tumor triglycerides of chow-fed animals, although the data suggest that the direction of changes were similar in animals consuming a high carbohydrate diet and in animals consuming a carbohydrate-free diet when compared to the chow-fed animals. The livers of these tumor-bearing animals were also examined for individual fatty acids. In the animals receiving the high carbohydrate diet, the composition of the free fatty acid fraction showed a decrease in the amounts of C_{16} and an increase in the levels of $C_{18:1}$ and $C_{18:2}$, but no change in the fatty acid composition of the triglyceride fraction. In contrast, livers from animals consuming the zero carbohydrate diet demonstrated an increase in C_{16} and a decrease in $C_{18:1}$, whereas an increase in C_{14} and $C_{18:2}$ fatty acids was also noted in the triglyceride fraction compared to the control (chow) animals. Thus, the influence of altered carbohydrate diets was seen in both normal and abnormal tissues.

Discussion. The present data confirm the earlier finding of Hilf *et al.* (5) on the influence of dietary carbohydrates on lipid levels in the R3230AC mammary tumor and extend these studies to demonstrate that the mammary tumor responded to an increase in car-

TABLE II. Effect of Diet on Fatty Acids in R3230AC Mammary Adenocarcinomas and Livers.

	Free fatty acids (moles/100 moles)			Fatty acids from triglycerides (moles/100 moles)		
Fatty acid	Control	Hi-carb	Zero-carb	Control	Hi-carb	Zero-carb
R3230AC Tumor						
C_{10}	4.9 ± 1.5	9.8 ± 1.0[a]	6.1 ± 0.9	8.5 ± 2.9	16.3 ± 1.4[a]	10.2 ± 4.5
C_{11}	0.6 ± 0.1	1.1 ± 0.2[a]	1.8 ± 0.2[a]	0.2 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
C_{12}	14.7 ± 2.1	23.3 ± 1.9[a]	23.5 ± 3.4[a]	37.2 ± 3.0	47.1 ± 1.5[a]	45.1 ± 2.4
C_{14}	7.6 ± 0.6	8.4 ± 0.4	9.8 ± 0.6[a]	19.7 ± 0.8	15.2 ± 1.7	18.0 ± 2.1
C_{16}	18.7 ± 1.0	16.1 ± 1.0	14.8 ± 0.9[a]	16.6 ± 1.0	10.0 ± 1.7[a]	14.3 ± 1.8
$C_{16:1}$	10.6 ± 0.6	9.5 ± 0.5	15.0 ± 1.2[a]	3.8 ± 0.2	2.8 ± 0.5	4.1 ± 0.4
C_{18}	12.4 ± 1.1	10.5 ± 1.0	8.9 ± 0.8[a]	2.9 ± 0.4	1.9 ± 0.4	1.8 ± 0.2[a]
$C_{18:1}$	26.6 ± 1.9	20.2 ± 1.0	20.4 ± 2.3	8.5 ± 0.6	5.3 ± 0.9[a]	7.1 ± 1.0
$C_{18:2}$	3.8 ± 0.7	1.9 ± 0.8	1.0 ± 0.4[a]	2.9 ± 0.3	0.7 ± 0.1	0.6 ± 0.1[a]
Liver						
C_{14}	2.6 ± 0.2	2.0 ± 0.1	1.4 ± 0.6	1.4 ± 0.2	1.4 ± 0.2	2.8 ± 0.3[a]
C_{16}	43.1 ± 1.0	26.6 ± 1.3[a]	53.8 ± 3.2[a]	39.9 ± 1.3	40.9 ± 1.4	35.2 ± 1.8
$C_{16:1}$	4.5 ± 0.6	8.4 ± 2.1	3.7 ± 0.6	2.8 ± 0.4	5.2 ± 1.2	3.5 ± 1.4
C_{18}	16.7 ± 0.6	15.4 ± 2.3	15.2 ± 0.2	6.2 ± 1.8	2.1 ± 0.1	16.2 ± 6.0
$C_{18:1}$	18.8 ± 1.2	26.0 ± 1.3[a]	13.4 ± 1.2[a]	35.8 ± 1.4	33.1 ± 0.8	19.3 ± 4.3[a]
$C_{18:2}$	14.3 ± 1.3	22.4 ± 1.4[a]	11.3 ± 3.4	13.9 ± 2.5	17.1 ± 3.0	22.9 ± 2.2[a]

[a] Differs significantly ($p < .05$) from the control (chow-fed) animals.

bohydrate in the diet with an elevation in the levels of the shorter chain fatty acids. Since the shorter chain fatty acids, C_{10} and C_{12}, are characteristic of mammary tissue (6), it would appear that this mammary carcinoma contains cells that have retained a metabolic capacity similar to that demonstrated by normal mammary cells. These results are further indication of the "lactation-like" response of the neoplasm, which was shown to occur after the administration of estrogens; a secretory fluid containing casein and lactose was induced in the carcinoma by treatment with estradiol valerate (7).

It is of interest that in the case of this neoplasm, a lipogenic response to dietary carbohydrate alterations occurred, whereas the virginal mammary gland demonstrated little or no response to the ingestion of a glucose supplement (5). A somewhat similar lack of responsiveness of the virginal mammary gland was reported by Smith *et al.* (6) who studied the effects of dietary fat in the C3H mouse. These results are quite opposite to the studies of hepatomas, in which the hepatomas show little or no responsiveness to the same dietary changes that markedly influence the normal liver (8, 9). The lack of responsiveness in these hepatomas cannot be due to differences in pathways for fatty acid synthesis nor to differences in blood supply (10). It would appear that the R3230 AC mammary tumor is quite sensitive to regimens that alter lipogenesis, a fact that may also account for the heightened secretory response observed after hormonal treatment. This response is not due simply to an increase in endogenous insulin secretion, as might be expected as a result of ingestion of a high carbohydrate diet, since administration of 2 IU of insulin daily for 3 wk had no effect on the lipid composition of the neoplasms (11).

Summary. The R3230AC mammary carcinoma demonstrated an increase in free fatty acids and triglyceride levels in animals consuming a diet high in carbohydrate content. Examination of the fatty acid composition of the lipid fractions in the tumor revealed that the dietary response was due primarily to an increase in the levels of the shorter chain fatty acids, C_{10} and C_{12}, fatty acids that are characteristic of mammary tissue.

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